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Interaction between threonine and glycine at low dietary crude protein and the effect on production performance, meat quality and plasma metabolites in broiler chickens

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PLENARY LECTURES

PROTEIN DIGESTION RE-VISITED BY PROTEOMICS

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Abstract

Further understanding of the mechanisms underlying protein digestion by poultry species is a prerequisite for a more efficient use of diverse protein rich feedstuffs. In this respect, proteomics methods are valuable tools, which can be applied to the feedstuffs or their digestion products. Basically, they rely on the combination of separation techniques (gel based or gel free) with mass spectrometry (MS) based identification, both of which have tremendously progressed in the last two decades and have also benefited of the increasing availability of sequence databases and bioinformatics. A number of studies applied to food products highlighted the power of these methodologies to identify the diversity of proteins in foodstuffs, of their digestion products *in vitro* or *in vivo*, and of undigested terminal products, including peptides with functional activities. Data about relevant feedstuffs and their digestion by poultry, or other livestock species, are still scarce. This paper reviews published data and ongoing research and perspectives arising from these.

Keywords: poultry, nutrition, broiler, protein

Introduction

Feed is the main contributor to economic and environmental costs of poultry production schemes. This applies in particular to the protein fraction of the diet, as fast growing broilers require high amount of protein in their diet, with optimal amino-acid profiles. They usually perform best when fed diets based on corn and soybean meal whose composition and nutrient availability are well known and relatively constant, and which are classically used for the breeder's selection schemes. Depending on the global and local contexts of the feedstuff production and markets, legislation, and consumer's demand, broiler producers consider incorporating alternative protein sources, with a lesser digestibility of the nitrous fraction, a suboptimal amino acid availability, which may reduce the performances. Although numerous alternatives have been tested and for a long time now, only few studies considered the actual mechanisms of protein digestion. This paper intends to review the available literature in poultry and other livestock species, and to consider how the advent of proteomics opens new perspectives for a better understanding of protein digestion and its future optimisation through the combination of alternative feedstuffs, improved technological process and incorporation of additives in the diets.

The principle of proteomics and its application in the food/feed sector

Proteomics is a global approach of the set of proteins present in a biological sample. It usually relies on the extraction of the proteins from the sample, their separation using gel based (1D or 2D SDS PAGE) or gel free (HPLC: reverse phase, ion exchange or size exclusion chromatography) methods, protein digestion, followed by mass spectrometry analysis. Depending on the mass spectrometer used the obtained data are protein or peptides molecular masses, or information on amino acid sequence. The interpretation depends on the quality of the available protein database in the biological organism where the sample originate. Further information can be obtained in a recent review article by Cunsulo et al, 2014. Proteomics has been applied to the study of food or feed protein digestion, and the terminology "protein digestomics" has been put forward by Picariello et al, 2013. These authors identified a growing activity in this field since the year 2000. Samples range from undigested food (feed) to digestion products collected *in vitro* (static or dynamic models) or *in vivo* (gastro intestinal tract contents). It is a valuable tool to understand how proteins are digested (or not) along the digestive tract, and to identify the host proteins involved in this process. The power of the methodology relies on the availability of protein (or genomic) sequence data from the organism providing the food (feed) stuff and the host. A further step of integration is the classification of proteins using Gene Ontology terms to appreciate the major biological processes involved.

Illustrations on the power of proteomics in the food sector are available in the review by Cunsulo et al, 2014, and allow to speculate on future perspectives for the feed sector. Protein profiling can be used as a way to assess food quality. Examples are available for wheat and for milk. As wheat is also an important feedstuff, it is worth mentioning that the identification of high molecular weight gluten subunits has been cited as a method for the identification of wheat varie-

ties, whether for quality assessment or authentication (Cunsulo et al, 2014). The identification of protein modifications has been cited as a way to evaluate the consequences of food processing and storage conditions (Cunsulo et al, 2014).

Peptidomics (Dallas et al, 2015), an adaptation designed for the identification of peptides, has also been used to detect digestion products and especially those having functional roles. Soybean, sunflower, cereals, maize and algae proteins have been cited as sources of such bioactive peptides, which could also benefit livestock's performances and health (see review by Cunsulo et al, 2014).

Studies on protein digestion in animals, including broilers

The European Union, which is largely dependent upon importation of proteins for animal feeding has long considered alternative feedstuffs from home-grown species (see review by Crévieu-Gabriel, 1999). Pea is rich in proteins (18 – 30 %) and in lysine, thus being a good complement to cereals. It can be incorporated to poultry diets up to 10 % without adverse effects on performances and meat quality, although the digestibility of N is lower than for soybean meal based diets. The apparent faecal digestibility of N lies between 67 and 83 % (Carré & Conan, 1989, cited by Crévieu-Gabriel, 1999), lower than in pig. An experimental approach has been conducted to identify the mechanisms underlying digestion of pea proteins in broilers. The globulin fraction was observed to be well digested (87 %), despite its globular structure and low solubility. The combination of polyacrylamide gel electrophoresis and immunoblotting (IB) with antibodies directed against specific pea proteins allowed to obtain important results. Undigested polypeptide fractions of albumin (PA2), lectin and legumin were detected at the terminal ileum (Crévieu et al, 1997a and Gabriel et al, 2008). An endogenous protein of MW 57 kDa also accumulated in the ileum of birds fed pea or N free diet (Crévieu et al, 1997 a and b).

As a first example of using proteomics for feed digestion by monogastrics, Le Gall et al (2005) conducted a study in pigs fed diets incorporating different pea cultivars. Ileal proteins were separated by 1D gel electrophoresis and subsequently identified by immunoblotting or by LC-MS-MS. The MS-MS strategy was found appropriate to identify endogenous protein losses while immunoblotting was more suited to the detection and identification of dietary proteins. This might have resulted from the limited amount of protein sequences available for pea at the time. The endogenous protein losses corresponding to porcine enzymes and to antibodies varied between pea cultivars, which the authors linked to their relative fibre contents.

In an ongoing study (Recoules et al, 2015), protein digestion was followed in 21 day-old broilers fed semi-synthetic diets based on a single protein source (S: soybean meal, R: rapeseed meal, P: pea or C: corn DDGS). The soluble fractions of jejunal contents were analysed by 1D gel electrophoresis and LC-MS-MS, allowing for the identification of proteins with molecular weights between 15 and 75 kDa. Five bands (25, 26, 27, 36 and 55 kDa) were present in all samples, regardless of the protein source. The proteomics approach allowed identifying a number of chicken functional proteins, with a high proportion involved in digestive processes (protein digestion, carbohydrate metabolism, nucleic acid metabolism or intestinal homeostasis). Of the 59 chicken proteins identified, 18 were found in all diets, while 15 were diets specific (12 for S, 3 for P), suggesting a specific induction in response to the diet. These proteins, or fragments, were detectable in all common bands, where they were also mixed with plant proteins, and not only the 57 kDa, which likely corresponded to the 55 kDa band by Crévieu et al, 1997 as endogenous, by collecting samples from birds fed a N free died. For the pea based diet, two specific bands of 16 and 24 kDa were identified and contained pea proteins with equal or lower theoretical MW: lectin, legumin and Bowman Birk Inhibitor (BBI), a trypsin inhibitor. This suggested that these proteins were hardly degraded. The observations concerning lectin and legumin were consistent with those made by Crévieu et al, 1997 and Gabriel et al, 2008, in chickens or Le Gall et al, 2005 in pigs, for lectin, using IB with specific antibodies. Similarly, soybean or rapeseed derived proteins were detected in samples collected from birds fed the corresponding diets. By contrast, no residual product was detected in the Corn DDGS based diet. As the corn protein database is the most documented, and assuming that technical problems such as extraction and solubilisation of proteins from different materials can be ruled out, this would suggest that corn proteins are highly digestible. By contrast, the occurrence of indigestible products may be underestimated in feedstuffs originating from species where data are scarce. Indeed, available protein sequences were about 3700 for Pisum (Pea), 18500 for Brassica (Rapessed), 105000 for Glycine (Soybean) and 175000 for Zea (Corn).

Conclusions and perspectives

The available and coming data show the interest of proteomics for a better characterisation of feedstuffs and for understanding the mechanisms underlying protein digestion. The following outcomes may be expected:

- A more detailed description of the products of digestion of plant proteins in the different parts of the digestive tract and their kinetic of appearance, the identification of resistant proteins or peptides, the understanding of their interaction with endogenous proteins.
- A better identification of endogenous proteins: i.e. a full list of enzymatic activities in the GI tract of the chicken, including the evaluation of the activity, by zymography for example. This will be completed by genomics studies for the identification of the expressed genes, thanks to the relatively rich genomics databanks in the Gallus gallus species.
- The obtained knowledge may ultimately lead to innovations for improving protein digestion, such as selection of more adapted cultivars, development of new feed processing schemes, or addition of appropriate additives.
- In a longer term, the feed sector could benefit from new ideas coming from the food sector. For example, the identification of bioactive peptides in raw materials could contribute to combine improved animal health and reduced medicinal inputs.

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RELEVANCE OF GUT MICROBIOTA FOR DIGESTION

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Summary

Microbes contribute to the digestive process in chicken. Bacterial fermentation of non-digestible carbohydrates and dietary fibre helps to generate energy for the host. Thus, microbial fermentation can be considered as trophic factor especially when high fibre ingredients and by-products are used. Protein fermentation yields a spectrum of undesirable metabolic products such as ammonia that can have negative effects on animal health and performance. However, the impact on the host is not yet clear and deserves more in depth work in chicken. Fat digestion is affected by microbial activities in the small intestine, especially bacteria with the ability to deconjugate bile acids can reduce the apparent digestibility of fat in the small intestine. Besides the gross composition of the diet, minor components such as amino acids and other feed additives have been shown to affect the microbial composition, metabolic activity and may impact animal health. Manipulation of the intestinal microbiota and optimizing the interaction with the host organism is a promising scientific field to improve animal performance, health and well-being. This is of high interest as the use of antibiotics should be as far as possible minimized in chicken production.

Key words: microbiota, digestion, fermentation, health, performance

Introduction

The intestinal microbiota has important functions in the digestive process in all animal species including humans. This was intensively studied in ruminants, but it became clear that monogastric animals such as poultry have a highly diverse microbial colonisation of the gastrointestinal tract (Waite and Taylor 2014) which is of utmost importance for the digestive process, animal health and finally also well-being. Ideally, nutrition has to support the "best possible fitness in a given environment" (Rinttila and Apajalahti 2013). Concentration, diversity and metabolic activity of the intestinal microbiota increase significantly from the upper to the lower gastrointestinal tract (Rehman et al. 2007). Studies in humans and many other species show that the intestinal microbiota affects and supports the digestive process, but it is also interacting in various ways with the host organism (Tellez et al. 2006). This interaction has on the one hand trophic functions; on the other hand, the intestinal microorganisms also widely affect the gastrointestinal epithelium, the intermediary nutrient metabolism and play a vital role for the immune system. While the understanding of the importance of the intestinal microbiota is constantly evolving, it has become clear that animal health and wellbeing are supported by a balanced microbial composition. This condition is often referred to as "*eubiosis"*. An undesirable status of the microbial system would be a "*dysbiosis"*, which could be described as atypical composition and/or metabolic activity of the intestinal microbiota. Dysbioses can pave the way for gastrointestinal disorders, and may prepare the colonisation by pathobionts or pathogens, as the colonization resistance can be adversely affected (Klis and Lensing 2007). In this overview, the composition of the gastrointestinal microbiota and their metabolic activity will be discussed in the context of nutritional interactions. It has to be emphasised, that the interpretation of studies and the comparison of different findings is complicated by methodological factors. Many of the available data comes from traditional cultural microbiological studies, on the other hand, in recent years almost all the work is based on molecular methods, allowing deeper insights into a very complex ecosystem with genetic, age and spatial related particularities, and exogenous factors. Nutritional impact seems to be of utmost importance for the intestinal microbial-host relation and has a high potential to modify animal health and performance.

Colonisation of the avian gastrointestinal tract with microorganisms

The intestinal microbiota and its diversity is subjected to age effects in broilers (Lu et al. 2003). The crop is the first storage organ for ingested feed and is colonised intensively with microorganisms, especially lactobacilli (Collado and Sanz 2007). Consequently, microbial fermentation activity is already high, which manifests in an intensive formation of lactic acid and other short-chain fatty acids. In addition to lactobacilli, which were detected in high concentrations, enterobacteria, enterococci and anaerobic bacteria (clostridia, bacteroides, bifidobacteria) have been determined more or less regularly (Rehman et al. 2007). Physiologically it can be assumed that the microbial fermentation in the crop results in a first fermentative digestion of feed compounds (Goodarzi Boroojeni et al. 2014). Furthermore, the formation of organic acids could be a first barrier against environmental bacteria (Rehman et al. 2008). A selection process with the

reduction of acid intolerant bacteria can be assumed. There are only few systematic studies on dietary influences on the microbial colonisation and metabolic activity in the crop. The glandular stomach has very low pH values, and thus is of paramount importance for nutrient digestion, especially the hydrolysis of proteins. Despite the relatively low pH value, comparatively high concentrations of lactobacilli, enterococci and lactose-negative enterobacteria have been determined. In addition, *Escherichia coli* and coliform bacteria occur in higher concentrations (Rehman et al. 2007). To what extent this is resident bacteria or bacteria that originate from the digesta flow out of the crop, is not yet clearly established. Also, few studies on the microbial metabolites in the gizzard have been published (Goodarzi Boroojeni et al. 2014). In poultry, the microbial density of the small intestine is subject to strong fluctuations. In proximo-distal direction, an increasing diversity of the microbiota and higher bacterial numbers have been determined. Low concentrations of microorganisms are likely to be more favourable for the efficiency of the digestive processes in the small intestine, because the host-microbiota competition for nutrients is lower. In the duodenum, relatively low pH values, and the high inflow of bile and pancreatic secretions are of critical importance as regulatory factors, reducing the microbial density. In the distal small intestine, total bacterial counts are 7-9 log/g digesta (Rehman et al. 2007). Mostly lactobacilli, enterobacteriaceae and enterococci have been determined. The microbial metabolites show amazing variations between individual studies: it was found that the levels of lactic acid can reach 10-53 µmol/g, the concentrations of short chain fatty acids are given with 4-47 µmol/g. Acetic acid is dominating, propionic and butyric acid were found more irregularly (Rehman et al. 2007). The caecum has an intense microbial colonisation and is considered as anaerobic fermentation chamber. Recent studies show a significant microbial diversity (Choi et al. 2014), where anaerobic bacteria predominate in contrast to the upper gastrointestinal tract. The fermentation activity is clearly different to the small intestine. The total concentrations of short chain fatty acids are higher and acetic, propionic and butyric acid are predominant, while lactic acid occurs in low concentrations only (Rehman et al. 2007). The microbial colonisation of the hindgut has similarities to the caecum.

Nutrition and intestinal microbiota

The gastrointestinal microbiota is subject to influences from the production technology, the feed composition, wherein both, the nutrient profile and the components used are of importance, and feed additives. The availability of feed is important, as fasting leads to a significant restructuring of the intestinal microbiota with increasing levels of enterobacteria (Holt et al. 2006, Moore and Holt 2006). At the same time, a significant reduction of microbial diversity was observed (Hinton et al. 2000, Thompson et al. 2008). The underlying reasons are the change in the substrate supply and stress-dependent effects. Strong influences of feed particle size have been described. Pelleted finely ground diets induced higher concentrations of volatile fatty acids in the crop and in the caecum (Huang et al. 2006). Feeding whole-wheat grains induced higher concentrations of volatile fatty acids in the crop of broilers (Bjerrum et al. 2005). Both, in broilers and laying hens feed structure has a significant impact on the microbial metabolic activity in the crop and in the gizzard. Apparently, a coarser structure stimulates a higher motor and secretory activity. This goes hand in hand with higher weights of the gizzard, the pancreas and the small intestine in animals fed with coarse diets. Under these conditions, a decrease in microbial colonisation of the small intestine was observed. Fermented liquid feed with high levels of lactobacilli has been shown to have a positive impact on the colonisation of the gut with *Salmonella* (Heres et al. 2003). Pelleting and thermal conditioning treatments of feed improve the hygiene of feed, can alter the chemical and physical characteristics of feed ingredients and affect the nutrient digestion and availability. The knowledge on the impact in microbial colonisation and metabolic activity of the gastrointestinal microbiota in chicken is limited, however, available data indicate only moderate effects (Goodarzi Boroojeni et al. 2014).

Carbohydrates have a strong impact on the intestinal microbiota as they and their hydrolysis products can serve as nutritional substrate for microbes and due to their hydration ability affect intestinal viscosity. Fermentable carbohydrates can be metabolised by lactic acid bacteria and thus can be useful to reduce enterobacteria during periods of feed withdrawal (Hinton et al. 2002). Non-starch polysaccharides affect the viscosity of the digesta and thus the substrate availability for the intestinal microbiota. The relevant carbohydrates comprise (1,3;1,4)-β-glucans and arabinoxylans, which have a high water binding capacity and can be readily degraded by microorganisms. Administration of diets containing high levels of non-starch polysaccharides also results in an increase in the transit time as well as in reduced nutrient absorption. This can stimulate the growth of specific microorganisms in the small intestine. Corresponding studies have shown that an increased viscosity of the digesta in the small intestine is accompanied by increased levels of *Clostridium perfringens* (Branton et al. 1997), *Campylobacter* (Fernandez et al. 2000) and spirochetes (Phillips et al. 2004). Administration of viscous diets with wheat instead of corn is at an increased risk. The impact of viscosity enhancing ingredients seems to be affected by the parasite status of birds. Carboxymethyl cellulose, as a viscosity-enhancing additive, had

less pronounced effects in birds infected with coccidia compared to non infected controls (Waldenstedt et al. 2000). The data allow the conclusion, that carbohydrates affect the intestinal microbiota and vice versa, and support the host by providing substrates being useable for energy generation. Viscous types of carbohydrates seem to be at higher risk to have negative impact on health and performance.

The role of different dietary fibre sources has not been widely studied in chicken. The microbiota can under certain conditions improve energy availability from fibre rich feedstuffs (Hegde et al. 1982, Muramatsu et al. 1991). Carboxymethylcellulose as jellying fibre source interfered with digestive processes in the upper intestinal tract and increased the concentrations of clostridia, lactobacilli and *Bacteroides* (Smits et al. 1998). Cellulose and wood shaving had different effects on the intestinal microbiota and obviously, this is also dependent on the particle length of the fibre sources (Amerah et al. 2009). Increasing dietary fibre in combination with coarse grinding has been shown to be useful for stimulating microbial diversity, leading to improved colonisation resistance against *Salmonella* (Santos et al. 2007, Escarcha et al. 2012). Pine shavings showed some protective efficacy in chicken challenged with *Salmonella* (Branton et al. 1997).

The protein supply may have a significant impact on the intestinal microbiota, both qualitatively and quantitatively. High protein diets increase the concentrations of proteolytic bacteria, especially clostridia. From the viewpoint of animal health, it is interesting in the context that there seems to be a link between the intestinal concentrations of *Clostridium perfringens* and the protein supply. Fishmeal is at higher risk compared to soya meal (Drew et al. 2004). Next to the protein itself, in particular the protein quality seems to be significant. The administration of feed with animal derived proteins led to a sharp increase in the concentrations of *Clostridium perfringens* and necrotic lesions in the intestinal mucosa. The amino acid methionine resulted in a reduction of the clostridia counts (Dahiya et al. 2007). In contrast, glycine caused an increase (Wilkie et al. 2005). In this respect, clear evidence is arising from these studies that the intestinal microbiota of the gastrointestinal tract is subject to strong effects by the protein composition, and that this can cause significant repercussions for animal health. Adjusting protein supply and amino acid profiles can be considered as essential to achieve optimal performance and to control the intestinal formation of metabolites such as ammonia and biogenic amines from protein fermentation, that are generally considered as detrimental (Rehman et al. 2009).

Fat intake seems to have a minor impact on the intestinal microbiota, although it could be shown that certain polyunsaturated fatty acids (Jozefiak et al. 2014) and medium chain fatty acids can reduce microbial concentrations including *Campylobacter* (van Gerwe et al. 2010, van der Hoeven-Hangoor et al. 2013). Bacterial bile acid deconjugation can interfere with the fat digestion in the small intestine (Knarreborg et al. 2003, Knarreborg et al. 2004). This aspect would deserve clearly more research to study the impact of fat concentration and qualitative aspects of different dietary fat qualities.

Many feed additives have an effect on the intestinal microbiota and thus influence on the digestive process. Corresponding effects are important under the terms of common feeding practice, also for *Salmonella* control (Totton et al. 2012). In practical terms, organic short and medium chain fatty acids, their salts, pre- and probiotics, enzymes and phytogenic additives have been widely studied with variable but generally positive effects (Huyghebaert et al. 2011). Relation to the intestinal microbiota is variable, but an impact on microbial concentrations and metabolic activity seem to be of utmost importance for performance enhancement and health effects, that have often been reported.

Conclusion

The gastrointestinal microbiota is of utmost importance for feed digestion and supports the host by providing energy yielding substrates from non or low digestible feed compounds. On the other hand, it generates potentially harmful metabolites from undigested proteins, and the importance for animal health and performance has not yet been fully addressed in poultry. New technological developments for assessing intestinal microbiology will help to foster the understanding of the role of the microbiota for nutrient utilisation, host physiology and finally animal health and well-being.

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DEVELOPMENT OF THE GUT ASSOCIATED IMMUNE SYSTEM

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Introduction

The mucosal surface of the gut is a fascinating structure. A single epithelial barrier separates the intestinal content from the body. The intestinal content does not only consist of digested feed but harbors a highly complex microflora called the microbiome. Consisting of bacteria, fungi and archaea and some poorly defined viruses the microbiome has been estimated to be made up of more than 1000 different species collectively representing more than 100-fold more cells than the vertebrate host itself. The microbiota is well known for its capability to digest plant products which otherwise were inaccessible for the vertebrate as a nutrient source. However, research in recent years has demonstrated that the microbiome affects not only metabolic processes but also the development and function of the immune system (Belkaid and Hand 2014). Conversely, the composition of the microbiome seems to be modulated by the immune system. This cross-talk between the bacterial flora, the intestinal epithelium and the immune system has become a research focus in immunology over the last 10 years. Immunologists have used germ free mouse models, gnotobiotic mice and mice treated with antibiotics to get completely sterile animals or mice with a defined microbiome. This work has led to a wealth of information in mouse models (Kamada and others 2013) while knowledge in humans and domestic animal species is still highly limited.

The avian immune system

The avian immune system is largely similar to its mammalian counterpart, even though some striking differences are found such as the absence of lymph nodes and the presence of a unique organ for B-lymphocyte development only existing in birds named the bursa of Fabricius (Schat and others 2014). Two major arms of the immune system have been defined which are called the innate and the adaptive immune system. The innate immune system provides a rapid response to pathogenic challenge while the adaptive immune system is responsible for sustained pathogen control and immunological memory. Both systems are made up of cellular and soluble components some of which have been known for a long time including macrophages and heterophils, complement components, and antibodies. Others have been characterized more recently in mice but are still poorly defined in birds (e.g. T-lymphocyte subsets or innate lymphoid cells). The two arms of the immune system closely interact with each other to provide adequate protection and communicate with each other through cell surface molecules and soluble factors collectively called cytokines. Cytokines play a critical role in the regulation of immune response to pathogens, vaccination, in immuno-regulation, and in the control of inflammation. Since the initial publication of the chicken genome significant progress has been made in avian immunology. Genomic data provides sequence information for genes involved in immuno-regulation and the function of the well-defined lymphoid tissues of the spleen, thymus, and the bursa of Fabricius. In contrast, mucosal tissues of the lung, reproductive tract, and the gut are less well understood.

Development of the gut associated innate immune system of chickens

Using immunohistology, holistic gene expression analysis, and functional assays we have investigated the development of the gut immune system from hatch to seven weeks of age in both broilers and layers. Macrophages are readily found in large numbers from the day of hatch throughout the gut. Their number increases in parallel with gut growth (Figure 1). Macrophages are located in the lamina propria and are thus well positioned to rapidly detect invading pathogens or epithelial damage. Studies in Salmonella enterica infection models of day old chicks have revealed that these macrophages are fully functional and respond to pathogen invasion with the secretion of inflammatory cytokines and chemokines to attract more phagocytes into the mucosal tissue which help to clear the infection. Within hours of infection the chemokines interleukin-8 (IL-8) and CCL-16 are produced. They attract heterophils and macrophages from the blood, respectively. In addition, effector cytokines regulating the anti-microbial response such as interferon-γ have been detected by RT-PCR. Thus macrophages and other components of the innate immune system are present and functional at hatch when the bird first encounters microbial organisms in its environment.

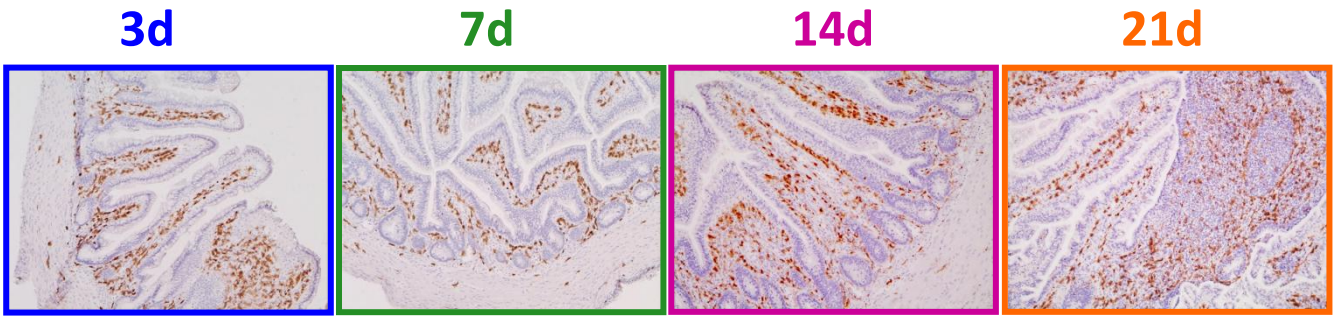

Figure 1: Immunohistological staining of macrophages in the caecum of layer chickens at different ages. Macrophages are found in the lamina propria but not in the epithelium.

In addition to the cellular components of the innate immune system, soluble components are found early after hatch. These include several acute-phase molecules of the pentraxin family and anti-microbial peptides of the defensin family (Cuperus and others 2013), which have been demonstrated by RT-PCR analysis. However, their functional role in the avian intestine has not been studied due to the lack of appropriate animal models deficient on one or several of these factors. From work in mice it has become clear that anti-microbial peptides are produced by epithelial cells of the crypts called paneth-cells and are secreted into the mucus layer. Bacteria invading the mucus barrier get in contact with these factors and are efficiently lysed. Most authors agree that paneth-cells (Nile and others 2004) are absent in chickens and even though defensin specific mRNA is found in gut tissue samples it is largely unclear where they are produced and which mechanisms elicit their synthesis and secretion.

Development of the gut associated adaptive immune system of chickens

While the innate immune system seems to be functional at hatch the components of the adaptive immune system are absent at that time point. Developmental studies have shown that B-lymphocytes, the producers of antibodies, start to emigrate from the bursa of Fabricius around hatch. Similarly, emigration of T-lymphocytes from the thymus starts shortly before hatch but major waves of cell migration are only observed days after hatch (Ratcliffe 2006). Importantly, both populations of lymphocytes are rare in the gut tissue during the first two weeks after hatch (Figure 2). Subsequently, more and more lymphocytes colonize the mucosal tissues and formation of lymphoid aggregates is found. Lymphocytes can be demonstrated in the lamina propria and in the epithelium as intra-epithelial lymphocytes. The latter population is made up of T-cells specialized in the control of virally infected cells. They are called cytotoxic T-lymphocytes (CTL) and are characterized by the expression of the cell surface protein CD8. T-helper cells (expressing the CD4 molecule) are more restricted to the lamina propria and are found in close proximity to lymphoid aggregates. B-lymphocytes start to colonize the gut at nearly the same time. They form organized lymphoid structures called Payer's patches and caecal tonsils.

Studies performed more than 50 years ago with birds raised under germ free conditions have shown that bacterial colonization has a significant influence on the maturation of the gut associated immune system (Thorbecke and others 1957) This observation has been confirmed recently in germ free mouse models and in our own studies with germ free chickens. To investigate the role of the microbiota, we hatched groups of birds which were maintained sterile or received one (*E.coli* Nissle) or a mixture of four strains of bacteria (*E.coli*, Lactobacillus, Enterococcus, Clostridium). These birds were compared with chickens housed under SPF conditions.

Even in the complete absence of microbiota the innate immune system (as far as investigated) developed normally. No difference was observed with regards to colonization by macrophages and micro-array studies showed undisturbed expression patterns for several genes of the innate immune system. In striking contrast, the development of the adaptive immune system was strongly impaired. B-cells were absent in the lamina propria of four weeks old birds despite normal B-cell development in the bursa of Fabricius. B-cell regions of the caecal tonsils were hugely underdeveloped and did not contain germinal centers (Figure 3), which are essential for the production of IgA and IgY antibodies. Consequently, IgA production was absent in germ free birds in the gut and in the serum (Figure 4). A similar picture was observed in the T-cell system with the absence of both $CD4^+$ and $CD8^+$ T-cells in germ free animals. Colonization with *E.coli* alone did not revert the histological picture but lead to the production of IgA in serum. These antibodies were, to a large extend, *E.coli* specific. Birds colonized with four bacterial strains showed significant maturation of the adaptive immune system in the gut. This was most pronounced in the caecal tonsils were germinal centers formation and IgA production was observed.

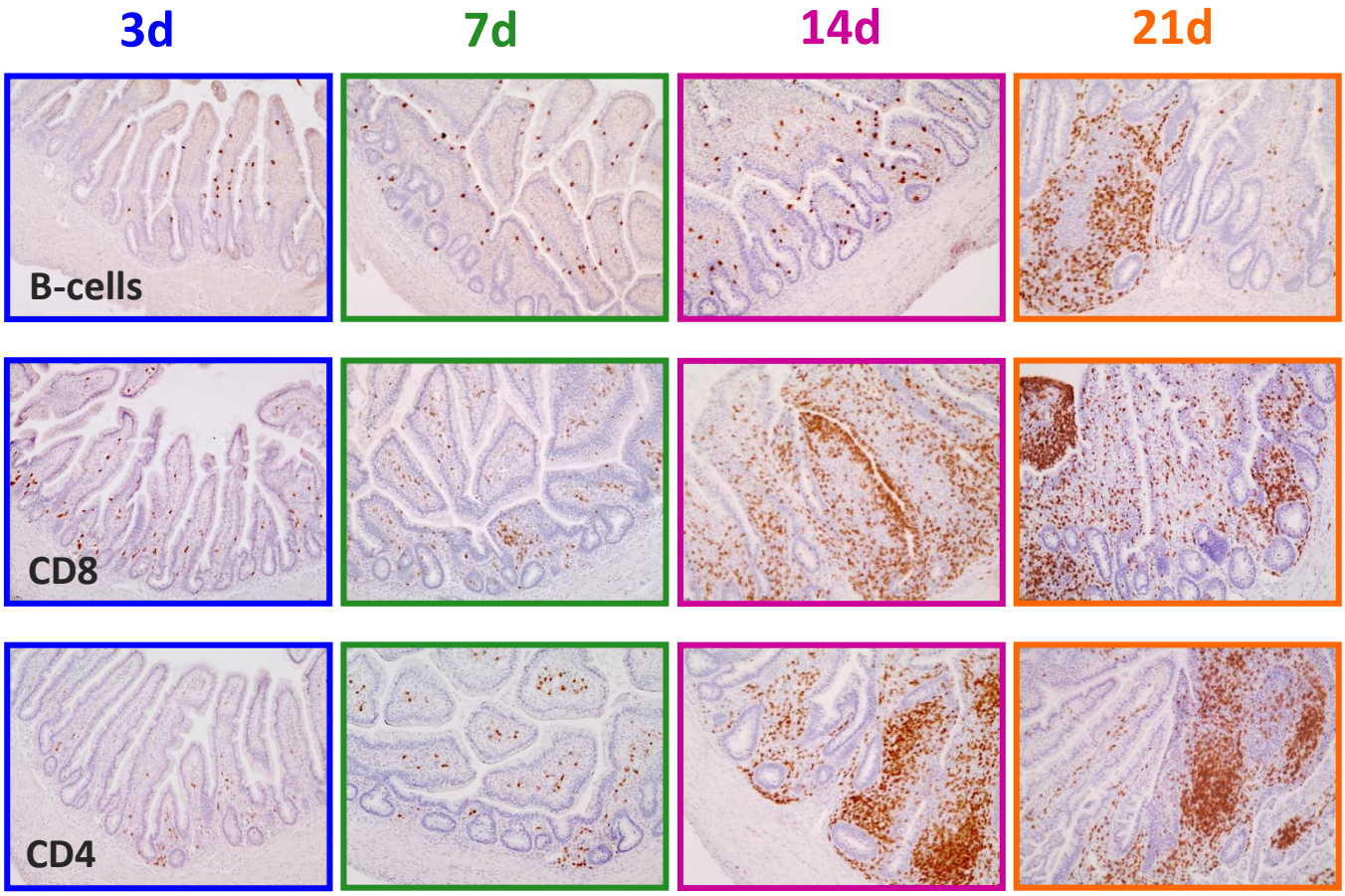

Figure 2: Immunohistological staining of B-cells, CD8+ and CD4+ T-cells in the caecum of layer chickens at different ages. Small numbers of lymphocytes are found during the first two weeks of age but colonization progresses rapidly thereafter. At three weeks formation of lymphoid aggregates is observed.

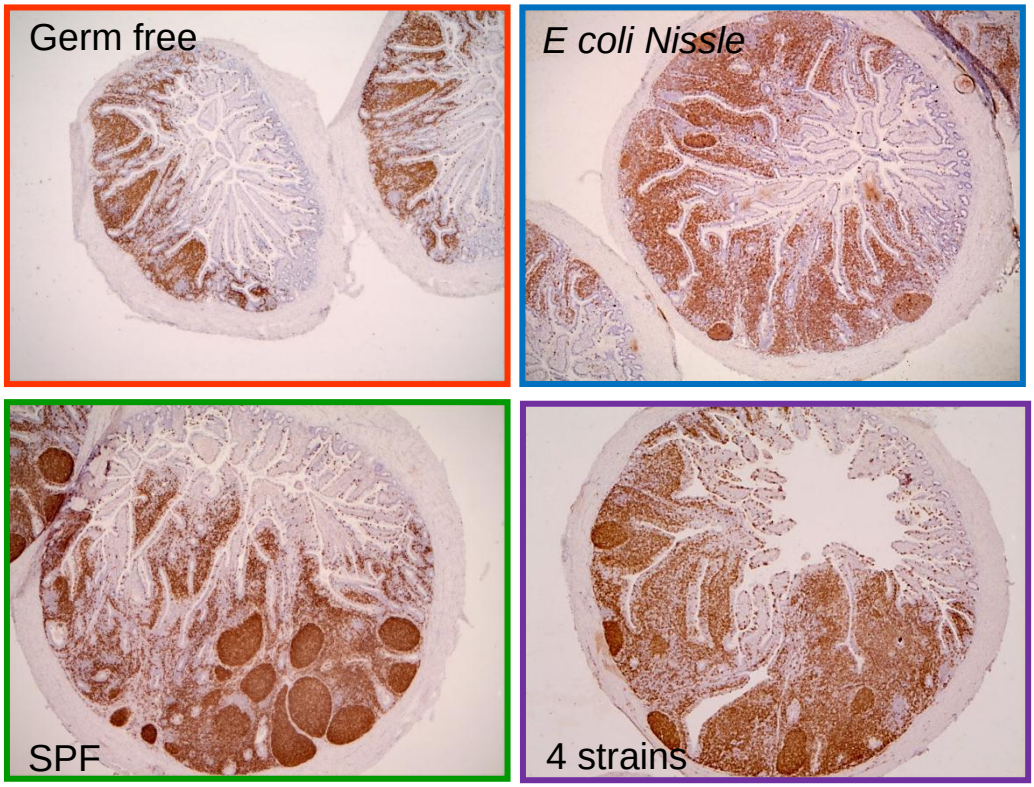

Figure 3: Immunohistological staining of B-cells in the caecal tonsills of layer chickens raised under germ free, gnotobiotic or SPF conditions. The absence of a microbiota (germ free) lead to a highly underdeveloped structure of the caecal tonsil. This phenotype was only partially reverted in birds mono-colonized (E.coli). Colonization with four strains further supported maturation and induced the formation of few and small germinal centers in comparison with the SPF controls.

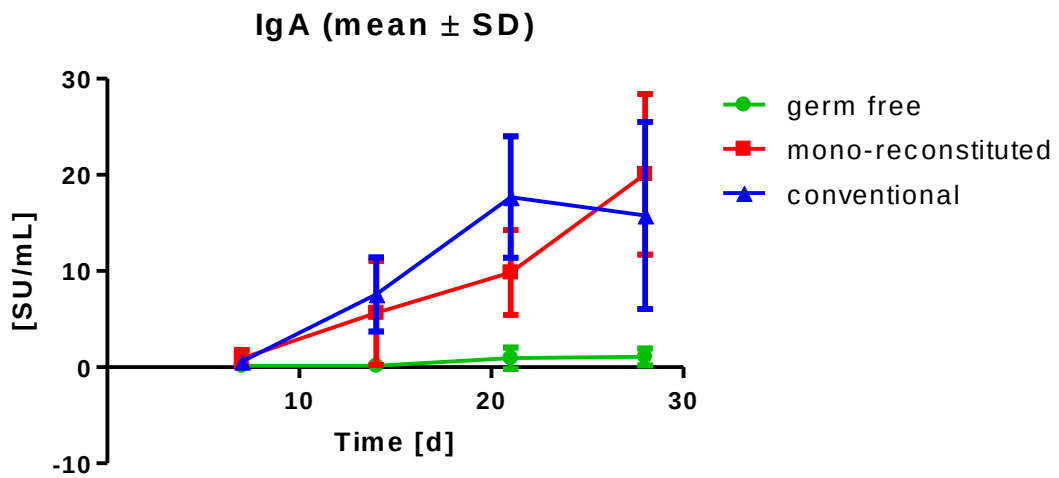

Figure 4: Immunoglobulin A levels in the serum of germ free, gnotobiotic and SPF (conventional) birds (n=5). Note the complete absence of IgA in birds without a microbiom.

The described observations were further supported by our gene expression analysis. Comparison of micro-array data from germ free and SPF birds showed higher expression of a large number of genes associated with immune function and developmental processes in SPF chickens. Most strikingly, genes known to be critical in B-cell maturation were found to be far less abundant in germ free birds reflecting the phenotype observed by immune-histology. AID, an enzyme essential for immunoglobulin class switching from IgM to IgA and IgY was weakly expressed in germ free birds but significantly higher expressed in mono-reconstituted chickens. A further increase was detectable in tetra-reconstituted animals which showed expression levels equivalent to SPF birds.

Conclusion

The development of the gut immune system is controlled by both genetic and environmental factors. The innate immune system develops early in ontogeny and is present at hatch and thereby able to protect birds during the critical phase of sudden encounter with microbes after hatch. Its subsequent development is not strongly influenced by the microbiota but it becomes strongly activated in response to pathogen invasion. In contrast, development of the adaptive immune system is significantly influenced by bacterial colonization of the gut. Absence of a microbiom leads to severe developmental defects with a lack of B- and T-lymphocytes in the gut mucosa and the absence of antibody formation. Our studies strongly indicate that full maturation of the gut immune system requires a complex flora. However, the precise composition of an optimal flora is still unknown. Under natural conditions hatchlings get in immediate contact with the maternal flora while birds hatched under commercial poultry production conditions are hatched essentially sterile. It is still unclear how these conditions influence the development of the mucosal immune system, the capability to control pathogenic microorganisms, and animal health and welfare. Interestingly, mouse studies have shown that nutrients and fermentation products also influence the functional status of the mucosal immune system either directly or through mediators secreted by the epithelial cells. The role of the epithelium, the mucus barrier, and macro- and micro-nutrients on the avian immune system has not been investigated to date but should be considered in future studies.

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RELEVANCE OF GLYCINE IN LOW PROTEIN BROILER FEEDS

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Summary

Lowering the crude protein (CP) content of broiler diets is an effective tool for decreasing N emission related to meat production and for decreasing costs by more efficient use protein in feedstuff. Maintaining an adequate concentration of essential amino acids (AA) in diets was previously shown to be a successful tool for reducing the CP concentration to a certain extent without negative effects on growth of broilers.

Considering nonessential AA as a sum of nonspecific nonessential AA in diets with a CP concentration below 20% has not consistently achieved growth performance of currently common CP concentrations of 21 to 22% of broilers at the age of 1 to 21 days post hatch. Consideration of the requirement for specific nonessential AA is necessary. Increasing the concentration of glycine in diets enables a CP reduction below 20% without compromising growth and feed efficiency. Feather development, skin strength and fat digestibility are also affected by glycine deficiency. It is generally accepted that on a molar basis serine is equally effective in meeting the functions of glycine. Therefore, glycine and serine should be assessed together by evaluating the response to glycine equivalents (Gly_{equi}) as the sum of glycine and the molar equivalent of serine. The extent of responses to dietary Gly_{equi} is inconsistent in literature. Consideration of factors influencing the response to Gly_{equi} is necessary. The dietary concentration of cysteine has an influence on the response to Gly_{equi} because the conversion of cysteine from methionine requires serine. Gly_{equi} sparing effects have been shown for threonine and choline which can be metabolized to glycine endogenously.

Because the concentration of Gly_{equi} in vegetable protein sources varies only little, altering the Gly_{equi} concentration independent from the CP concentration is hardly feasible. Usage of some animal byproducts, or free glycine or L-serine would overcome this problem but those ingredients currently are not approved in the European Union.

Introduction

The increasing demand for meat and other animal products and concomitant limitation of land for cropping results in a shortage of protein-rich feedstuff to be expected. Consequences of increased crop prices have been shown to especially affect affordability of food in developing countries (FAZENI and STEINMÜLLER, 2011).

The excretion of nitrogenous compounds can have negative effects on the environment because the excreted N can be dispersed into water, soil and air. Nitrogen contained in manure can be applied as fertilizer, but in too high amounts it risks N leakage into the groundwater (VERSTEGEN and JONGBLOED, 2003). Ammonia emissions have been associated with a number of harmful effects on the environment, which include soil acidification, eutrophication, formation of fine particulates, and secondary emissions of nitrous oxide (MARTÍNEZ-LAGOS et al., 2013). In addition, high ammonia levels in the animal house can affect human and animal health as well as the acceptance of livestock farming by the public due to unpleasant odors (ANEJA et al., 2009). Ambient temperature and manure characteristics like pH and moisture have an influence on ammonia emissions, but total excretion of N by livestock is most determining (LIU et al., 2007; NAMROUD et al., 2008).

Excretion of N in parts is inevitable because of characteristics of feed, such as digestibility of nitrogenous compounds, or due to metabolic processes leading to formation of substances which are subject to urinary excretion (BERG et al., 2007; RODEHUTSCORD, 2008). However, reduction of N excretion by using highly digestible feed ingredients and adjusting the supply of nitrogenous compounds to the requirement of the animal is accomplishable (BAKER, 2009) and often done.

Experiments, however, showed that low protein diets may have undesirable effects on performance of farm animals and carcass quality although the requirement of essential AA was met. Various potential reasons have been suggested to explain the undesirable effects of low protein diets (AFTAB et al., 2006). Glycine plays a special role in feeding low protein dies in poultry and the role of glycine has been better understood recently.

This contribution in the first part summarizes relevant approaches being discussed to overcome negative consequences of lowering CP concentrations in broiler diets. The physiological background and current state of research concerning the importance of glycine in low CP broiler nutrition is presented in the second part.

Approaches of lowering crude protein concentrations in broiler diets

Optimal ratio of essential amino acids

It is often discussed that the optimal ratio of essential AA might be different between standard and low CP diets and therefore represent one possible reason for diminished growth when low CP diets are fed to broilers. Low CP diets supplemented with free amino acids to raise the level of all essential AA failed to achieve growth performance as with standard CP diets (e.g. DESCHEPPER and DEGROOTE, 1995; JIANG et al., 2005; BREGENDAHL et al., 2002). In other studies combinations of some essential AA were varied with the result of improving growth performance partially but without fully overcoming the impaired growth induced by a low CP diet (e.g. HUSSEIN et al., 2001; NAMROUD et al., 2008; 2010; SI et al., 2004a; b). Interactive effects of some essential AA with nutrients reduced along with CP reduction such as nonessential AA may also have led to differences in growth. Such interactive effects have been mentioned e.g. for glycine and arginine by AKINDE (2014), and for glycine and threonine by CORZO et al. (2005).

Utilization of supplemented free amino acids compared to peptide-bound amino acids

Different utilization of supplemented free AA compared to peptide-bound AA in the metabolism of animals has often been suspected (e.g. NAMROUD et al., 2008). Differences are conceivable because peptides and free AA are absorbed via different transport mechanisms. An imbalance between AA in the systemic circulation as a consequence of faster passage of free AA through the intestinal wall might occur and lead to an increased amount of certain AA catabolized in the enterocytes (WU, 2009). Studies investigating different proportions of peptides and free AA without basically varying dietary AA concentrations in feed of farm animals in general are rare and not available for poultry. Recent investigations from our institute found no differences in N efficiency when AA from soy protein isolate were substituted with free AA (SIEGERT et al., 2015a). It appears likely that potential differences are less pronounced in poultry than in other farm animals that are not fed with continuous access to feed because the crop portions the feed delivered to the subsequent digestive tract. This may reduce concentration peaks of certain AA in the systemic circulation.

Consideration of nonspecific nonessential amino acids

In literature, the effect of nonspecific nonessential AA was evaluated by varying either the ratio of the sum of the concentrations of essential AA to the sum of the concentrations of nonessential AA or by altering the sum of the concentrations of nonessential AA. These ways of proceeding could not reproducibly prevent undesirable effects of low CP diets. To give examples: When added to low CP diets, mixtures of free aspartic acid and glutamic acid (BREGENDAHL et al., 2002; LECLERCQ et al., 1994) and a mixture of free aspartic acid, glutamic acid and alanine (NIESS et al., 2003) had no effect on growth performance. However, mixtures of free glutamic acid and glycine (DESCHEPPER and DEGROOTE, 1995; NAMROUD et al., 2008; 2010) and tyrosine and serine (THORNTON et al., 2006) increased performance, but not to the level of diets with standard CP concentrations.

Consideration of nonspecific nonessential AA probably is not sufficient because an unlimited interconversion of nonessential AA is necessary. Results of the aforementioned studies suggest that adequate supply of specific nonessential AA is important for prevention of decline in growth performance of broilers fed diets with low CP concentrations.

Specific nonessential amino acids

Studies aiming to increase nonessential AA nitrogen by adding free glutamic acid failed to prevent growth depression caused by feeding low CP diets (amongst others HUSSEIN et al., 2001; KERR and KIDD, 1999). CORZO et al. (2005) and DEAN et al. (2006) found that diets with 18 and 16.2% CP, respectively, supplemented with glycine to the level of 22% CP control diet caused no difference in growth and feed efficiency compared to the control diet. In both studies free aspartic acid, glutamic acid, alanine and proline failed to overcome negative effects of the low CP diet. PARR and SUMMERS (1991) also reported no difference in growth between a diet with 20% CP supplemented with glycine and a diet with 23% CP and additionally disproved similar effects of free glutamic acid, alanine and aspartic acid. Other studies showed a growth promoting effect of free glycine added to low CP diets (amongst others CORZO et al., 2004; JIANG et al., 2005; SCHUTTE et al., 1997).

Since a few decades the gain to feed ratio (G:F) in broiler production continuously increased and this caused specific nonessential AA becoming increasingly relevant. This can in part be explained by the increasing probability that not enough metabolic precursors for the respective AA are available or because endogenous metabolic processes are too slow (AFTAB et al., 2006; BERRES et al., 2010). The potential of glycine to increase growth has been known for decades (ALMQUIST and MECCHI, 1940). Since publication of the study of DEAN et al. (2006), it is broadly accepted that

a deficiency of dietary glycine is one factor limiting reduction of CP concentration in broiler diets and that glycine is the first-limiting nonessential AA (OSPINA-ROJAS et al., 2012). WAGUESPACK et al. (2009) described glycine as the fourth-limiting of all proteinogenic AA after methionine, lysine, and threonine in a diet based on corn and soybean meal for broilers from 1 to 18 days post-hatch. OSPINA-ROJAS et al. (2014) described valine and glycine as equally limiting after methionine, lysine, and threonine in a corn-soybean meal-based diet for broilers from 1 to 21 days post-hatch.

Relationship between glycine and serine

Glycine can be metabolized from serine by splitting the hydroxmethyl group of serine. This reaction can be reversed by adding CH_3 from tetrahydrofolic acid (VELÍŠEK and CEJPEK, 2006). For poultry, it is generally assumed that the interconversion of glycine and serine is not limited in metabolism (AKRABAWI and KRATZER, 1968; SUGAHARA and KANDATSU, 1976) and therefore usually they are assessed together.

As currently common practice, most authors including NRC (1994) take the analogue effect of dietary glycine and serine as the sum of both AA (Gly+Ser) into account, neglecting the fact that serine only has the same effect as glycine on an equimolar basis. DEAN et al. (2006) proposed to describe the physiological value of a diet by calculating the glycine equivalent (Gly_{equi}) as the sum of the concentration of glycine and the molar equivalent of Ser:

Gly_{equi} (g/kg) = glycine (g/kg) + [0.7143 × serine (g/kg)]

where 0.7143 is the ratio of the molar weight between glycine and serine.

The Gly_{equi} appears more appropriate than the currently common Gly+Ser unit; the advantage of rendering the physiological value of diets more precisely should justify the additional calculation effort.

Physiological functions of glycine and serine

As any other proteinogenic AA, glycine and serine are incorporated in proteins. Whole body accretion of glycine and serine in broilers from d 8 to d 21 was determined between 7.8 and 11.4 g glycine accretion and between 4.2 and 5.5 g serine/16 g N accretion(FATUFE et al., 2004; FATUFE and RODEHUTSCORD, 2005). The lack of a side chain of glycine causes some physical characteristics like size, imparting charge, and hydrophobicity. These features allow for a possible accommodation in the hydrophobic interior of proteins which causes flexibility in the folding of proteins with a propensity to form helices and causes versatility in the structure of receptor sites as well as flexibility for active sites of enzymes (HALL, 1998).

<u>Proteins incorporating glycine and serine</u>

Collagen and elastin: The proteins richest in glycine are collagen and elastin, where glycine is incorporated at every third position in the primary structure. In broiler slaughter processing, low skin strength due low collagen content in consequence of low dietary supply with glycine can have economic implications (CHRISTENSEN et al., 1994).

Keratin: Keratin is rich in both glycine and serine. In avian species, keratin is mainly present in feathers and claws. Feather development of birds fed with glycine deficient diets is impaired (ROBEL, 1977).

Mucin: Mucin proteins are rich in serine because serine provides attachment sites for the oligosaccharide chains which have a high proportion in mucins. The physiological functions of mucin are described as lubrication of the gut epithelium, protection of the epithelium against acidic conditions and proteases and selective diffusion barrier for nutrients. Further microbiota-associated functions are fixation of commensal bacteria, protection of the epithelium from pathogens, and substrate for bacterial fermentation. OSPINA-ROJAS et al. (2013) found that intestinal mucin secretion of broilers linearly increased with the proportion of glycine and serine in diets.

<u>Processes using metabolization products of glycine or serine</u>

Uric acid: In uricotelic species like birds, ammonia is detoxified and excreted as uric acid being the main excretion product of N metabolism. The formation of each molecule of uric acid requires one molecule of glycine to build the purine ring. In addition, protein synthesis and cell proliferation depends on DNA synthesis, which requires glycine to form purines (WANG et al., 2013).

Creatine: Glycine is an integral part of creatine along with arginine. Creatine can either be directly supplied by feed derived from animal products or produced by endogenous synthesis. In a first step arginine reacts with glycine to form guanidino acetic acid. In a second step, guanidino acetic acid is methylated to form creatinine.

Cysteine synthesis: Most species cannot synthesize cysteine de novo but can metabolize cysteine from methionine. Serine is required when cystathionine is formed from homocysteine. Cystathionine further reacts to cysteine and ammonia (VELÍŠEK and CEJPEK, 2006).

Bile salts: Primary bile salts are synthesized from cholesterol in the liver and then conjugated with either glycine or taurine. In different animal species, 75 to 85% of the produced bile salts were found to be glycine conjugates (HAFKENSCHEID and HECTORS, 1975). Glycine deficiency was found to affect fat digestibility and therefore the AME_N concentration in feed (OSPINA-ROJAS et al., 2013).

Response to dietary glycine and serine

Since the importance of glycine and serine in poultry nutrition has been recognized, the publication rate of studies enlarging knowledge about effects of glycine and serine has increased. Several dose-response studies investigating the effect of dietary glycine and serine were published. A compilation of some studies shows that the response to dietary Gly_{equi} was inconsistent (Figure 1). Therefore, factors influencing the response to Gly_{equi} in reduced CP diets should be better understood. Such factors are addressed in the following section.

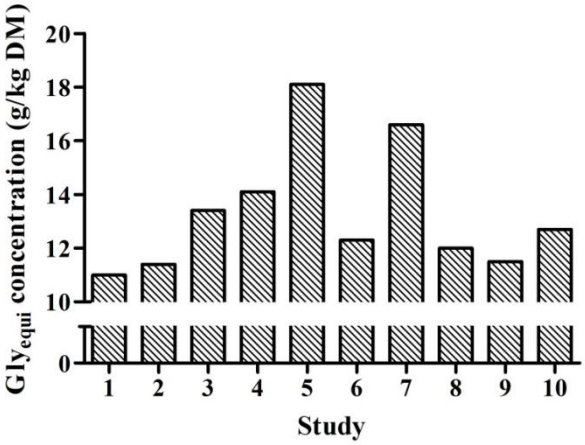

Figure 1. Dietary Gly_{equi} needed to achieve 95% of the maximum gain:feed response (based on the 10 studies used by SIEGERT et al., 2015c)

Effect of cysteine

POWELL et al. (2011) found that the impact of glycine and serine on G:F is explained by the conversion of methionine to cysteine, for which serine is required. They found an improved G:F after addition of Gly to a diet adequate in total sulfur AA (TSAA) but deficient in cysteine. Further inclusion of methionine showed no improvement in growth performance, but the addition of cysteine above the requirement reduced the positive effects of glycine supplementation. Low CP concentrations are often associated with the inclusion of pure ingredients, such as DL-methionine. Because cysteine is usually not added, the ratio between methionine and TSAA (Met/TSAA ratio) in diets is increased upon supplementing DL-methionine disregarding a specific requirement of cysteine.

The dietary concentrations of both methionine and cysteine have a substantial impact on the requirement for dietary Gly_{equi} (Table 1). Fulfilling the requirement for both methionine and cysteine in birds reduces the necessity of the conversion of methionine to cysteine. Each molecule of methionine not converted to cysteine reduces the requirement for Gly_{equi}.

Table 1. Dietary Gly_{equi} concentrations at 95% of maximum G:F in a meta-analysis compiled from 10 peer-reviewed studies investigating growth response of about 1 to 21 days old broilers (derived from SIEGERT et al., 2015c)

		Gly_{equi} (g/kg)
Met/TSAA ratio	0.5	19.9
	0.8	13.8
Cys concentration	2 g/kg	14.8
	5 g/kg	21.1

Effect of endogenous precursors of glycine

Several substances can be metabolized to glycine or serine. Glycine can be metabolized from threonine, which can either be metabolized directly to glycine via the enzyme threonine aldolase with acetaldehyde as a byproduct or via threonine dehydrogenase with 2-amino-3-ketobutyrate as an intermediate step, which further reacts to glycine, acetyl-

CoA, and aminoacetone. Another precursor is choline which, with betaine as an intermediate step, is metabolized to glycine if homocysteine is available. Other endogenous precursors of glycine are glyoxylate in combination with alanine, and trimethyllysine where glycine is produced as a byproduct of carnitine synthesis. However, glyoxylate has no relevance in broiler feeding, and the potential quantity of glycine metabolized from trimethyllysine is low (MELÉNDEZ-HEVIA et al., 2009).

Threonine: Different levels of G:F and average daily gain (ADG) can be achieved with distinct combinations of Gly_{equi} and Thr concentrations (Figure 2). An increase in Thr concentration reduces the Gly_{equi} concentration required to achieve certain response levels. One molecule of threonine can be converted to one molecule of glycine. Given the molar weights of both molecules, the replacement value due to endogenous conversion of 1 mass unit of threonine cannot exceed 0.63 mass units of glycine. Much higher replacement can be derived from the graphs in Figure 2.

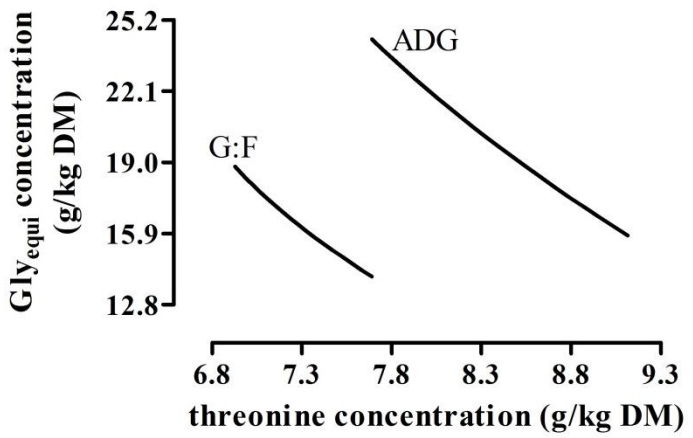

Figure 2. Dietary Gly_{equi} necessary to achieve 95% of maximum G:F and ADG of broilers from day 7 to 21 post hatching depending on dietary threonine (SIEGERT et al., 2015b)

A replacement value of dietary threonine to dietary glycine exceeding the possibility to be explained by endogenous conversion was also found in other studies (e.g. OSPINA-ROJAS et al., 2013). The high replacement value originates most likely due to an excess supply of other AA. If threonine limited growth performance, then the excess essential AA had to be catabolized. Increasing the threonine concentration probably reduced the amount of catabolized AA (other than threonine), thereby reducing the need for Gly_{equi} to be used for uric acid formation.

Choline: Choline can considerably affect the Gly_{equi} concentration required to achieve a certain level of response in G:F and ADG. The replacement effect of dietary choline for Gly_{equi} is lower than the replacement effect of threonine (SIEGERT et al., 2015b). To our knowledge, interactive effects between glycine and choline were not reported in other literature.

Concentrations of Gly_{equi} in feedstuffs

The concentration of Gly_{equi} varies considerably between and within types of feedstuffs (Table 2). However, the variation in the proportion of Gly_{equi} in CP is low and ranges between 7.3 and 8.3 g/100g CP in most cereals, cereal byproducts, brewery byproducts, oil seeds and pulses. Proportion of Gly_{equi} in CP is highest in meat meal and meat and bone meal.

Because the variation of the proportion of Gly_{equi} in CP is low, using only vegetable ingredients in feed formulation causes the dietary concentration of Gly_{equi} to depend mainly on the CP concentration of the diet. It is hardly possible to increase or decrease the proportion of Gly_{equi} in CP of plant-based diets. Some animal byproducts are useful to elevate the Gly_{equi} concentration in diets but feeding of animal-derived proteins to poultry currently is generally prohibited in the European Union (EUROPEAN COMMISSION, 2001). Feed additives potentially suitable for elevating the Gly_{equi} concentration in feedstuff are free glycine and L-serine, which are currently also not approved in the European Union (EUROPEAN COMMISSION, 2014). Consequently, current legislation in the European Union is not in support of reducing the CP concentration in poultry diets.

Table 2. Concentrations of Gly_{equi} and proportion of Gly_{equi} in the protein of selected feedstuffs relevant for animal feeding (extracted from EVONIK, 2010)

Feedstuffs		Gly_{equi} (g/kg DM)	Gly_{equi} (g/100g CP)
Cereals	Winter wheat	8.3	7.4
	Corn	6.6	7.3
	Triticale	9.5	7.3
Oil seeds	Rapeseed meal	31.7	7.9
	Soybean meal	41.6	7.8
	Sunflower expeller	44.4	8.3
Pulses	Field peas	18.5	7.6
	Lupins	30.4	7.4
Non-animal byproducts	Wheat bran	11.4	8.2
	Brewer's dried yeast	35.3	7.8
Animal byproducts	Fish meal	64.3	6.4
	Meat meal	11.4	14.8
	Meat and bone meal	14.9	17.7

Perspectives of crude protein reduction

Current research mainly aims to find ways to reduce the CP concentration without compromising growth and feed efficiency achieved with nowadays common CP concentrations (amongst others CORZO et al., 2005; DEAN et al., 2006), which is at about 21 to 22% in diets.

DEAN et al. (2006) summarized that in literature growth and feed efficiency was reduced in broilers fed with diets containing less than 19 to 20% CP even when the requirement for essential AA was met. If the concentrations of glycine and serine were adequate, growth and feed efficiency of broilers fed diets containing 17 to 18% CP were at the level of diets containing more than 20% CP (CORZO et al., 2004; DEAN et al., 2006; SIEGERT et al., 2015c).

The Met/TSAA ratio, the concentrations of endogenous precursors, and other nutrient concentrations influencing the response to Gly_{equi} probably were suboptimal in the studies mentioned above. Consequently, further reduction of the CP concentration in diets without negative effects on performance should be possible through optimization of both the dietary Gly_{equi} concentration and factors influencing the response to Gly_{equi}. According to a personal estimate, this would enable to reduce the CP concentration in diets to about 16% in feed of broilers from 1 to 21 days of age. This estimate needs to be verified by targeted investigations.

Glycine and serine are the first nonessential AA of which requirement values were quantified. The possibility to further reduce the CP concentration in diets without adverse effects on performance can be expected when the role of other nonessential AA is better understood and their requirement values are assessable.

Conclusion

Because protein sources for animal feeding will become more expensive, further CP reduction and therefore optimization of nutrient composition will be necessary in future. Glycine and serine are very likely to become growth-limiting when diets with a CP concentration below 20% are fed. Consideration of Gly_{equi} enables to reduce the CP concentration remarkably without adverse effects on performance compared to the current standard. The requirement for Gly_{equi} depends on other dietary characteristics. The role of cysteine and threonine has already been investigated but other influencing factors are still to be addressed.

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REVISITING AMINO ACID NUTRITION

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Summary

Amino acids are recognized as anabolic factors that induce protein gain by stimulating protein synthesis while inhibiting proteolysis, and they are important substrates for the synthesis of various nitrogenous compounds. There is also increasing evidence that amino acids act as key regulators of metabolism through the control of major metabolic pathways in various tissues and organs. Elucidating the mechanisms involved in such regulation can provide a basis to optimize amino acid requirements and to control protein mass, body composition and meat quality while ensuring greater efficiency of production systems. However, with the aim of improving understanding of the response of chickens to nutrition, new approaches and technology (e.g. comparative physiology, genomics and modeling) are also available to decipher mechanisms which could not be adequately analyzed a few years ago. This review presents the recent advances in this area generating original scientific knowledge and optimizing dietary amino acid provision (quantitative and qualitative) over time in relation to production targets. Emphasis is also placed on the limits of the information available for broiler chickens.

Keywords: amino acid; nutrition; low crude protein diet; combined approaches; broiler

Introduction

One major issue in poultry production is the development of feeding strategies that are more efficient and more eco-friendly by reducing waste. Among these strategies, one aim is reduction of the crude protein content in a diet fortified with adequate amounts of currently available free amino acids (e.g. L-Lys, DL-Met, L-Thr, L-Val and L-Trp) in order to achieve increased protein utilization efficiency while maintaining animal growth performance and reducing nitrogen excretion.

Amino acids have a major effect on the control of protein metabolism. They are components of tissue proteins and provision at insufficient levels leads to reduced protein synthesis. Provision of the different essential amino acids is therefore a prerequisite for maintaining optimal rates of protein synthesis, an unquestionably major concept in protein nutrition. However, optimizing amino acid nutrition requires consideration of the different functions of amino acids, which are recognized to have several significant influences. Amino acids are precursors of important molecules. For example, cysteine is required for the synthesis of glutathione and taurine, which are essential compounds for host defense against oxidative stress (METAYER et al., 2008). Methionine is a source of the methyl groups needed for all biological methylation reactions, including methylation of DNA and histones involved in epigenetic regulation (TESSERAUD et al., 2009; WATERLAND, 2006; WATERLAND and MICHELS, 2007). Moreover, studies conducted during the last decade indicate that amino acids act as regulators of various metabolic pathways (see TESSERAUD et al., 2011; WU, 2009 & 2013 for reviews). The mechanisms through which amino acids control tissue metabolism in poultry are not discussed here. Following an overview of the role of amino acids and setting out the interest of revisiting amino acid nutrition in poultry production, we then describe the strategies developed to adjust amino acid provision in low crude protein diets and discuss the recent advances in amino acid nutrition, with the aim of optimizing broiler growth performance and product quality.

Overview of the role and importance of amino acids in controlling growth, body composition and product quality

Amino acids exert their actions in various tissues and organs, including splanchnic tissues that control the distribution of nutrients between tissues since they are the first tissues exposed to newly absorbed nutrients due to their anatomical position (first-pass splanchnic extraction; see NIETO and LOBLEY (1999) for a review). The nutritional regulation of protein metabolism by amino acids has also been intensively studied in skeletal muscles since such nutrients are recognized as anabolic factors, which induce protein gain by stimulating protein synthesis while inhibiting proteolysis. The aim of the various studies performed to date has thus been to improve and control muscle growth and meat quality in animal production and to preserve muscle growth in critical situations (e.g. stress, infection). Indeed, due to the differences between the amino acid composition of the acute-phase and muscle proteins, a considerable amount of muscle protein must be degraded to provide the amino acids used in the acute-phase response (REEDS et al., 1994). Under-

standing the mechanisms by which amino acids regulate protein metabolism is therefore essential to improve control of the use of nutrients and to optimize dietary amino acid provision.

Dietary amino acids are major determinants of the carcass components of broilers, as abdominal fat content and breast meat yield are influenced by their levels (MORAN and BILGILI, 1990; LECLERCQ, 1995). Lysine is known to have specific effects on carcass composition and, for instance, lysine deficiency greatly reduces breast muscle proportion (TESSERAUD et al., 1996a, b). It should be noted that neither threonine nor valine exhibits as pronounced an effect on body composition as that of lysine, as demonstrated in experiments in which these three amino acids were studied together in similar conditions (LECLERCQ, 1998). Interestingly, increasing the level of lysine in the diet of broilers (beyond the requirement for growth) reduced drip loss of breast muscle during storage by increasing its ultimate pH (pHu; BERRI et al., 2008). This result was consistent with the general hypothesis that breast muscle hypertrophy in broilers is accompanied by a decrease in glycogen storage (BERRI et al., 2007). Breast meat traits and pHu have also been reported to be affected by distributing diets varying in amino acid content over a very short period (3 days) before slaughter (GUARDIA et al., 2014). Provision of various amino acids (and the resulting amino acid balance or imbalance) may indeed affect the proportion of nutrients used for glycogen storage and hence the pHu. It should be noted that changes in pHu caused by variations in dietary amino acid profiles were fairly moderate in the latter study. However, they significantly affected breast meat lightness that itself is strongly correlated with the water holding capacity of meat and processing yield (QIAO et al., 2002; PETRACCI et al., 2004; ZHUANG and SAVAGE, 2010).

Amino acid provision has been shown to have an impact on the chemical composition of tissues, with a potential effect on meat quality. In particular, Met and/or Cys deficiency affects lipid and protein content, and also amino acid composition of tissue proteins in chickens (CONDE-AGUILERA et al., 2013), as previously reported in piglets (CONDE-AGUILERA et al., 2010 & 2014). Sulfur amino acid provision has also been reported to modify meat susceptibility to oxidation. For example, according to the Met source provided in the diet (DL-Met or hydroxy analog of methionine, DL-HMTBA), oxidative stress and lipid peroxidation will be greater or less significant (MARTIN-VENEGAS et al., 2006; SWENNEN et al., 2011; WILLEMSEN et al., 2011). Fully or partially replacing DL-Met by its hydroxyl-analog HMTBA has indeed been shown to reduce breast meat susceptibility to oxidation (leg and breast meat) and also to increase its pHu, with positive consequences on storage and processing ability (MERCIER et al., 2009).

Why revisit amino acid nutrition?

In chickens, as in other farm animals, amino acids have to be provided in the diet in balanced proportions to optimize performance and feed utilization (D'MELLO, 2003). This necessitates precise evaluation of amino acid requirements, whereas they are influenced by many environmental and dietary factors as well as by the animal itself, including age, sex and strain. Moreover, it has been established that dietary amino acid requirements vary according to the performance criteria chosen for optimization. For example, higher dietary Lys requirement is generally found for the feed conversion ratio (FCR) and breast meat yield compared to body weight gain (HAN and BAKER, 1994; LECLERCQ, 1998; MACK et al., 1999; DOZIER et al., 2009 & 2010). These findings together involve reinforcing research into the responses to amino acid provision in terms of performance, body composition and quality of products. Furthermore, modern broiler genotypes, which have been greatly improved in terms of weight gain and feed efficiency, may require higher dietary amino acid content to optimize performance and breast meat yield compared to broilers of past years (SI et al., 2001; DOZIER et al, 2010). Indeed, there is evidence that the response to dietary amino acid provision is modified by genetic selection. For instance, genetically lean chickens are more sensitive than fat chickens to diets containing less crude protein or fewer essential amino acids (LECLERCQ et al., 1993 & 1994; MORAN, 1994; LECLERCQ, 1995; JLALI et al., 2012). There is better use of dietary amino acids by lean genotypes. Similarly, an experimental line selected for high breast development and low abdominal fat percentage exhibited a more efficient utilization of Lys for body weight and *Pectoralis major* muscle protein deposition compared to the control line maintained without selection (TESSERAUD et al., 1999). The different levels of responsiveness to changes in dietary Lys intake can be explained by differences in protein metabolism (TESSERAUD et al., 2001).

Revisiting amino acid nutrition stems from the need to reduce the crude protein content of diets, which is a major issue for sustainable poultry production from economic, environmental and social points of view. For example, formulating diets with the constraint of too high crude protein levels results in massive incorporation of soybean meal. This raw material is controversial as a feedstuff because of the price volatility, its environmental impact (deforestation, transport) and the GMO (genetically modified organisms) issue. Decreasing dietary crude protein levels can reduce dependency on soybean but also leads to the incorporation of larger amounts of free amino acids to maintain dietary amino acid

balance. It also reduces nitrogen excretion resulting from excessive amounts of undigested nitrogen or excess provision of amino acids (i.e. beyond requirements). This reduction of waste also allows improvement of litter quality (e.g. lower water consumption and excretion, lower litter humidity), and contributes to a better health status of livestock (MEDA et al., 2011; VAN CAUWENBERGHE and BURNHAM, 2001). Nevertheless, formulation of low crude protein diets requires good understanding of the nutritional characteristics of raw materials and controlling the provision of digestible essential amino acids (Lys, Met (+ Cys), Thr, Val, Ile, Leu, Trp, Arg, Phe (+ Tyr), His), the need for the most limiting essential amino acids being satisfied by incorporating free amino acids. To balance the diet, practical formulation is generally based on an 'ideal profile' of essential amino acids, for instance maximizing growth performance. The requirements for essential amino acids in ideal protein are usually expressed as a proportion of Lys (HAN and BAKER, 1994; NRC, 1994; EMMERT and BAKER, 1997). Although many profiles have been provided and updated (MACK et al., 1999; ROSTAGNO et al., 2011; WU, 2014), decreasing dietary crude protein content is still difficult to achieve without negatively affecting broiler performance (DEAN et al., 2006; WAGUESPACK et al., 2009; BELLOIR et al., unpublished data). What makes these findings surprising is that decreasing dietary crude protein content does not result in any particular problems in pigs (see the next paragraph).

Limits of available data

Reducing dietary crude protein content by using free amino acids in pigs maintains performance as long as the amino acid requirements are met. For example, it has been known for a decade that supplementation with Lys, Met, Thr and Trp in free form allows a reduction in dietary crude protein level of approximately 4% units (FIGUEROA et al., 2003; KERR et al., 2003). A recent study even showed that it is possible to formulate very low protein diets that maintain piglet growth with the use of free Val, Leu, Ile, His and Phe, on top of the usual free amino acids Lys, Met, Thr and Trp (GLOAGUEN et al., 2014). Moreover, soybean meal can be totally replaced by cereals and free amino acids.

To determine the extent to which low crude protein diets can be used in poultry, it is important to synthesize and analyze reliable scientific information. The information available is indeed sometimes fragmented (for example, the majority of studies have been performed in young chickens between 1 and 21 days of age), not always consistent and corresponding to very different experimental designs and conditions, in particular with regard to the extent of protein level decrease and the modalities applied to achieve it. A bibliography database has recently been established for that purpose for broilers (BELLOIR et al., 2015a). The first database included 56 trials originating from 29 publications focusing on a decrease in dietary protein published after 1990. The information computed in the database included the experimental design, broiler performance levels and the composition of the diets which were recalculated on a common basis (SAUVANT et al., 2004). The database therefore included the levels of total amino acids, and also levels of digestible amino acids to avoid any experimental bias due to differences in amino acid digestibility between raw materials. Briefly, formulating low crude protein diets was systematically associated with the incorporation of L-Lys, DL-Met and L-Thr. L-Val, L-Ile and L-Arg were also often used in addition to these amino acids. The preliminary analysis showed impaired growth and feed efficiency in many trials. This loss of performance was thought to be due to the incorporation of large amounts of free amino acids in replacement of the protein fraction from the raw materials or to inadequate amino acid provision. The first hypothesis was recently tested in an experimental trial where the impact of the form of provision (protein vs free amino acids) on broiler performance was evaluated (BELLOIR et al., unpublished data). In the trial, 30% of the dietary crude protein fraction was gradually replaced by either a highly digestible protein (casein) or a mixture of free amino acids whose amino acid profile was exactly the same as for casein. When chickens were fed more than 3.8% dietary free amino acids as replacement for casein protein, the feed conversion ratio and breast meat yield were impaired. Chickens were able to adapt their digestive physiology to increasing provision of free amino acids, at least for levels lower than 3.8% (e.g. increased surface of villi and increased expression of amino acid transporters). Beyond this level, the digestive absorption capacity may be affected, limiting the use of nutrients by the animal, which could partly explain poorer performance. However, in practice, the use of amino acids in free form should not be a problem, since the quantities reached for formulating low crude protein diets (15% CP) may not exceed 1.7% free amino acids (BELLOIR et al., unpublished data).

From the bibliography database presented above, fifteen trials were then selected according to two 'selection criteria': 1) the level of digestible Lys that had to be sub-limiting and constant when dietary crude protein was decreased, and 2) the dietary digestible essential amino acid profile in that had to be approximately consistent with the 'ideal' protein (MACK et al., 1999; ROSTAGNO et al., 2011). After selection, trial results were compiled and analyzed graphically to assess the effects of protein decrease on levels of performance. On average, low protein diets corresponded to a

reduction in dietary crude protein content of approximately 2% units (compared to the positive control recalculated for each trial). In these conditions, low protein-related variability of performance levels was observed above a digestible Lys/crude protein ratio of 6.5. The majority of trials tested only two levels (i.e. positive control and experiment low protein diets) which did not allow modeling of the chicken performance responses to decreases in dietary crude protein. However, this meta-analysis approach already indicates specific features of trials in which production performance is maintained and emphasizes certain assumptions concerning the impact of low protein diets in broiler chickens. In particular, it appears necessary to pay attention to the Thr:Lys ratio used and to take into account the next limiting essential amino acids (Val, Ile and Arg). It should be noted that these assumptions are supported by recent studies (BELLOIR et al., 2015b & c). Moreover, this meta-analysis indicates that a further reduction in dietary crude protein level (below 17%) may necessitate including an adequate level of certain non-essential amino acids.

Recent advances in amino acid nutrition

The above assumptions highlight the inadequate provision of certain amino acids when using low crude protein diets. Another issue in amino acid nutrition originates from formulating balanced diets with the increasing number of production criteria (e.g. including meat quality in addition to growth performance), and improving animal health (importance of 'functional' amino acids). For example, Thr is sometimes deficient in low crude protein diets (see above), despite its crucial importance for maintaining intestinal integrity and immune function (WU, 2009). A recent meta-analysis reported that the digestible Thr:Lys requirement of broilers was estimated to be at least 67% and was thought to be even higher in critical environmental conditions (LAMBERT et al., 2015a). With regard to the optimization of meat quality, it has been shown that dietary Lys and amino acid levels affected the quality traits of breast meat, including color, drip loss and pHu (BELLOIR et al., unpublished data). For example, deficient amino acid provision (i.e. essential amino acids other than Lys) combined with a reduced Lys content favored the production of meat with high pHu (> 6.0), while more acid meat (< 5.85) was produced with higher levels of amino acids (essential amino acids other than Lys) combined with a low Lys content. In this trial, maximum changes in pHu induced by dietary treatments reached 0.29 pH unit. This change in pHu was quite high compared to previously released studies: maximum extent of 0.20 pH unit with dietary Lys variations (BERRI et al., 2008), 0.14 pH unit with short term variations in dietary amino acids profiles (GUARDIA et al., 2014) and 0.13 pH unit between different genotypes selected for growth criteria (BERRI et al., 2005).

Recent advances in amino acid nutrition have benefited from combined approaches including meta-analyses, nutritional and metabolic studies. The major challenges for the future are integrating the data obtained at various levels from the cell to the animal and the complete understanding of amino acid metabolism for rational use in nutrition. An example of such difficulties was reported in a review of the role of sulfur amino acids in controlling nutrient metabolism and cell functions, with the emphasis placed on the implications for nutrition (TESSERAUD et al., 2009). A large number of studies have indeed been devoted to exploring the key roles of sulfur amino acids, but further experiments are still necessary to obtain direct evidence regarding some of their effects on nutrient metabolism, cell functions and physiological responses in animals on physiological (e.g. early nutrition) or physiopathological conditions (disease, environmental stress). In particular, the effects of sulfur amino acids should be analyzed under circumstances affecting oxidative status and gene expression. Recommendations for Met and Cys intake have to be revisited in the light of their roles and the intricacies of sulfur amino acid metabolism. Further experiments are also necessary to improve the integration of the various roles of sulfur amino acids at the whole-body level. These findings are particularly interesting in birds due to the high Met and Cys requirements for feather synthesis. The content of Cys has to be taken into account when adjusting the provision of Met, while also taking into account their potential interactions with Gly-Ser, as summarized in Fig. 1. Glycine (or Gly+Ser because of equimolar and reversible conversion between them) must also be present in the diet in sufficient quantities, since Gly is not synthesized quickly enough to meet the metabolic needs (i.e. precursor for the synthesis of uric acid). There is evidence of a positive effect on chicken performance of Gly supplementation in low protein diets (DEAN et al., 2006). Nevertheless, there are conflicting reports in the literature and a recent meta-analysis indicates that it is still difficult to establish reliable Gly+Ser requirements for broilers due to the low number of dose responses available, and probably limiting factors or bias in these dose responses (LAMBERT et al., 2015b). In addition, the interaction between Gly and Thr (Fig. 1) has to be investigated, the quantitative importance of Gly synthesis from Thr being probably dependent on Thr dietary availability (WANG et al., 2013; LAMBERT et al., 2015c).

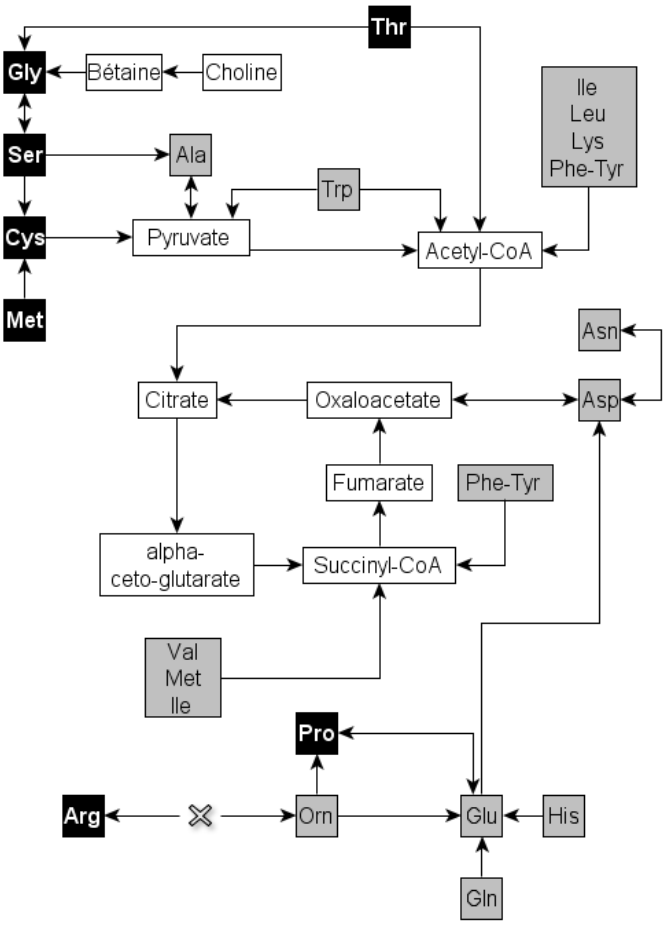

Figure 1. Metabolism of amino acids, interactions and particular features in avian species. General outline of metabolic pathways involving amino acids with a focus on some of them of particular interest in birds (underlined in black): sulfur amino acids (Met and Cys) due to the high requirement for feather synthesis; Thr, sometimes supplied in inadequate amounts in low crude protein diets, especially in challenging environmental conditions; amino acids considered as 'nutritionally essential' in birds. For example, Arg cannot be synthesized via the urea cycle; Pro, synthesized from Arg, Glu or Gln in most mammals, cannot be synthesized at sufficient levels in birds; Gly, a precursor for the synthesis of uric acid (nitrogen excretion in uric acid form instead of urea) is not synthesized quickly enough to meet metabolic needs. It (alone or in combination with Ser because of conversion between these amino acids) must also be present in sufficient quantities in the diet.

Conclusions

Amino acid nutrition is an old story and the issue is always improving the control of nutrient utilization and optimizing dietary amino acid provision for animal production purposes. Reducing the crude protein content of diets for broilers appears to have major benefits for the sustainability of animal production, but is difficult to achieve in chickens despite the knowledge available on amino acids. This can be partly explained by the complexity of mechanisms involved in the control of metabolism and also interactions between amino acids whose importance and implications for nutrition need to be examined and even hierarchized. Authors often focus on particular points and the difficulty is to integrate all the data available to propose recommendations. A major challenge for future studies will be to improve the integration of data without oversimplification, while taking into account the findings/concepts which are generalizable or dependent on a particular condition such as the experimental design, physiological situations, including early life, etc. Such studies will certainly provide a rationale for nutritional advice in the future.

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THE NUTRITIONAL VALUE OF YELLOW LUPIN (LUPINUS LUTEUS) FOR BROILERS

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Abstract

Yellow lupine seeds, 7 varieties were harvested in 2013. The experiment was conducted with 240, 16-day old Ross 308 male chickens. The reared birds were randomly located in cages and allotted 7 dietary treatments (10 replications in each, three birds per replication). For seven days, birds were fed experimental diets (30 % - yellow lupine seeds) and basal diet (70 % - maize-soybean). On day 20 and 21, excreta were individually collected twice per day and immediately frozen. On the 22nd day of the experiment, 30 chickens from each group were sacrificed and the ileum was removed. To allow digestibility to be determined, 3 g/kg TiO_2 was included as a marker. Apparent ileal digestibility (AID) was calculated using the difference method. Total NSP content in seeds ranged from 261 to 288, soluble NSP ranged from 24 to 95 mg/g (as-is basis). There were differences (P<0.05) in ileal digesta viscosity (4.97 to 8.61 mPas·s) and feed intake (115 – 121 g/d/bird) between yellow lupine cultivars. The obtained correlation coefficients (r) for raffinose content explained over 85% DM AID variability (r = -0.85, P<0.05) and 58% ether extract AID variability (r = -0.58, P<0.05). The relationship between soluble NSP content and the ether extract AID (r=0.26) was linear (P<0.05).

Keywords: yellow lupin, viscosity, ileal digestibility, non-starch polysaccharides,

Introduction

It is well known that nutrient composition and anti-nutritional factors in yellow lupin, generally, depend on the cultivar and growing conditions. The published data on nutrient and amino acid utilization and apparent metabolisable energy (AME) of yellow lupins for broilers are limited and inconsistent (GDALA and BURACZEWSKA, 1996, ALLOUI et al., 1994). In our previous study (KACZMAREK et al., 2014), it was found that raffinose content in blue lupine seeds was affecting AME_N and nutrient utilization. ZALEWSKI et al. (2001), LAHUTA (2011) showed that lupin seeds carbohydrates composition is affected by environmental conditions and it could be different across batches. Whole lupin seeds still contain approximately 35% NSP, of with over 5% are water soluble limiting the application in poultry feeding (GDALA and BURACZEWSKA, 1996).

The aim of the study was to explain causes of variations in feed intake, nutrient digestibility of Polish cultivars of yellow lupin meal (YLM) for broiler chickens.

Material and Methods

The basal diet was mixed in proportion 70:30 (w/w) with corresponding YLM. To allow digestibility to be determined, 3 g/kg titanium dioxide was included as a non-absorbable marker. All diets were offered in mash form *ad libitum*. The experiment was conducted on 240, 16-day old Ross 308 male chickens. The birds were randomly allocated to 8 dietary treatments (10 replications in each). Birds were kept in individual cages. In 7 treatments, diets containing a lupin meal (Perkoz, Lord, Parys, Baryt, Dukat, Mister, Taper *cv.*) and basal diet in proportion 70:30 were fed. Birds in one treatment were fed only the basal diet. Each bird had unlimited access to water and feed. For five days, birds were fed experimental diets for accommodation. On day 19 and 20, excreta were individually collected twice per day and immediately frozen for future analyses (n=10). On day 21 of the experiment, all chickens from each group were sacrificed and the ileum was removed. Digesta were flushed from the terminal ileum (15 cm, adjacent to the ileo-caecal junction) and pooled (3 birds/sample) to provide sufficient material for chemical analyses (n=10).

Results

Total NSP content in seeds ranged from 261 to 288 and soluble NSP ranged from 24 to 95 mg/g (as-is basis). There were differences (P<0.05) in viscosity of ileal digesta (4.97 to 8.61 mPas·s) and feed intake (115 – 121 g/d/bird) between lupine cultivars. The obtained correlation coefficient (r) for raffinose content explained over 85 % DM AID variability (r = -0.85, P<0.05) and 58 % ether extract AID variability (r = -0.58, P<0.05). The relationship between soluble NSP content and the ether extract AID (r=0.26) was linear (P<0.05). There was no effect of the investigated traits (raffinose and soluble NSP content or ileal viscosity) on average feed intake. However, a tendency (P=0.09) was observed for decreased FI as raffinose content increased in the diets (r = -0.23). In our previous trial (KACZMAREK et al., 2014), we found that raffinose affected negatively AME_N of blue lupine seed meals. Additionally, COON et al. (1990) and LESKE et

al. (1993) demonstrated that stachyose and raffinose content affected negatively TME_N of soy protein concentrate or SBM. Generally, it is suggested that legume oligosaccharides have hygroscopic properties and may affect transit of GIT digesta by elevating intestinal osmolarity (SMITS and ANNISON, 1996).

Table 1. Pearson's correlation coefficients between lupin seed traits ileal digesta and digestibility or feed intake of broiler chickens.

Traits	Response	r	p-value
Raffinose content	EE AID	-0.58	<0.05
	DM AID	-0.85	<0.05
	CP	-0.46	<0.05
	FI	-0.23	0.09
Soluble NSP content	EE AID	-0.26	0.06
	DM AID	-0.39	<0.05
	CP	-0.15	0.26
	FI	0.06	0.64
Ileal viscosity	EE AID	-0.54	<0.05
	DM AID	-0.46	<0.05
	CP	-0.35	<0.05
	FI	-0.04	0.77

EE – ether extract, AID – apparent ileal digestibility, DM – dry matter, CP – crude protein, FI – feed intake.

Conclusions

It can be concluded that the inherent variability of seeds may partially explain why birds responded differently across trials (including this experiment) to the inclusion of similar YLM doses. It seems that the nutritional value of yellow lupin seeds for broilers depends, to a considerable extent, on raffinose content and ileal viscosity (soluble NSP content).

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EVALUATION OF A LYS TO ARG ANTAGONISM IN LOW CRUDE PROTEIN DIETS FOR BROILER CHICKENS

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Abstract

A trial was conducted to evaluate the Lys and Arg interactive effect on production performance and slaughter characteristics. A total of 414 eight-day-old chicks were divided into 6 treatment groups (6 replicates). Diets 1 and 2 were high in dietary crude protein (CP) and differed in Lys (21% CP and 1.05% or 0.9% true digestibility of an amino acid (TD) Lys in grower; 19% CP and 0.95% or 0.8% TD Lys in finisher). Diets 3 to 6 were low in CP (19.5% in grower; 17.5% in finisher), differed in TD Lys (1.05% or 0.90% in grower; 0.95% or 0.80% in finisher) and TD Arg:Lys ratio (85% or 105% in grower and finisher). Differences between treatments were tested by ANOVA for diets 1 to 6. In an additional two-way ANOVA differences between diets 3 to 6 were evaluated.

Reducing CP did not affect zootechnical or slaughter performance (except abdominal fat) if the diet was high in both TD Lys and Arg:Lys ratio. High Lys diets showed a higher production performance, carcass weight, carcass yield, and breast meat yield at both high and low CP diets compared to low Lys diets ($p<0.01$). High Arg:Lys ratio improved F:G and resulted in higher carcass weight, breast meat weight and carcass yield ($p<0.05$). An interaction between Arg and Lys was only observed for carcass weight and breast meat yield ($p<0.05$). In conclusion, reducing CP in broiler diets does not impact performance when the animals are fed adequate amount of Lys and Arg. No Lys x Arg antagonism at low CP diets was observed except for tissues rich in meat.

Keywords: broiler, Lys to Arg antagonism, performance

Introduction

The knowledge of the requirement of essential amino acids helps to optimise the different nutritional inputs at various stages of broiler production. In low dietary crude protein (CP) diets for broiler chickens, a deficient Arg supply cannot be excluded. Furthermore, the performance reducing effect of high Lys diets in the case of a non-adequate Arg supply is described in literature (Jones 1964, Brake 2001). Hence, the present study should evaluate a potential Lys x Arg antagonism in modern diets for broiler chickens.

Material and Methods

Grounded feed and water were provided *ad libitum*. One day old broiler chicks (ROSS 308) were divided evenly into 36 pens and were fed a commercial starter feed (13.5 MJ AME_N, 18.0% CP) in the first week (preperiod). On life day 8, chicks of each replicate (6 pens) were weighed combined, to determine average body weight (BW). Subsequently, chicks which had less than 80% of the average BW were removed from the trial.

The study started with 414 eight-day old broiler chicks and was divided in a grower (0 to 21 days) and a finisher period (21 to 36 days). Four trial groups (TG) differing in Lys and Arg content (Table 1, TG 3-6) were created. Additionally, two diets (TG 1 and 2) higher in CP were involved in the study, to assess a dietary CP reducing effect on performance. All other essential amino acids were formulated according to the ideal protein (Lys:Met+Cys:Thr:Trp:Ile = 100:75:67:21:67). Grower and finisher diets (13.2 MJ AME_N) were based on corn, wheat, soy bean meal with hulls, DDGS, corn gluten meal, vegetable oil and grass green meal.

Table 1: Study design based on analysed (CP,AA content) and calculated (TD values) nutrient values

	Trial groups (TG)											
Traits	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
	Grower: *from day 8 to 21*						*Finisher: from day 21 to 36*					
CP, g/kg	209	205	197	190	190	192	194	190	180	182	175	177
TD Lys, g/kg	10.5	9.0	10.5	10.5	9.0	9.0	9.5	8.0	9.5	9.5	8.0	8.0
TD Arg, g/kg	11.3	9.4	8.9	11.0	7.6	8.5	10.0	8.4	8.1	10.0	6.8	8.4
TD Arg:Lys	*107.6*	*104.4*	*84.8*	*104.8*	*84.4*	*105.9*	*105.3*	*105.0*	*85.3*	*105.3*	*85.0*	*105.0*

The digestibility of free AA was assumed to be 100% (CHUNG and BAKER, 1992). True digestibility (TD) coefficients obtained from published tables (SAUVANT et al., 2004) were applied to the analysed amino acid levels in each ingredient to formulate diets on a TD amino acid basis.

Daily feed intake (dFI), average daily gain (ADG) and feed:gain (F:G) were corrected for mortality. Means separation for each variable was performed by Tukey-Kramer-Test. The tables present the least square means (LS-means) of the dietary treatments derived from the analysis of variance as well as the pooled standard error of means (SEM). Significant differences among means ($P < 0.05$) are indicated by superscripts.

Results

The results of zootechnical performance are presented in Table 2.

Table 2: Results of the growth performance

CP content	high	high	low	low	low	low	SEM	p-value
TD Lys content	high	low	high	high	low	low		
Arg to Lys ratio	105%	105%	85%	105%	85%	105%		
BW at day 8, g	179	176	178	178	177	172	1.81	0.923
BW at day 21, g	848^{a}	739^{b}	817^{a}	843^{a}	737^{b}	740^{b}	9.44	<0.0001
BW at day 36, g	2173^{a}	1760^{c}	1992^{b}	2078^{ab}	1735^{c}	1731^{c}	31.94	<0.0001
Grower period								
ADG, g	48^{a}	40^{b}	46^{a}	47^{a}	40^{b}	41^{b}	0.65	<0.0001
dFI, g	72^{b}	67^{c}	73^{a}	72^{ab}	68^{c}	68^{c}	0.52	<0.0001
F:G, kg/kg	1.50^{b}	1.68^{a}	1.60^{ab}	1.53^{b}	1.73^{a}	1.67^{a}	0.02	<0.0001
Finisher period								
ADG, g	83^{a}	64^{c}	73^{b}	77^{ab}	63^{c}	62^{c}	1.44	<0.0001
dFI, g	138^{a}	113^{c}	129^{ab}	132^{ab}	121^{b}	113^{c}	1.94	<0.0001
F:G, kg/kg	1.67^{b}	1.76^{b}	1.78^{ab}	1.71^{b}	1.93^{a}	1.83^{ab}	0.02	<0.0001

The study started with an average bird weight of 177 ± 1.81 g. After 14 trial days, significant differences between treatments were observed. In the grower period, birds applying Lys enhanced diets reached higher BW, ADG, dFI and an improved F:G ($P < 0.05$). No differences between treatments were observed regarding TD Arg:Lys ratio in the low CP diets. In the finisher period, similar effects were observed regarding Lys content. Birds receiving the low protein low Lys high Arg diet (TG 6) showed a significantly lower dFI compared to the low protein low Lys low Arg diet (TG 5) ($P < 0.05$), while in the low protein high Lys high Arg (TG 4) diet a numerically increased ADG was observed compared to the low protein high Lys low Arg (TG 3) diet ($P > 0.05$). In both periods, no significant effect of increased Arg was recorded but the F:G was numerically improved by 3.5 to 4.4% in growers and by 3.9 to 5.2% in finishers when feeding 105% TD Arg:Lys compared to 85%.

The results of slaughter performance are listed in Table 3. Animals receiving the high protein high Lys diet (TG 1) and the low protein high Lys and high Arg diet (TG 4) recorded the highest carcass weights. In contrast to this, the low Lys diets showed lower carcass weights in both high and low CP ($P < 0.05$). The abdominal fat content was reduced by the high protein diets ($P < 0.05$). Dressing percentage was comparable to the results of the live weights. Furthermore, an interaction ($P < 0.05$) between Lys content and Arg ratio in low CP diets, resulting in heavier carcass weights for birds receiving the high Lys high Arg diet (TG 4) compared to high Lys low Arg diet (TG 3), was detected. Higher Lys contents resulted in a higher relative proportion of breast meat, and reminder of carcass as well as a lower proportion of legs compared to low Lys diets ($p<0.05$). Regarding the parameter breast meat proportion an interaction ($P < 0.05$) was observed, resulting in larger breast meat proportion for birds receiving the high Lys high Arg diet (TG4) compared to high Lys low Arg diet (TG3).

Table 3: Results of the slaughter performance and relative proportion of carcass parts compared to the carcass for grilling

CP content	high	high	low	low	low	low	SEM	p-value
TD Lys content	high	low	high	high	low	low		
Arg to Lys ratio	105%	105%	85%	105%	85%	105%		
Carcass for grilling, g	1546[a]	1210[c]	1396[b]	1504[a]	1171[c]	1170[c]	12.88	<0.0001
Abdominal fat, g	34[d]	35[cd]	44[a]	40[abc]	40[ab]	37[bcd]	0.54	<0.0001
Heart, g	9.9[a]	8.2[bc]	8.9[b]	9.1[b]	7.8[c]	7.7[c]	0.09	<0.0001
Liver, g	44[a]	33[b]	42[a]	42[a]	35[b]	32[b]	0.43	<0.0001
Gizzard, g	29[a]	26[bc]	27[ab]	26[bc]	25[b]	25[bc]	0.19	<0.0001
Dressing, %	79.8[a]	77.7[c]	78.7[b]	79.8[a]	77.4[c]	78.4[b]	0.08	<0.0001
Relative proportion of carcass parts compared to the carcass for grilling								
Breast meat, %	30.7[a]	25.5[d]	27.6[bc]	29.4[ab]	26.3[cd]	25.7[cd]	0.32	<0.0001
Legs, %	27.9[c]	30.5[a]	29.3[abc]	28.4[bc]	29.9[abc]	30.1[ab]	0.23	0.001
Remainder of carcass, %	31.5[b]	33.2a	32.3[ab]	31.4[b]	33.1[a]	33.0[a]	0.15	<0.0001

Conclusion

In general, the well known performance improving effect of Lys in low and high CP diets was confirmed with the present study. A higher TD Arg to Lys ratio improved F:G, carcass weights and dressing percentage. Furthermore, an interaction between Lys and Arg was recorded for the parameters carcass weight and breast meat percentage. However, no Lys x Arg antagonism was detected for growth performance of broiler chickens in this trial.

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FIBER IN POULTRY NUTRITION: BONUS OR BURDEN?

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Abbreviated title: Fiber in poultry nutrition

Summary

The generic term fiber encompasses a very diverse group of polymers, with varying physicochemical properties. It is well known that dietary fiber can contribute significantly to the nutritive value of poultry diets both directly, as energy source, and indirectly, through its effects on digestive and metabolic processes. In order to more accurately predict the nutritive effect of fiber from raw materials, a better characterization of fiber fractions, their degradation in the chicken, and their physiological effects are required. Traditional analytical methods to analyze fiber, as crude fiber (CF) and neutral detergent fiber (NDF), recover only a variable part of the fiber fraction and are hence unfit to evaluate fiber fractions in raw materials and poultry diets. For scientific purposes, the enzymatic-chemical (Englyst or Uppsala) methods are more appropriate, whereas for routine analyses the AOAC (2009.01/2011.25) method for total, insoluble, and soluble dietary fiber can be used. In the chicken, solubilization is a prerequisite for fermentation, but even if solubilized during the digestive processes, a substantial part of non-starch polysaccharides (NSP) may remain undegraded. Coefficients of apparent total tract digestibility of NSP in chicken range between 0 and 0.4 and generally reflect differences in solubility of the fiber fraction. Besides, physical entanglement of polysaccharides in the cell wall matrix also time available for fermentation and the absence of appropriate enzyme activities as determined by the microbial colonization in the gastrointestinal tract are possible limiting factors for NSP degradation. Although fiber in poultry nutrition is often associated with reduced energy availability due to its minor role in energy supply and interference with digestive processes, low to moderate amounts of fiber (up to 50 g/kg) might be beneficial for gastrointestinal development, function, and health, thereby enhancing nutrient digestibility and growth performance. A better understanding on the relation between specific fiber fractions and factors as GIT development, digesta retention time, and microbial colonization will help to develop nutritional strategies using specific fiber fractions to steer on GIT health and function to enhance performance, especially under suboptimal environmental conditions.

Key words: analytical methods, fermentation, fibre, gastrointestinal health, non-starch polysaccharides,

Introduction

Although abundantly present in many feed ingredients used for poultry diets, dietary fiber (DF) has received little attention poultry nutrition for many years. It is well known that dietary fiber can contribute significantly to the nutritive value of diets both directly, as energy source (JAMROZ et al., 2002, JØRGENSEN et al., 1996), and indirectly, through its effects on digestive and metabolic processes (CHOCT et al., 1996, JØRGENSEN et al., 1996, SMITS et al., 2000, SMITS et al., 1998, SMITS et al., 1997, ANNISON, 1993, MONTAGNE et al., 2003). The generic term fiber, however, encompasses a very diverse group of polymers, with varying physicochemical properties, which hampers prediction of the nutritive value of fiber fractions. Not only the quantity of fiber, but maybe even more importantly, the type of fiber will determine the digestive utilization of the diet and, hence, affect its energetic value, either positively or negatively. In order to more accurately predict the nutritive effect of fiber from raw materials, a better characterization of fiber fractions, their degradation in the chicken, and their physiological effects are required.

How to track and trace the dietary fiber fraction?

CHALLENGES IN THE ANALYSIS OF DIETARY FIBER

Albeit extensive debates on the appropriate definition and adequate analytical methods, DF is generally defined as the non-digestible fraction of plant cell walls in food and feed ingredients[1] and typically includes oligosaccharides, pectic polysaccharides, hemicellulose, cellulose, lignin, gums, and some minor associated plant cell wall substances (DE VRIES, 2004). Although the chemical constitution of these cell wall fractions varies widely, they can be considered alike

[1] The currently most widely accepted definition of DF includes Dietary fiber means carbohydrate polymers with three or more monomeric units, which are not hydrolyzed by the endogenous enzymes in the small intestine of humans (JONES, 2014, CODEX ALIMENTARIUS COMMISSION, 2013).

from a nutritional viewpoint, as they are not enzymatically digested and exert similar physiological properties. Resistant starch – which is by definition resistant to enzymatic digestion, but mostly not originating from the cell wall (ASP and BJÖRCK, 1992) – is often included based on its similar behavior in the gastrointestinal tract.

Several methods to analyze DF – or its specific components as non-digestible oligosaccharides non-starch polysaccharides (NSP), RS, or lignin – are available (DE VRIES, 2004). Historically in animal nutrition, the gravimetrically based crude fiber (CF), and later neutral (NDF) and acid detergent fiber (ADF) methods, have been used. Although these methods, based on chemical extraction with alkali and acid solutions, are robust to analytical variation and might correlate well with degradability of specific fiber fractions in the animal (MERTENS, 2003), they recover only part of the fiber fraction (Figure 1). Depending on the material, a variable, but considerable part of the fiber fraction – such as cereal β-glucans and some pectic polysaccharides – can be solubilized and hence excluded from the detergent and crude fiber methods, whereas some other pectic polysaccharides that precipitate in strong acid will be included in the ADF fraction (MERTENS, 2003). In addition, also the physical characteristics of the sample, such as particle size, are of importance, because particles can be lost during the filtration step of these gravimetric methods. For CF, for example, this means that depending on the fiber structure of the material, 80-85 % of the hemicellulose, 0 to 60 % of the cellulose, and 10 to 95 % of the lignin are not recovered in this fraction (MERTENS, 2003). It follows that comparison between diets or feed ingredients with distinct fiber structures and physicochemical properties based on these analytical methods is specious. Moreover, this hampers not only comparisons between fiber sources, but also evaluation of fiber degradation in the animal, as physicochemical properties of the ingested feed will be modified by digestive processes along the gastrointestinal tract (GIT).

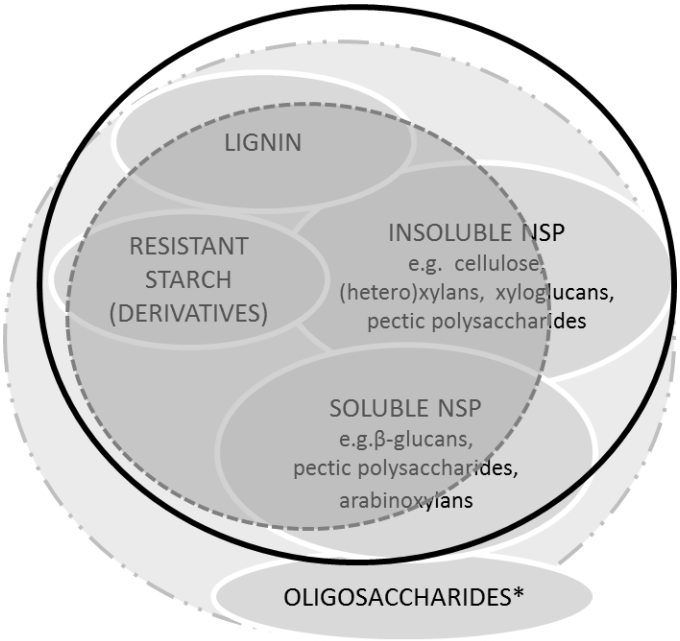

Figure 1. Indicative composition of total dietary fiber* (solid black line), fiber fractions analyzed by gravimetric methods† (dashed grey line), or calculated as residual fraction (calculated NSP)‡ (dashed-dotted grey line).*Analyzed by 'rational' methods as sum of non-starch polysaccharides (enzymatic-chemical methods) and lignin. Oligosaccharides can be separately quantified by liquid chromatography (MCCLEARY et al., 2011). †Empiric gravimetric methods, such as crude fiber or neutral detergent fiber (VAN SOEST et al., 1991). Recovery of various fractions depends on method and physical characteristics of sample (e.g. particle size). ‡Residual organic material after subtraction of ash, crude protein, crude fat, starch, and ethanol (40%)-soluble sugars from DM (CVB, 2011), including NSP, lignin, residual components not analyzed by the proximate system, and accumulated analytical error. Calculated NSP (dashed-dotted grey line) minus crude fiber (dashed grey line) approximates nitrogen-free extract minus digestible starch and sugars. Adapted from ZIELINSKI ET AL. (2013).

Alternatively, in feed formulation the fiber fraction is often calculated as the residual fraction remaining after subtraction of several components analyzed by the proximate or Weende analysis system (AOAC, 2009). In this system, the nitrogen-free extract (NFE) is defined as the organic material remaining after subtraction of ash, crude protein (CP), crude fat, and crude fiber from the dry matter (DM), representing the carbohydrate fraction that is potentially degraded in the animal without distinguishing between easily digestible starch and sugars and the (partly) fermentable fiber fraction.

Due to the lack of specificity of the CF-method as described above, NFE also may contain considerable part of the lignin and poorly degradable NSP. The calculated NSP fractions as reported in the Dutch CVB tables (CVB, 2011, Figure 1), are obtained by subtraction of ash, CP, crude fat, starch, and ethanol-soluble sugars, and hence include NSP, lignin, and some low-molecular-weight carbohydrates that precipitate in 40%-ethanol. In both NFE and NSP fractions, components not analyzed by the proximate system – such as some nitrogenous components and vitamins – and analytical errors associated with the analyses accumulate in these residual fractions. Hence, the exact composition of these fractions depends on the raw material and its physicochemical properties.

For scientific purposes, a more appropriate alternative, is the use of enzymatic-chemical methods, as the Englyst or the Uppsala procedures (BACH KNUDSEN, 2001, ZIELINSKI et al., 2013). In these, so-called, "rational"[2] methods, NSP are measured as the sum of constituent monosaccharides released by acid hydrolysis of samples after removal of starch and small saccharides. Not only do these methods provide detailed information on the chemical composition of the fiber fraction, they also have the advantage that they are not sensitive to physical factors such as solubility and particle size of samples, which makes them more appropriate to evaluate fiber degradability in the animal. In addition, they are species-independent as opposed to the empirical DF methods that are typically optimized for their application in human. These sophisticated methods, are, however, not suitable for routine analysis as they are expensive, both in terms of labor and equipment, and are less robust to analytical errors due to the multiple subsequent analytical steps, as well as the small amount of sample material. Furthermore, the small amount of sample material hampers representative sampling in inhomogeneous feed materials. With the recent advances and consensus on optimization of analytical methods for DF in the human nutrition field (ZIELINSKI et al., 2013), the enzymatic-gravimetric methods for total, insoluble, and soluble DF recognized by AOAC (2009.01/2011.25) have evolved in robust methods that may be used for routine analyses of fiber fractions for day-to-day feed formulation practices. These methods could be further optimized in terms of filtration procedures, to avoid confounding effects of particle size. Additionally, near-infrared reflectance spectroscopy (NIRS) might be used to predict contents of DF and specific DF fractions (KAYS and BARTON, 2002, KAYS et al., 1996)).

MEASURING THE FATE OF DIETARY FIBER ALONG THE GASTROINTESTINAL TRACT

As discussed above, the nature of the gravimetric fiber methods makes them unfit to monitor degradation of fiber along the GIT, as changes in physicochemical properties of ingested fiber fractions during digestion confound the results. Hence, the use of "rational" methods is preferred for digestibility studies. When evaluating fiber degradation along the GIT, not only methods to correctly analyze the fiber constituents, but also obtaining a representative sample of the GIT-contents can be challenging. In chickens, soluble and solid digesta fractions are separated and retrograde digesta flows occur. Often, fiber-rich diets have pronounced effects on physical characteristics of the feed matrix, thereby affecting a.o. digesta flow and transit time (ROBERTSON, 1988). These processes may complicate digestion studies, particularly, but not exclusively, when fiber degradation is the matter of interest (DE VRIES et al., 2014a). Unrealistic high ileal and cecal digestibility values for NSP, sometimes exceeding total tract digestibility (PETTERSSON and ÅMAN, 1989, JAMROZ et al., 2002, BRENES et al., 2003, DE VRIES et al., 2014a), clearly indicate that that the marker method is unsuitable to measure fiber degradation along the GIT in chicken. In future research, a dual phase marker system combined with mathematical modelling of digesta flow pathways, quantitative digesta collection, and stable isotope methods can be helpful to keep track of fiber fractions along the GIT. When apparent total tract degradation of NSP is measured, separation of digesta fractions does not have to be an issue provided that the collection time is sufficiently long and a representative sample of the excreta is collected. Ceca empty at irregular intervals; approximately 2 to 4 times a day, majorly in the morning, just after onset of light, and in the afternoon (HERRICK and EDGAR, 1947, GASAWAY and WHITE, 1975). Hence, short collection periods at single time points may promote selective recovery of excreta, resulting in considerable over- or underrepresentation of cecal contents and erroneous digestibility estimates for fermentable matter.

[2] Rational refers to methods in which an identifiable chemical or analyte is quantitated, as opposed to empirical, non-analyte specific, methods (ZIELINSKI et al., 2013).

Fate of fiber in the gastrointestinal tract

FIBER DEGRADATION IN THE CHICKEN

Degradation of NSP in the animal depends to a large degree on the original cell wall matrix in which the polysaccharides are embedded (DE VRIES et al., 2012). In chickens, ceca access is restricted to fluids and small particles (<0.2mm) and consequently solid digesta won't reach the major site of fiber degradation (JÓZEFIAK et al., 2004). It follows that NSP should be solubilized in order to be fermented. However, a substantial fraction of canola meal NSP that were solubilized during transit through the GIT, remained undegraded in broilers (PUSTJENS et al., 2014) indicating that also the time available for fermentation or the lack of appropriate enzyme activities are possible limiting factors in NSP degradation.

Coefficients of apparent total tract digestibility (CATTD) of NSP in chicken, as reported in literature range between 0 and 0.4 and generally reflect differences in solubility of the NSP fractions of the various feed ingredients (Table 1). Degradation of NSP is particularly low (0-0.1) in diets containing poorly solubilizable NSP from ingredients as maize or some sources of canola meal (MENG and SLOMINSKI, 2005, SLOMINSKI et al., 1994b, DE VRIES et al., 2014b), whereas higher CATTD of NSP (0.2-0.4) are found for diets that contain more soluble NSP, originating mainly from cereal grains as barley, oats, wheat, and rye. Degradability of NSP from the wheat-soybean meal (SBM) diets, with and without inclusion of several fiber-rich byproducts, in the studies of BOROS et al. (2004) and MENG et al. (2005) are lower than expected based on NSP solubility. In these studies excreta were collected for a period of only 3 h, and selective recovery of ceca contents due the irregular emptying of the ceca may have resulted in erroneous CATTD estimates. Also the CATTD of NSP from diets containing pea fiber in the study of JØRGENSEN et al. (1994) are lower than expected based on the NSP solubility. Apparently, the soluble NSP of this product, which is produced by water extraction of peas and has a high water-binding capacity, were poorly degraded and likely pea fiber even impeded degradation of barley NSP.

In common feed practices, NSP-degrading enzymes are usually added to broiler and laying hen diets. The use of such enzymes can facilitate degradation of specific NSP structures, when either appropriate enzyme activities are lacking or when time is limiting their full operation. Depending on e.g. the NSP source and the type of enzymes used, CATTD of NSP can be improved over 0.2 units in broilers (PUSTJENS et al., 2014, PETTERSSON and ÅMAN, 1989, MENG and SLOMINSKI, 2005, MENG et al., 2006, BOROS et al., 2004, JIA and SLOMINSKI, 2010, MARSMAN et al., 1997), whereas the effect in adult birds can be even greater (MENG et al., 2006, LÁZARO et al., 2003, SLOMINSKI and CAMPBELL, 1990). Effects of common feed processing technologies are typically smaller, and generally mainly affect easily solubilizable NSP such as cereal β-glucans (DE VRIES et al., 2012).

FIBER AS NUTRIENT: DOES IT MAKE A DIFFERENCE?

Non-starch polysaccharides can be partly fermented by the microbiota residing in the gastrointestinal tract of the animal. The end products of NSP fermentation, short chain fatty acids (SCFA), can be absorbed and used as energy source (DIERICK et al., 1989, JØRGENSEN et al., 1997, JUST et al., 1983). Given that less than 40% of NSP are degraded, the lower efficiency of energy utilization obtained from microbial fermentation compared with enzymatic digestion, and the potential higher energy requirements for fiber-rich ingredients (DIERICK et al., 1989), it follows that, generally, the contribution of NSP to the energy supply in the bird is expected to be minor (JØRGENSEN et al., 1996). In addition, DF can interfere with digestive processes, thereby often reducing digestion and absorption of other nutrients from the diet (FENGLER and MARQUARDT, 1988, CHOCT and ANNISON, 1992b, CHOCT and ANNISON, 1992a, MONTAGNE et al., 2003, SMITS et al., 2000, SMITS et al., 1997, SMITS et al., 1998). Hence, DF is often considered as diluent or even anti-nutritional factor, in poultry nutrition. However, as reviewed by MATEOS et al. (2012), DF play an important role in GIT development and functioning and when fed at low to moderate levels (up to 50 g/kg) may enhance GIT health, nutrient digestibility, and bird performance. Especially insoluble, recalcitrant, fiber fractions that resist fermentation in the gut may be important from this perspective due to their effects on gizzard function and digesta retention time in the GIT (HETLAND et al., 2004). A better understanding on the relation between specific fiber fractions and factors as GIT development, digesta retention time, and microbial colonization will help to develop nutritional strategies using specific fiber fractions to steer on GIT health and function to enhance performance, especially under suboptimal environmental conditions.

Table 1. Coefficient of apparent total tract digestibility (CATTD) of non-starch polysaccharides (NSP) in chickens[1].

Diet characteristics				CATTD	Bird type	Reference[6]
Main NSP sources[2]	Adaptation[3]	NSP content[4]	NSP Solubility[5]			
Barley (100)	0 to 63 d[7]	45	0.29	0.31	Male broilers (2-13 wk)	1
Barley (73), oat bran (27)	0 to 63 d[7]	91	0.43	0.35	Male broilers (2-13 wk)	1
Barley (65), oat bran (35)	0 to 63 d[7]	127	0.39	0.25	Male broilers (2-13 wk)	1
Barley (47), SBM (31), wheat (22)	41 d	127	0.31	0.39	Male broilers (6 wk)	2
Wheat bran (51), barley (49)	0 to 63 d[7]	132	0.27	0.19	Male broilers (2-13 wk)	1
Wheat bran (61), barley (39)	0 to 63 d[7]	203	0.18	0.16	Male broilers (2-13 wk)	1
Wheat (70), SBM (30)	37 d	97[8]	0.24[9]	0.05	Male broilers (7 wk)	3
Wheat (45) SBM (27), canola meal (12), wheat screenings (10), peas (6)	12 d	101[8]	0.25[9]	0.06	Male broilers (2-3 wk)	4
Wheat (37) SBM (33), barley (16), canola meal (9), wheat screenings (5)	37 d	120[8]	0.31[9]	0.15	Male broilers (7 wk)	3
Rye (58), SBM (26), wheat (16)	20 d	121	0.26	0.40	Mixed sex broilers (5-6 wk)	5
Pea fiber (54), barley (36)	0 to 63 d[7]	149	0.40	0.06	Male broilers (2-13 wk)	1
Pea fiber (64), barley (46)	0 to 63 d[7]	251	0.40	0.12	Male broilers (2-13 wk)	1
Maize (54), peas (49)	12 d	88	0.07	0.05	Male broilers (2-3 wk)	6
Maize (100)	12 d	57	0.08	0.08	Male broilers (2-3 wk)	6
Maize (100)	11 d	68	0.10[9]	0.14	Female broilers (4-5 wk)	7
Maize (52), SBM (48)	12 d	100	0.09	0.09	Male broilers (2-3 wk)	6
SBM (51), maize (35), canola meal (14)	12 d	136[9]	0.16[9]	0.12	Male broilers (2-3 wk)	8
SBM (51), maize (35), canola seed (14)	12 d	136[9]	0.16[9]	0.11	Male broilers (2-3 wk)	8
Canola meal (75), maize (25)	11 d	176	0.25[9]	0.20	Female broilers (4-5 wk)	7
Canola meal (56), maize (44)	12 d	106	0.09	0.08	Male broilers (2-3 wk)	6
Canola meal, brown seeded (100)	7 d	91	0.10	0.03	Adult laying hens	9
Canola meal, yellow seeded (100)	7 d	113	0.09	0.09	Adult laying hens	9
Canola meal (100)	4 d	71	0.14	0.02	Adult laying hens	10

[1] Only studies that reported CATTD of total NSP in (semi-purified) complete diets were included. Studies in which CATTD measurements were preceded by fasting were excluded.
[2] Values between brackets indicate contribution of NSP from raw materials to total NSP content of diet (%) calculated using data from original publications or BACH KNUDSEN (1997).
[3] Time in days (d) birds were allowed to adapt to the diet before CATTD measurements.
[4] NSP content of diet (g/kg DM) as reported or calculated from analyzed NSP contents of raw materials, unless indicated otherwise. Dry matter contents diets were assumed to be 900 g/kg, if not provided.
[5] Fraction of soluble NSP in total NSP (g/g), calculated from analyzed NSP contents, unless indicated otherwise.
[6] Data from: 1. JØRGENSEN et al. (1996); 2. JAMROZ et al. (2002); 3. BOROS et al. (2004); 4. MENG et al. (2005); 5. PETTERSON and ÅMAN (1989); 6. MENG and SLOMINSKI (2005); 7. DE VRIES et al. (2014b); 8. MENG et al. (2006); 9. SLOMINSKI et al. (1994) ; and 10. SLOMINSKI and CAMPBELL (1990).
[7] CATTD was measured during two blocks of five and four weeks from 12 d of age onwards, without preceding adaptation.
[8] Calculated based on data from BACH KNUDSEN (1997) and SLOMINSKI et al. (2004).
[9] Calculated based on data from BACH KNUDSEN (1997).

Conclusion

Traditional analytical methods to analyze fiber, as crude fiber (CF) and neutral detergent fiber (NDF), recover only a variable part of the fiber fraction and are hence unfit to evaluate fiber fractions in raw materials and poultry diets. For scientific purposes, the enzymatic-chemical (Englyst or Uppsala) methods are more appropriate, whereas for routine analyses the AOAC (2009.01/2011.25) method for total, insoluble, and soluble dietary fiber can be used. Coefficients of apparent total tract digestibility of NSP in chicken range between 0 and 0.4 and generally reflect differences in solubility of the fiber fraction. Besides, physical entanglement of polysaccharides in the cell wall matrix also time available for fermentation and the absence of appropriate enzyme activities as determined by the microbial colonization in the gastrointestinal tract are possible limiting factors for NSP degradation. Although fiber in poultry nutrition is often associated

with reduced energy availability due to its minor role in energy supply and interference with digestive processes, low to moderate amounts of fiber (up to 50 g/kg) might be beneficial for gastrointestinal development, function, and health, thereby enhancing nutrient digestibility and growth performance. A better understanding on the relation between specific fiber fractions and factors as GIT development, digesta retention time, and microbial colonization will help to develop nutritional strategies using specific fiber fractions to steer on GIT health and function to enhance performance, especially under suboptimal environmental conditions.

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COMPARATIVE INTERESTS AND LIMITS OF METABOLIZABLE ENERGY AND NET ENERGY FOR EVALUATING POULTRY AND PIG FEEDS

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Summary

The evaluation of the energy content of pig or poultry feeds has been most commonly based on their DE or ME contents. However, the closest estimate of the "true" energy value of a feed should be its NE content, which takes into account differences in metabolic utilization of ME of nutrients. This review considers some methodological aspects of NE determination. Experimental data in pigs indicate that the NE/ME ratio varies greatly with the chemical composition of diets and nutrient, with ratios for fat (90%) and starch (80%) that are higher than for protein and dietary fibre (60%). This has marked consequences on the relative energy values of ingredients according to the energy system that is used. The NE system is also better in predicting the performance of pigs. With regard to poultry, the ranking between nutrients for NE/ME is similar to what is observed in pigs but with smaller differences between nutrients. However, complementary data are required to propose a convincing and functional NE system for poultry. In any case, the accuracy of the NE value is highly dependent on the accuracy of DE or ME values or digestible nutrient contents that are used as predictors of NE values. Overall, there is an obvious advantage in using NE systems for pigs while further investigations are required for implementing a reliable NE system for poultry.

Introduction

The cost of feed is the most important cost of pig or broiler meat production (#60-70%) and the energy component represents the greatest proportion. Therefore, it is important to estimate precisely the energy value of feeds, either for least-cost formulation purposes or for adapting feed supply to energy requirements of animals. Evaluation of energy content of pig or poultry feeds was firstly and most commonly based on their DE or ME contents (only ME for poultry). However, the closest estimate of the "true" energy value of a feed corresponds to its NE content which takes into account differences in metabolic utilization of ME between nutrients. In addition, in a NE system, energy requirements and diet energy values are expressed on a same basis which should theoretically be independent of the feed characteristics. The objectives of this paper are then to present 1/ the available energy systems for pig feeds with emphasis given to NE systems and 2/ the corresponding available data on energy utilization in poultry (broilers). An overview of the methodological aspects of energy evaluation of pig and poultry feeds will introduce the topics.

Methodological aspects

Not all gross energy (GE) of a feed is available for meeting the requirements of animals since variable proportions of GE are lost in excreta (faeces and urine in pigs), as fermentation gases (i.e., methane, hydrogen) and as heat (or heat increment, HI). The DE content of a feed (in pigs) is equal to its GE content minus faecal energy losses after digestion in the digestive tract. The ME content of a feed corresponds to the difference between the DE content and energy losses in urine and gases in pigs; for poultry, it is simply the difference between feed gross energy and energy losses in excreta and fermentation gases. Most of the energy lost in gases is due to methane production, which typically is very small in growing pigs and poultry and most ME values in literature and tables for growing pigs and poultry ignore energy losses as methane. As illustrated later on, it is not the case in adult pigs (Le Goff et al., 2002). In addition and unlike amino acids evaluation, most energy values do not consider energy losses related to the gut activity (i.e. endogenous losses) and DE and ME values are then apparent values. Finally, in the case of poultry, it is still rather common to consider that digestible N is totally excreted as uric acid and then to adjust the ME values for a zero N balance, even though that situation is unrealistic.

Net energy is defined as the ME content minus HI associated with feed utilization (i.e., energy cost of ingestion and digestion and HI related to metabolic utilization of ME) and the energy cost corresponding to a "normal" level of physical activity. However, the HI of a given feed (as % of ME) depends on several physiological/animal factors and may not be constant over a large range of ME intakes for a given animal. For instance, the HI tends to be lower below than above maintenance energy supply (Noblet et al., 1993; 1994a; 1994b) or when ME is used for fat deposition compared with protein deposition (Noblet et al., 1999). Therefore, for comparing different feeds for their HI or the efficiencies of ME utilization, it is necessary to calculate these values under similar conditions (e.g., feeding levels), at protein

and amino acid supplies meeting the requirement and/or constant composition of the gain and/or at a given physiological stage.

While DE (pig) and ME (pig, poultry) contents are rather easy to measure, NE is more complex to evaluate, especially the so-called HI which corresponds to the rate of increase of heat production (HP) per unit of ME intake increase. In practice, the HI is calculated as the difference between HP in a "standard" fed state and HP in a fasting state (FHP) and related to the ME intake in the fed state. For a growing animal, the NE intake is then calculated as the sum of retained energy (RE) at this "standard" feeding level and fasting heat production at zero activity (FHP) (Noblet et al., 1994a; Noblet and van Milgen, 2004; 2013). The corresponding NE value or the efficiency of ME for NE (k as NE/ME) then correspond to a combined utilization of energy for meeting requirements for maintenance and for growth. Different methods are used in order to measure either HP (indirect calorimetry in respiration chambers, for instance; and RE equals ME intake minus HP) or energy gain (comparative slaughter method, for instance). The indirect calorimetry method is the most used, especially for large animals, since it allows measurements over a short period of time (i.e. a few days) and it is far more flexible and informative in order, for instance, to partition the total HP between different components (van Milgen et al., 1997). The value of FHP can be calculated by extrapolating HP measured at different feeding levels to zero ME intake. However, this method, even though it has been widely used in the past and is still used nowadays, has important limitations, mainly because the growing animal adapts its FHP to its feeding/energy level prior to fasting (Labussière et al., 2011) and questions severely the use and meaning of extrapolated HP at zero ME intake for getting an estimate of FHP. Consequently, direct measurements of FHP according to appropriate methods are highly preferable (Noblet et al., 1994a; 2015). In practice, the use of literature measurements of FHP is an alternative solution. Finally, the mode of expression of FHP is also quite critical. In brief and for being practical, it is now accepted that FHP averages 750 kJ per kg $BW^{0.60}$ in growing pigs (Barea et al., 2010) and 450 kJ per kg $BW^{0.70}$ in broilers (Noblet et al., 2015).

From a practical point of view and to avoid bias in the calculation of NE for a series of feeds, it is necessary to carry out energy balance measurements in similar animals (i.e., same sex, same breed and the same body-weight range), to keep these animals in a temperature-controlled environment within their thermoneutral zone, to minimize variation in behaviour, and to feed the animals at the same energy level with balanced diets so that the animals can express their production/growth potential. Under these circumstances, an erroneous estimate of FHP will affect the absolute NE value but not the ranking between feeds. This also means that NE should not be measured in animals fed a single ingredient for which the chemical characteristics are usually quite different from those of a complete balanced diet.

Measurements of DE and ME are easy to implement and can be undertaken on a large number of feeds at a reasonable cost. On the other hand, the measurement of NE is more complex and expensive and cannot then be implemented routinely, either for complete feeds or ingredients. The alternative is to keep measuring DE or ME contents and to use literature NE prediction equations that were obtained from experiments carried out under strict and standardized conditions with DE or ME values as predictors of NE. As illustrated later, the situation is rather operational for pigs; it would not be the case for broilers or poultry.

Energy utilization in pigs

For most pig diets, the digestibility coefficient of energy (DCe) varies between 70 and 90% but the variation is larger for feed ingredients (10 to 100%). Most of the variation of DCe is related to the presence of dietary fiber (DF) which is less digestible than other nutrients (#50% vs 80-100% for starch, sugars, fat or protein) and which also reduces the apparent fecal digestibility of other dietary nutrients such as crude protein and fat. Consequently, DCe is linearly and negatively related to the DF content of the feed (Le Goff and Noblet, 2001; table 1). The coefficients relating DCe to DF are such that DF essentially dilutes the diet, at least in growing pigs (table 1). In other terms, even though DF is partly digested by the young growing pig, it provides very little DE to the animal. Digestibility of energy can be modified by technological treatments. Pelleting, for instance, increases the energy digestibility of feeds by about 1% (Le Gall et al., 2009). However, the improvement is more important for some ingredients such as full fat rapeseed or (high oil) corn for which pelleting improves the digestibility of fat with subsequent marked differences in their DE value between mash and pellet forms (Noblet, 2006).

Table 1. Effect of diet composition (g/kg DM) on energy digestibility (DCe, %), ME:DE coefficient (%) and efficiency of utilization of ME for NE of mixed diets for growth (k_g, %)[a]

	Equation	RSD	Source[b]
1	DCe = 98.3 - 0.090 x NDF	2.0	1
2	DCe = 96.7 - 0.064 x NDF	2.2	1
3	ME/DE = 100.3 - 0.021 x CP	0.5	1
4	k_g = 74.7 + 0.036 x EE + 0.009 x ST - 0.023 x CP - 0.026 x ADF	1.2	2

[a] CP: crude protein, NDF: Neutral Detergent Fibre, EE: ether extract, ST: starch, ADF: Acid Detergent Fibre; RSD: Residual standard deviation.

[b] 1: Le Goff and Noblet, 2001 (n=77 diets; equations 1 and 3 in 60 kg growing pigs and equation 2 in adult sows, respectively); 2: Noblet et al., 1994a (n=61 diets; 45 kg pigs).

Energy digestibility is also affected by animal factors. In growing pigs, DCe increases with increasing BW (Noblet, 2006; Noblet et al., 2013) and the largest effect of BW is observed when adult sows either pregnant or lactating and (close to) ad libitum growing pigs are compared (Le Goff and Noblet, 2001; Le Gall et al., 2009). The difference due to BW increase is the most pronounced for high fiber diets or ingredients. The negative effect of DF on DCe is then lower in adult pigs than in growing pigs (table 1). In the case of adult sows and "60 kg" growing pigs, the DE value is 1.8, 4.2, 6.0, 10.3 and 16.6% higher in sows for wheat, corn, soybean meal, wheat bran and corn gluten feed, respectively (Sauvant et al., 2004). This improvement of energy digestibility with BW is mainly related to an improved digestive utilization of DF (Noblet and Le Goff, 2001). Little information concerning comparative digestibility in piglets and growing pigs is available. Considering that piglets are usually fed low-fiber diets for which the effect of BW is minimized, piglets can, from a practical point of view, be considered as growing pigs concerning the digestive utilization of energy.

The ME content of a feed for pigs is the difference between DE and energy losses in urine and gases (methane). In growing pigs, average energy loss in methane is equivalent to 0.4% of DE intake and is 2-3 times this amount in adult sows. Energy loss in urine represents a variable percentage of DE since urinary energy depends greatly on the urinary nitrogen excretion. At a given stage of production, urinary nitrogen excretion is mainly related to the (digestible) protein content of the diet (table 1). On average, it represents about 4% of the DE value. However, this mean value cannot be applied to single feed ingredients. The most appropriate solution is to estimate urinary energy (kJ/kg feed DM) from urinary nitrogen (g/kg feed DM) according to the following equation (in growing pigs):

$$\text{Urinary energy} = 192 + 31 \times \text{urinary nitrogen}$$

with urinary nitrogen representing 50% of digestible nitrogen or 40% of total nitrogen (Noblet et al. 2004).

Net energy is defined as ME minus heat increment associated with metabolic utilization of ME and to the energy cost of ingestion, digestion and some physical activity. The NE content, as a percentage of ME content (k) corresponds to the efficiency of utilization of ME for NE. In the studies of Noblet et al. (1994a) conducted on 61 diets fed to growing pigs, the variations in k were due to differences in efficiencies of ME utilization between nutrients with the highest values for fat (~90%) and starch (~82%) and the lowest (~60%) for DF and crude protein; k as obtained in growing pigs is then directly dependent on diet chemical composition (table 1) with positive effects of starch and fat on k and negative effects of DF and protein. Additional measurements conducted in pigs which differed for their BW and the composition of BW gain suggest that the efficiency of ME for NE is little affected by the composition of BW gain, at least under most practical conditions (Noblet et al., 1994b). Similarly, the ranking between nutrients for efficiencies is similar in adult sows fed at maintenance level and in growing pigs (Noblet et al., 1993). Finally, the heat increment associated with protein utilization, either retained as protein or catabolized, is constant (van Milgen et al., 2001) which means that the NE value of dietary CP is not dependent on its final utilization (deposition *vs*. deamination).

Table 2. Equations for prediction of NE in feeds for growing pigs (NEg; 61 diets; MJ/kg dry matter and % of DM; adapted from Noblet et al., 1994a)

N°	Equation[a]
5	NEg2 = 0.121 DCP + 0.350 DEE + 0.143 ST + 0.119 SU + 0.086 DRes
6	NEg4 = 0.703 DE - 0.041 CP + 0.066 EE - 0.041 CF + 0.020 ST
7	NEg7 = 0.730 x ME - 0.028 x CP + 0.055 x EE - 0.041 x CF + 0.015 x ST

[a] CP: crude protein, EE: ether extract, ST: starch, DCP: digestible CP, DEE: digestible EE, DRes: digestible residue (i.e., difference between digestible organic matter and other digestible nutrients considered in the equation).

The NE measurements conducted on 61 diets by Noblet et al. (1994a) were used to establish prediction equations relating dietary NE to predictors such as DE or ME and chemical indicators or digestible nutrients; the most practical ones have been used for the establishment of the INRA & AFZ feeding tables (Sauvant et al., 2004) or the EvaPig software (www.evapig.com); they are listed in table 2. Comparable equations have been produced by Schiemann et al. (1972) and further adapted in the Dutch feeding tables (CVB).

Energy systems for pig feeds

Apart from direct measurement on pigs, the DE and ME values of raw materials can be obtained from feeding tables (Sauvant et al., 2004; EvaPig; NRC, 2012). The utilization of these tabulated values should be restricted to ingredients having chemical characteristics similar or close to those in the tables. But, practically, the chemical composition and therefore the energy value of most raw materials differ from those listed in the feeding tables. It is then advised to correct the energy value for differences in chemical composition between ingredients proposed in the tables and those actually available for least-cost formulation; the EvaPig tool has been proposed for such a purpose (www.evapig.com). As illustrated in the previous section, DCe is affected by BW of the animals. It is therefore appropriate to use DE and ME values adapted to each BW class. However, from a practical point of view, it is suggested to use two values, one for "60 kg" pigs which can be applied to piglets and growing-finishing pigs and one for adult pigs applicable to both pregnant and lactating sows. The INRA & AFZ feeding tables propose such a differentiation (Sauvant et al., 2004) and the re-calculation of both energy values according to the chemical characteristics of ingredients is possible from the EvaPig software (www.evapig.com). Finally, a rather frequent difficulty in feed evaluation consists in estimating the energy value of compound feeds when their ingredients composition is unknown. The best solution is then to use prediction equations based on chemical criteria (Le Goff and Noblet, 2001; EvaPig) or estimates from near infrared or *in vitro* methods (Noblet and Jaguelin-Peyraud, 2007).

All published NE systems for pigs combine the utilization of ME for maintenance and for growth or for fattening. The system proposed by Noblet et al. (1994a) and applied in the INRA & AFZ feeding tables (Sauvant et al., 2004) is based on NE measurements on 61 diets and the NE prediction equations that have been generated from these measurements (table 2) are applicable to ingredients and compound feeds and at any stage of pig production (Noblet, 2006). It is important to point out that different DE values or digestible nutrient contents should be used in growing-finishing pigs and adult sows with two subsequent sets of NE values. Similarly, reliable information on digestibility of energy or of nutrients is absolutely necessary for an accurate prediction of NE content of pig feeds. In fact, this information represents the most limiting factor for predicting energy values of pig feeds. From that point of view, the lack of comprehensive information on effects of technology (pelleting, extrusion, enzymes addition, etc.) is a major limiting factor for getting accurate estimates of energy values for swine whatever the system used.

Assuming that NE represents the best estimate of the "true" energy value of feeds and according to differences in efficiencies of nutrients ME for NE, the energy value of protein-rich or fibrous feeds is overestimated when expressed on a DE (or ME) basis. On the other hand, fat or starch sources are underestimated in DE and ME systems (Table 3). With regard to NE for pigs, several systems have been proposed over the last 40-50 years. The INRA proposal (Noblet et al., 1994a; 2004) is probably the most advanced system which has been validated by calorimetry measurements (Noblet and van Milgen, 2013) and growth trials (table 4).

As previously mentioned, it is extremely important to use the same energy system for expressing the diet energy values and the animal energy requirements. From that point of view, the only energy system in which the requirements are the most independent on diet characteristics should be the NE system. This is illustrated by several growth trials

conducted with variable dietary fat or CP levels that show that the energy cost of growth or the daily energy requirement are independent on diet composition when expressed on a NE basis. On the other hand, on DE or ME bases, the energy cost is increased when CP content is increased or decreased when fat content is increased (Table 4). In other terms, relative to the NE system, the DE and ME systems are less able to predict the performance of growing pigs and reproductive sows.

Table 3. Relative DE, ME and NE values of ingredients for growing pigs[a]

	DE	ME	NE	NE/ME, %
Animal fat	243	252	300	90
Corn	103	105	112	80
Wheat	101	102	106	78
Reference diet	***100***	***100***	***100***	***75***
Pea	101	100	98	73
Soybean (full-fat)	116	113	108	72
Wheat bran	68	67	63	71
Soybean meal	107	102	82	60

[a] From Sauvant et al. (2004). Within each system, values are expressed as percentages of the energy value of a diet containing 68% wheat, 16% soybean meal, 2.5% fat, 5% wheat bran, 5% peas and 4% minerals and vitamins.

Table 4. Performance of growing-finishing pigs according to energy system and diet characteristics [a]

Energy system	DE	ME	NE
Trial 1: Added fat (%)			
0	***100***	***100***	***100***
2	100	100	100
4	99	99	100
6	98	98	100
Trial 2: crude protein content (30-100 kg)			
Normal	***100***	***100***	***100***
Low	96	97	100
Trial 3: crude protein content (90-120 kg)			
Normal	***100***	***100***	***100***
Low	97	98	100

[a] Energy requirements (or energy cost of BW gain) for similar daily BW gain and composition of BW gain; values are expressed relative to the energy requirement (or energy cost of BW gain) in the control treatment (considered as 100; values in bold characters); from Noblet (2006), Wu et al. (2007) and unpublished data.

Most energy recommendations for pigs were obtained according to DE and ME estimates for feeds and conclusions were expressed as DE or ME values. These recommendations were obtained with rather conventional feeds, i.e. cereals-soybean meal based diets whose efficiency of ME utilization in growing pigs is close to 74%. It is then proposed to estimate the NE recommendations (diet energy density, daily energy requirements, components of energy requirements, etc.) as DE or ME requirements multiplied by 0.71 or 0.74, respectively. This proposal is applicable at any stage of pig production, including pregnant or lactating sows, since NE value is calculated for any stage from one single set of equations obtained in growing pigs (Table 2). However, DE or ME or digestible nutrients may differ between stages of production, i.e. growing-finishing pigs *vs* reproductive adult sows, with subsequent different NE values.

Energy utilization and energy systems in poultry

Numerous studies have concerned the digestibility (in fact metabolizability) of energy in broilers or poultry. As for pigs, the main factor of variation of energy metabolizability is the DF content with even more pronounced effects since DF is almost undigested by broilers and the contribution of DF to ME supply is then even slightly negative. The recent paper of Carré et al. (2013) on 28 diets illustrates these observations. In addition, the (apparent) ME value of poultry feeds is frequently corrected for a zero N balance, assuming then that all absorbed amino acids are deaminated; even

such a correction eliminates the differences between ingredients due to their different CP contents, it is not representative of the reality for growing broilers, turkeys, etc. and even laying hens that retain 50% or more of the N that is absorbed. Therefore, this correction is questionable.

With regard to NE, several trials or theoretical assumptions over the last 70 years have been carried out to quantify the utilization of ME and its digestible nutrients for NE in poultry (review of Pirgozliev and Rose, 1999). Some studies were performed on ingredients or unbalanced diets with subsequent limitations in the interpretation of the results (cf. methodological section). The most comprehensive series of measurements were conducted in USA by Fraps (1946) and by the Rostock group (Schiemann et al., 1972), mainly focussing on feed ingredients. Unfortunately, most attention in these studies was focused on starch, DF and CP with little variability in fat content of diets or feedstuffs. A recent study of Carré et al. (2014) was conducted on quite variable complete feeds (n=28; 18.0 to 29.8 % CP, 5.6 to 12.3 % fat, 27.4 to 45.9 % enzymatic starch and 8.1 to 20.6 % NDF; expressed relative to DM) fed to 3 to 5 wk old broilers. The comparative slaughter method was used to quantify energy retention and NE was calculated according to a FHP value (500 kJ/ kg $BW^{0.60}$/d) measured in respiration chambers in comparable birds. A small concern in that study originates from an unexplained trial effect that has been included for interpreting the data and may have biased the final results.

As for pigs, literature values on poultry indicate that the lowest efficiency is observed for CP and the highest for fat (table 5). However, the difference between the most extreme values (i.e., CP and EE) appears to be lower in poultry (65 and 85%) than in pigs (60 and 90%). In addition, the values from Schiemann et al. (1972) were obtained in adult fattening birds which are in a physiologically different stage. From that point of view, the study of Carré et al. (2014) is probably more representative of modern broiler production. Preliminary results obtained in Australia (Barekatain et al., 2014) or in the USA (Cerrate Fernandez et al., 2012; Cerrate et al., 2013) on a limited number of diets would confirm these literature data. In addition, the data of Cerrate et al. (2013) indicate efficiencies of ME for NE in broilers that are remarkably close to those obtained in pigs (Sauvant et al., 2004) for a few typical ingredients used in pigs and broilers diets.

An alternative approach to study the effect of diet on the efficiency of ME for NE was tested in trials conducted at INRA (Noblet et al., 2003; 2007; 2009). The approach consisted in preparing diets focussing each time on one specific nutrient (in exchange for starch). The effects of CP, EE and DF contents were evaluated and the trials were conducted in respiration chambers on group housed and *ad libitum* fed growing broilers over the 3rd to the 6th week of age. The methodology allowed to quantify the HP related to physical activity of the birds and potential differences between diets due to changes in their behaviour. The most important results are presented in Table 6.

Table 5. Efficiency of ME utilization of digestible nutrients for NE in poultry (%)

Reference	Production[1]	CP	EE	CHO	Diet
Schiemann et al., 1972	M + fattening	61	84	75	73[2]
De Groote, 1974	M + growth	60	90	75	74[2]
Carré et al., 2014	M + growth	68	85	78	76

[1] M: maintenance; CP: crude protein; EE: ether extract; CHO: carbohydrates

[2] Assuming that 25, 20 and 55% of ME is provided as CP, EE and CHO

Table 6. Effect of diet composition on heat production and efficiency of ME for NE in broilers: compilation of INRA data[1]

		kJ/kg $BW^{0.70}$/d			NE/ME
Trial	Diets	ME	HP	AHP	%
1	18% CP	1609	853	146	75.1
	22.7% CP	1609	846	153	74.8
2	2.8% EE	1739	884	127	74.3[2]
	9.7% EE	1728	875	143	75.5[2]
3	9.5% NDF	1503	912	170	71.3
	17.7% NDF	1521	923	175	72.3

[1] Measurements carried out in groups of broilers weighing 1.3 to 1.5 kg BW on average for each trial (5 to 8 measurements per diet); the indirect calorimetry method in respiration chambers was used; AHP: Activity heat production; complementary details by Noblet et al. (2007) for trial 1 and Noblet et al. (2009) for trial 2; trial 3: unpublished data. In trials

1 and 2, the variation in CP or EE is associated with an inverse variation in starch content; in trial 3, the increased NDF level corresponds to a dilution by dietary fibre provided by wheat bran, maize bran and soybean hulls. In trial 1, data have been adjusted for a similar ME intake while observed values are given for trials 2 and 3. None of the differences between treatments within each trial were significant (P>0.05).

[2] Values adjusted for the same level of physical activity with the 2 diets

Table 7. Comparative effect of dietary crude protein on energy utilisation in growing pigs and broilers (adapted from Noblet et al., 2003)[1]

Species	Pigs		Broilers		
Dietary protein level	Normal	Low	Normal	Low	
Body weight, kg	57.6	57.2	1.47	1.46	
Energy balance[2], kJ/kg $BW^{0.60}$					
ME intake	2564	2566	1626	1642	
Heat production	1402[a]	1346[b]	862	861	Fasting heat
production	735	731	446	456	Heat incre-
ment	667[a]	614[b]	417	404	
NE/ME (x100)	73.9[a]	75.9[b]	74.8	75.0	

[1] The reduction of dietary CP consists in a replacement of protein from soybean protein concentrate by maize starch with supplementation of amino acids in order to meet the requirements.

[2] Energy balance data should be preferably expressed per kg $BW^{0.70}$ in broilers (Noblet et al., 2015)

The most surprising result of these trials is the absence of effect of dietary CP on HP and NE/ME in broilers; a parallel study conducted in both pigs and broilers confirmed this major difference between pigs and poultry (Noblet et al., 2003; Table 7). Table 6 also indicates that the replacement of starch by fat increased numerically the NE/ME ratio as it can be expected from similar trials in pigs (van Milgen et al., 2001) but this variation was not significant. Finally, the presence of high levels of undigested DF in broilers diets did not significantly change the HP and the NE/ME ratio of the diet. This latter result does not confirm the hypotheses of Emmans (1994) who suggested attributing an energy cost to undigested organic matter for its excretion in order to calculate the so-called "effective" energy. Overall and unlike our previous results on pigs or the preliminary results of Cerrate et al. (2013) in broilers, our studies indicate that major changes in diet composition do not affect markedly the efficiency of ME for NE in broilers. This result also means that the hierarchy between diets or ingredients for poultry would not be markedly affected by the energy system (ME vs. NE). Overall, additional studies conducted with adequately designed diets and discriminating methodologies and statistics are urgently required to solve these discrepancies between studies and to answer the question: is a NE system justified for poultry?

The NE equations from digestible nutrients contents proposed by Carré et al. (2014) represent a new and reliable basis for implementing a NE system in broilers feeds. Their evaluation on other NE data, either complete feeds or ingredients, is required. In a validation exercise conducted by Pirgozliev and Rose (1999), the NE value of a series of feedstuffs as measured by Fraps (1946) was compared to the values calculated according to different NE prediction equations. None of the literature NE systems for poultry was able to accurately predict the NE values measured on the feedstuffs; the biggest discrepancy was observed with the system proposed by Emmans (1994). This exercise might be repeated with the equations proposed by Carré et al. (2014). One may also question the values proposed by Fraps (1946)! Finally, to our knowledge, there is no clear and convincing study conducted on broilers for illustrating the relationship between their performance and the energy intake, either as ME or NE. Again, the preliminary results of Cerrate et al. (2013) suggest that BW gain is better predicted by NE intake than by ME intake; they deserve a confirmation.

Conclusions and perspectives

This review indicates that NE is a better predictor than DE or ME of the "true" energy value of poultry or pig feeds. In pigs, the interest of formulating on a NE basis is obvious and NE systems should be implemented for getting a reliable prediction of performance of animals, especially when quite numerous and variable ingredients are available. The system proposed by Noblet et al. (1994a) and implemented in feeding tables by Sauvant et al. (2004) or in the EvaPig software (www.evapig.com) has been widely used internationally. In the case of poultry, literature is less clear

with no convincing advantage of a NE system over a ME system for predicting the performance of broilers; further investigations are necessary to evaluate the potential interest of a NE system for poultry. For achieving that, most attention should be focused on the impact of dietary fat and protein levels with potential consequences on the relative energy value of fat- or protein-rich ingredients and results in least-cost formulation. Finally, even though NE is the final objective in energy evaluation of feeds, most attention should be paid to the accurate estimation of DE or ME values, which are the most important factors of variation of the energy value of poultry or pig feeds. The lack of comprehensive information on the effects of technology (e.g., pelleting, extrusion, enzymes addition) or about the differences in digestion between poultry species or physiological stages (growing vs. adult, etc.) is a major limiting factor for getting accurate estimates of energy values for pigs or poultry, irrespective of the energy system used. Improvements in energy evaluation will also come from proposals for rapid and non-invasive prediction methods such as in vitro or NIR methods (Bastianelli et al., 2015). Finally, even it has not been considered in detail in this review, the change from DE or ME systems to a NE system is usually associated with a shift in diet composition with lower crude protein contents and slightly higher fat levels and therefore different profiles of ingredients used in the diets.

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EVALUATING THE ENERGY CONTENT OF INGREDIENTS IN POULTRY DIETS – AN UPDATE

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Summary

The methodology currently in use to evaluate the energy content of ingredients, under practical situations, needs improvements to increase the accuracy of the estimation, reduce safety margins, and improve feed cost. Different methods can be used to estimate the energy values of the ingredients and diets. The method most widely used worldwide to estimate the energy content of the ingredients is the use of tabulated values but most companies involved in poultry production are moving towards the use of predictive regression equations based on chemical analyses and in vitro and in vivo data. More research to improve the accuracy of systems in use for the estimation of the energy content of poultry feeds is needed. Also, we need a better knowledge of the many factors that affect energy values. The final objective is to implement an easy method to predict more accurately the energy content of the feeds using on line NIR technology.

Introduction

Feed energy is one of the major cost in the production of eggs and poultry meat. Accurate evaluation of the energy content of the ingredients is fundamental to reduce feed cost. Apparent metabolizable energy corrected for nitrogen retention (AMEn) is widely used for feed evaluation in most countries (HILL AND ANDERSON, 1958; VOHRA, 1972; CHOCT, 2012) but the system might not work properly under all circumstances (VAN DER KLIS and FLEDDERUS, 2007). As a consequence, many scientists advocate for the use of net energy systems to better estimate the energy content of the ingredients (DE GROOTE, 1974; NOBLET et al., 2010; CHOCT, 2012). In practice, the 3 main approaches used to evaluate the energy content of ingredients in poultry diets are based on values obtained from a) tables, b) predictive equations (in vitro studies, wet chemistry, or NIR technology), and c) in vivo experiments (research farms). Each of them has advantages and disadvantages and at present time, it is not easy to make a fair recommendation on which one is best. In practice, many feed mills use table values to estimate the energy value of the ingredients. However, most of the nutritionists and feed mill managers from key European companies and broiler integrators are moving to evaluate ingredients energy by using predictive regression equations. Finally, the in vivo trials are time consuming and expensive, and not always the data are consistent and accurate overtime, especially in the case of soybean meal, wheat, and lipid sources (BOURDILLON et al., 1990; YEGANI and KORVER, 2012; FRIKHA et al., 2012; RAVINDRAN et al., 2014). Consequently, the use of in vivo trials is not common in commercial operations. In any case, none of these systems is free of problems. Many factors, including feed technology (i.e., particle size, heat processing, and feed form) (MCKINNEY and TEETER, 2004; SKINNER-NOBLE et al., 2005), diet composition including fat inclusion (MATEOS and SELL, 1980; MATEOS et al., 1982), type of cereal (YEGANI and KORVER, 2012), use of whole wheat (RAVINDRAN et al., 2006), and additives (ANNISON, 1991; MATEOS et al., 2002) modify in different ways, the energy content of the ingredients in practical diets. In the current presentation we will focus on practical problems encountered by the industry when using Institutional tables, predictive regression equations, and in vivo values for key ingredients, namely cereals, soybean meal, and fats.

Table values

In spite of the wide criticism, the use of table values is the main used worldwide to evaluate the energy content of ingredients and feeds. Table values provided by recognized, institutional sources, are of value. In fact, the approach might be the most adequate for ingredients with limited information published and not many own lab analyses available. However, the wide range in energy values proposed by the different Institutions for a given ingredient is of concern. Examples for SBM, corn, rapeseed meal, and DDGS are shown in Tables 1 to 4, respectively. In some cases, the variability in energy values can be justified by differences in the CP and antinutritional factors (ANF) content (i.e., rapeseed meal tables), the moisture of the grain (i.e., corn), or the nature of the heating process used (e.g., soybean meal). However, the wide range of values (e.g., 230 kcal AME/kg for SBM and 850 kcal AMEn/kg for corn DDGS) detected in some other cases deserves a thorough revision.

Table 1. AMEn content of soybean meal

Institution	Country	Year	CP (%)	AMEn (kcal/kg)
NRC	USA	1994	47.2	2,380
INRA	France[2]	2002	47.2	2,340
NARO	Japan	2009	47.1	2,450
FEDNA	Spain	2010	47.5	2,380
CVB	Neth.[1]	2011	47.5	2,220
ROSTAGNO	Brazil[2]	2011	47.0	2,320
MPA[3]	Russia	2014	47.0	2,300

[1]Estimated from offered values
[2]Average of broilers and laying hens
[3]Russian feed tables

Table 2. AMEn content of corn for poultry

Institution	Country	Year	Moisture (%)	CP (%)	EE (%)	AMEn (kcal/kg)
NRC	USA	1994	11.0	8.5	3.8	3,350
INRA	France[2]	2002	13.6	8.1	3.7	3,200
FEDNA	Spain	2010	13.8	7.7	3.6	3,280
Rostagno	Brazil	2011	12.5	7.9	3.7	3,380
CVB	Netherlands	2011	12.8	8.2	3.8	3,294

[1]HCl hydrolysis

Table 3. AMEn content of rapeseed meal expeller in poultry

Institution	Country	Year	CP (%)	AMEn (kcal/kg)
NRC	USA	1994	38.0	2,000
INRA	France[2]	2002	33.7	1,440[2]
Premier	UK	2008	33.9	1,670
NARO	Japan	2009	37.3	1,740
FEDNA	Spain	2010	33.8	1,700
CVB	Neth.	2011	33.5	1,580[2]
MPA[3]	Russia	2014	35.5	1,800

[1]2.5 to 5.0% ether extract
[2]Average
[3]Russian feed tables

Table 4. AMEn content of corn DDGS in poultry

Institution	Country	Year	CP (%)	EE (%)	AMEn (kcal/kg)
WPSA	Europe	1986	25.0	6.5	2,380
NRC	USA	1994	28.5	9.0	2,930
INRA	France[2]	2002	24.6	5.1	2,190
NARO	Japan	2009	30.8	11.0	2,170
FEDNA	Spain	2010	26.6	10.1	2,350
CVB	Neth.	2011	27.0	10.0	2,070

[1]HCl hydrolysis

Use of predictive regression equations

Regression equations are becoming popular for the evaluation of the energy content of feedstuffs and used consistently by major companies involved in animal feeding. The implementation of predictive equations reduces the workload for diet formulation under all circumstances, but it is of primary interest in these companies or integrators with different feed units and high number of recipes. Regression equations are easy to implement, allowing for the updating of feed ma-

trixes of the ingredients in use and facilitating the use of lab data into the feed formulation process. However, under practical conditions and certain circumstances, some problems might arise.

1) The regression equations were obtained using samples belonging to a different population or it is of "unknown" origin.

2) The summation of lab analyses of the major dietary components of the ingredient (moisture + ash + CP + EE + NDF + sugar + starch + soluble fiber) do not add to 100%.

3) The equations used include chemical values but potential differences because of effects of processing on nutrient digestibility (i.e., heat processing of SBM or DDGS) or method of analyses applied (i.e., polarimetry rather than enzymatic process for starch and HCl hydrolysis for fat) have not been taken into consideration.

4) Predictive equations in use had a small r and a high RSD values. This situation is frequent when the batches of the ingredient used have a narrow range of values within samples (i.e., trypsin inhibitor content of commercial SBM samples).

The use of predictive equations obtained with a set of samples of a different population is quite common, and will result in inaccurate estimation of the energy value. For example, in many instances the same equation is used for grain by-products that have been obtained by different processes (i.e., temperature and heating conditions). A situation that deserves special attention is the estimation of the energy value of SBM to be received in coming months from the values of samples collected in the previous month. The information needed is not the value of the SBM of the last vessel but to know in advance, the characteristics of the meal at the arrival to the port of the new vessel.

A problem often found, especially in small, local feed mills, relates to the use of chemical lab analyses in which the sum of all proximal analyses vary widely from 100% to predict the energy value of a novel or local ingredient. Under circumstances of poor lab analyses (below or higher, depending on potential errors), the misuse of the ingredient in diets is warranted, with important economic and/or production losses.

Evaluation of the energy content of cereals

Cereals are the main source of energy in commercial poultry diets worldwide. Consequently, the accurate determination of its energy content is of paramount interest. However, the discrepancies among authors, when estimating the energy content of cereals, such as corn and wheat, by using tabulated values, predictive regression equations, or in vivo data (FARRELL, 1999; YESANI and KORVER, 2012) are wide. For example, moisture content is probably the main constituent affecting the AMEn content of corn (See NRC, 1994 value in Table 2). However, not in all labs, the moisture content of the cereals is analyzed in a correct form. Moreover, not always moisture content is taken into consideration when estimating the energy content of the corn under practical conditions. For wheat, energy data is even more variable, with samples analyzed in the same lab varying often between 50 and 70% (ABDOLLAHI et al., 2011; YEGANI and KORVER, 2012). Factors such as variety (hard vs. soft), length of storage (new crop vs. old crop), ANF content (xylanase level and type), diet composition (level and type of fat), and age of the birds, might impact the energy content of the cereal. Consequently, the practical interest of determining the energy content of the cereals in vivo, is not always justified.

Evaluation of the energy content of soybean meals

A good example of the advantages and disadvantages of the use of predictive regression equations to evaluate the AMEn content of protein sources is that of the European table of energy values for poultry feedstuffs (WPSA, 1986) and its recommendation for SBM. This equation (AMEn (kcal/kg) = 37.5 x CP + 46.4 x EE + 14.9 x NFE) is widely used and recognized as a good tool to evaluate the energy content of the meals in poultry. However, this equation, created 25 years ago, might not be as precise as needed and thus, the interest of its use might be limited in commercial current situation. For example, the same equation is recommended for all SBM, independent of the origin of the beans. However, several reports (MATEOS et al., 2011; FRIKHA et al., 2012; RAVINDRAN et al., 2014) clearly indicate that the chemical composition, and therefore the energy content, varies with the country of origin of the meal, an effect probably related with day length (latitude), soil characteristics, and growing and storage conditions of the beans. In this respect, MATEOS et al. (2011) and FRIKHA et al. (2012) have reported that for meals with similar CP content, those of USA origin had more sucrose and oligosaccharides and less NDF than those from Brazil origin. Moreover, process conditions applied for the crushing of the beans will also affect the AMEn content of the meal. Under-heating of the beans will reduce the energy content of the corresponding meal, because of the presence of high amounts of TI in the meal,

whereas over-heating will reduce this level of TI but at the expense of a higher incidence of Maillard reactions. However, the European equation equalizes the energy content of SBM from different crushing plants, independent of the heating conditions applied during the processing of the beans and the final quality of the protein fraction.

A second problem related to the use of the WPSA (1986) equation for SBM, relates to the ether extract part of the equation. Energetically, EE is an important constituent of the bean and has to be taken into account when evaluating the energy content of a meal. However, the equation does not take into consideration several points of interest. For example, the real fat content of the EE fraction varies depending on the amount of lipids left in the meal. Also, different lipid fractions (e.g., acid soapstocks, lecithins, gums) might be used to increase the fat content of the SBM. Finally, the use or not of previous HCl hydrolysis by the lab, will give different EE content of the meal (up to 1% higher). A last concern with the use of the WPSA (1986) equation is the use of nitrogen free extract (NFE) as a part of the equation. This fraction does not mean much from a nutritional point of view. It obtained by difference between 100 and the proximal analyses contents. Therefore, it includes 2 sources of variation: a) no distinction among components, giving the same energy value to lignin, pectins, or other more digestible components and b) all mistakes that might occur during the calculation process, including errors in lab analyses will have a positive (or negative) effect on energy evaluation. Thus, NFE should not be included in predictive regression equations to estimate the energy value of any ingredient. Intuitively, a good equation to determine the AMEn of a given batch of SBM should include the amount of digestible protein (not good lab methods available yet) and the amount of sucrose of the sample. Also of interest, could be the inclusion in this equation of real fat content (quite similar for all solvent meals marketed in the EU-28 but of interest for expeller meals or solvent meals that add other lipid fractions) and the content in oligosaccharides (stachyose and raffinose). Oligosaccharides (around 7% of the meal on DM bases) are not digested by the bird but will be fermented at some extent on the cecum, being a good source of energy, especially in laying hens.

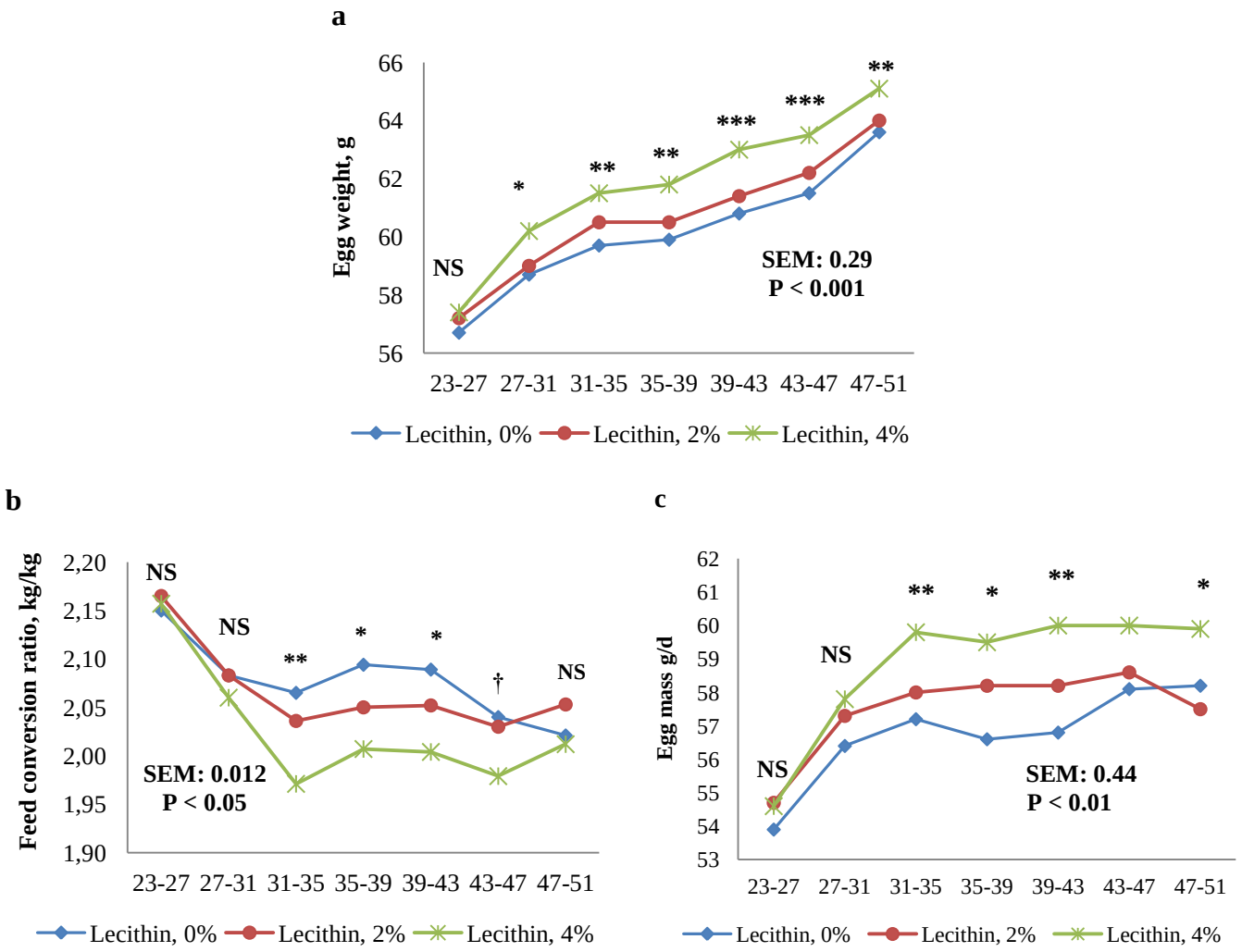
Evaluation of the energy content of lipid sources

The use of lipid sources have increased steadily in poultry diets in the last decade. The main factors affecting the energy content of oils and fats are the chemical quality (including among other variables, the gross energy, moisture, impurity, and unsaponificable content, and the peroxide value) and the characteristics of the molecule (namely, proportion of free fatty acids (FA), degree of unsaturation, and length of the carbon chain). Most of the data available on the energy content of fats and oils were obtained more than 30 years ago and consequently, the practical application of these values to the new standards of feed formulation and sources of fats (i.e., lecithins and blended fats) might not apply in some occasions.

Recent research conducted in our lab (MANDALAWI et al., 2015, unpublished data) has shown that the inclusion of lecithins in laying hen diets improved the AMEn content of the feed more than expected. In fact, the substitution of animal fat by soy lecithins, increased egg weight and improved feed efficiency (Figure 1). Probably, the lecithin, a lipid source rich in phospholipids and unsaturated FA, improved bile production and micelle formation, facilitating the incorporation of the FA into the yolk.

Fats are the most difficult ingredient to evaluate in poultry diets, the main reason being problems in the vivo determination of their energy content. Fats are incorporated into the experimental diets at low levels (usually less than 6-8%) and consequently, the experimental errors magnifies differences in energy values among lipid sources. Moreover, in many cases the control diet is based on corn that contains appreciable amounts of intracellular oil which might have different digestibility (usually lower) to that of the fat source tested (IRANDOUST et al., 2012). Consequently, the experimental error is large in fat digestibility studies, which in turn might explain, at least in part, the wide difference in values reported by different authors for a given source of fat and AMEn values of a fat beyond its GE content. In addition, it has been shown that the inclusion of fat in the diet might improve the digestibility and utilization of other components of the diet (MATEOS et al., 1980), which adds new confusion to energy determination. Consequently, new approaches are needed to better estimate the energy content of fat sources in poultry diets.

Figure 1. Effect of inclusion of soy lecithin in the diet on egg weight (a), feed conversion ratio (b), and egg mass (c) from 23 to 51 wk of age.



Interactions among dietary components

One of the main assumptions of the AMEn system is the additivity of the energy values of the ingredients within a given formula. However, this assumption might not be correct. For example, the inclusion of fat (level and type) and fiber, the presence of contaminants and ANF, and the use of additives, including enzymes (phytases, carbohydrases, and proteases), organic acids, and others might modify the energy value of ingredients and diets

Fat supplementation reduces the rate of passage of the digesta through the GIT, which in turn might favor the utilization of the components of the diets, including the lipid, carbohydrate, and protein fractions (MATEOS et al., 1980). For example, the inclusion of unsaturated fats might improve micelle formation and the utilization of the saturated fat present in the constituents of the diet, contributing to the "so called" extra caloric effects of the fat.

Dietary fiber has been considered as an ANF factor in diet for poultry, because of its negative effects on palatability and nutrient digestibility. However, recent research (HETLAND et al., 2003, 2005; GONZÁLEZ-ALVARADO et al., 2007; JIMÉNEZ-MORENO et al., 2009; MATEOS et al., 2012) has shown that this might not be the case, and that under certain circumstances, the inclusion of small amounts (2-3%) of insoluble fiber sources in diets low in fiber, might improve nutrient digestibility and growth in broilers and young pullets.

The presence of ANF, contaminants (i.e., mycotoxins), or toxic components (i.e., heavy metals) in an ingredient affects nutrient digestibility and therefore, its energy content. The presence of ANF in the diet affects not only the energy of the ingredient "per se" but also that of the remaining components of the diet, because of its negative effects on the digestive mucosa and GIT function.

Additives are widely used in poultry feeding, especially in diets for young chicks (MATEOS et al., 2002). In many instances, the companies responsible for the research on the potential benefits of these products create matrixes for the additive that include "energy equivalent values". These matrixes facilitate the work of the nutritionist, but are not free of problems. When several additives, each of them with its own matrix are incorporated into the diet, the "matrix approach" is probably not correct, because the potential benefits of the additives (i.e., exogenous enzymes, probiotics, prebiotics, essential oils, organic acids, etc) on energy are not additive.

Heat processing, mean particle size, and feed form

Grinding and heat processing of the cereals results generally in an improvement in the energy value of ingredients in young chicks but the effects tended to disappear with age (GRACIA et al, 2003; FRIKHA et al, 2013). Pelleting affects the AMEn of the diet, probably by modifying the structure of the fiber fraction (i.e., increasing the solubilization), releasing the lipids inside the cells (i.e., toasted soybeans and corn), and improving carbohydrate digestibility (i.e., pea starch) (ABDOLLAHI et al., 2012; SERRANO et al., 2013). Moreover, feed form of the diet and particle size of the ingredients, affect GIT development and function, mainly that of the gizzard. Also, diet characteristics modifies the rate of passage of the digesta through the GIT, which in turn might alter microbiota profile and the energy content of the feed (AMERAH et al., 2007; SVIHUS, 2011; MATEOS et al., 2012). However, the effects of feed form and particle size are not uniform. In fact, the benefits of fine grinding are less evident (and even negative) with the use of mash diets (ABDOLLAHI et al, 2011; SERRANO et al, 2013). Also, pelleting improves feed intake and broiler growth, but not always nutrient digestibility or AMEn content of the diet (ZELENKA, 2003). Recent research has shown that often the beneficial effects of pelleting reflects a lower feed wastage and feed intake and not necessarily an increase in the AMEn content of the ingredients (ABDOLLAHI et al, 2001; SERRANO et al, 2013). As a result, the AMEn of the diet might not be uniform and will depend not only on the ingredient composition and the physical and chemical characteristics of the diet but also on the health status of the bird. All these components are difficult to evaluate and add uncertainties to the real energy contribution of ingredients in poultry diets.

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IMPACT OF FEED MYCOTOXINS ON PERFORMANCE AND HEALTH OF POULTRY

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Introduction

Molds belong to the natural microflora which commonly infests feedstuffs. Under certain gross-environmental conditions, such as temperature, moisture content, air humidity and water availability, oxygen availability and micro-environmental conditions such as microbes competing for ecological niches the molds are stimulated to form secondary metabolites. If these metabolites exert toxic effects against the living environment, they are collectively termed as mycotoxins. Due to the ubiquitous occurrence of molds it is not surprising that mycotoxins belong to natural contaminants often detected in feedstuffs.

Owing to different chemical structures of mycotoxins, their toxicodynamics also varies considerably (Table 1). Mycotoxins might exert toxic effects in farm animals when feedstuffs are contaminated at levels that exceed the tolerance level by the animals. This tolerance is species specific, but is not unique among the mycotoxins. In addition, the clinical manifestation of a particular mycotoxin intoxication might also differ between species (e.g., fumonisins). By comparing the species-specific sensitivity together with the relevance for practical feeding conditions it becomes obvious that the pig is on special risk in spite of different chemical natures and toxicodynamics of different mycotoxins while poultry appears to respond less sensitive (Table 1).

Table 1. Overview on selected mycotoxins, their mechanisms and toxic effects and t heir relevance for practical feeding (literature compilation)

Mycotoxins	Mechanisms and toxic effects	Predisposed feed-stuffs	Affected livestock and relative sensi-tivity[1]	Rele-vance [2]
Zearalenone (**ZEN**)	Estrogen-like, endocrine disruptor / repro-ductive disorders	Maize, wheat	Pig > cattle > poul-try	++
T-2 Toxin	Inhibition of protein synthesis, immune-modulating / anorectic, cytotoxic, dermato-toxic, hepatotoxic	Oats, barley, maize, wheat	Pig > cattle ~ poul-try	+
Deoxynivalenol (**DON**)	Inhibition of protein synthesis, ribotoxic / immune-modulating, anorectic	Maize, wheat	Pig > cattle ~ poul-try	++
Fumonisins (**FUM**)	Disruption of sphingolipid metabolism / carcinogenic, neural tube defects, hepato-toxic, pneumotoxic (Porcine pulmonary edema), neurotoxic (Equine leukoen-cephalomalacia)	Maize	Horse ~ pig > cattle ~ poultry	+
Aflatoxin B1 (**AFB1**)	DNA- and protein adduct formation / car-cinogenic cytotoxic, hepatotoxic	Pea nuts, cotton seed, maize, sun-flower seed, soy beans	Cattle, pig, poultry	++
Ergot alkaloids (**EA**)	Vessel-active, pharmacological effects, performance depressive	Rye, triticale, wheat	Poultry, Cattle, Pig	+

[1]Farm animals which are not considered are less sensitive or there is insufficient information on toxic effects

[2] Relevance for practical feeding (occurrence of the toxins in relation to sensitivity of livestock):"**++** "highly important, "**+"** important," **-"** less important, "?" relevance unclear.

Current risk management

The practical relevance of a particular mycotoxin for a specific species results from its sensitivity to this toxin and from the occurrence of the toxin on feedstuffs commonly used for feeding of that species (Table 1). For risk management is it consequently necessary to consider both aspects, i.e., the sensitivity of the animal species and the most pre-disposed feedstuffs (Table 1).

Table 2. European Commission recommendation of 17 August 2006 on the presence of mycotoxins in products intended for animal feeding

Mycotoxin	Products intended for animal feed	Guidance value in mg/kg (ppm) relative to a feedstuff with a moisture content of 12 %
Deoxynivalenol	**Feed materials (*)**	
	- Cereals and cereal products (**) with the exception of maize by-products	8
	- Maize by-products	12
	Complementary and complete feedstuffs with the exception of:	5
	- Complementary and complete feedstuffs for pigs	0.9
	- Complementary and complete feedstuffs for calves (< 4 months), lambs and kids	2
Zearalenone	**Feed materials (*)**	
	- Cereals and cereal products (**) with the exception of maize by-products	2
	- Maize by-products	3
	Complementary and complete feedstuffs	
	- Complementary and complete feedstuffs for piglets and gilts (young sows)	0.1
	- Complementary and complete feedstuffs for sows and fattening pigs	0.25
	- Complementary and complete feedstuffs for calves, dairy cattle, sheep (including lamb) and goats (including kids)	0.5
Ochratoxin A	**Feed materials (*)**	
	- Cereals and cereal products (**)	0.25
	Complementary and complete feedstuffs	
	- Complementary and complete feedstuffs for pigs	0.05
	- Complementary and complete feedstuffs for poultry	0.1
Fumonisin B1 + B2	**Feed materials (*)**	
	- Maize and maize products (***)	60
	Complementary and complete feedstuffs for:	
	- Pigs, horses (*Equidae*), rabbits and pet animals	5
	- Fish	10
	- Poultry, calves (< 4 months), lambs and kids	20
	- Adult ruminants (> 4 months) and mink	50

(*) Particular attention has to be paid to cereals and cereals products fed directly to the animals such that their use in a daily ration should not lead to the animal being exposed to a higher level of these mycotoxins than the corresponding levels of exposure where only the complete feedstuffs are used in a daily ration.

(**) The term 'Cereals and cereal products' includes not only the feed materials listed under heading 1 'Cereal grains, their products and by-products' of the non-exclusive list of main feed materials referred to in part B of the Annex to Council Directive 96/25/EC of 29 April 1996 on the circulation and use of feed materials (OJ L 125, 23.5.1996, p. 35) but also other feed materials derived from cereals in particular cereal forages and roughages.

(***) The term 'Maize and maize products' includes not only the feed materials derived from maize listed under heading 1 'Cereal grains, their products and by-products' of the non-exclusive list of main feed materials referred to in the Annex, part B of Directive 96/25/EC but also other feed materials derived from maize in particular maize forages and roughages.

The European Commission responded to the risk characterization and evaluation of the presence of DON, ZON, OTA and FUM in feedstuffs by the European Food Safety Authority (EFSA 2004b, c, a, 2005b) with the release of a recommendation for critical orientation (guidance) values for these toxins in feedstuffs (see Table 2) and for monitoring of the simultaneous occurrence of these mycotoxins (CEC 2006).

A distinction must be made between guidance values for individual feedstuffs (feed materials) and for the total diet (complete feedstuffs) or parts of it (complementary feeding stuffs) (Table 2). From a toxicological viewpoint the guidance values for complete feedstuffs needs to be considered as total dietary toxin concentrations, together with the level of feed intake, determine the toxin exposure of the animal.

While the guidance values for critical feedstuff concentrations of DON, ZEN, OTA and FUM were derived based on animal health aspects, the upper limits for AFB1 consider mainly the high carry over into milk, but also animal health effects in young animals including poultry (Table 3).

Up to now, the risk management for ergot remains an unsolved issue. Toxicologically, ergot alkaloids (EA) potentially interact with serotoninergic, dopaminergic and adrenergic receptors depending on their specific chemical structure (EFSA 2005a). Moreover, the combined overall toxic effects of differentially acting individual alkaloids further depend on their absolute concentrations and proportions to each other (EFSA 2005a). In addition, the EA content of sclerotia from C. purpurea varies largely between nearly zero to approximately 10400 mg/kg (=1.04%) as reviewed recently depending on geographic region and harvesting year, cereal species, variety and genotype (EFSA 2012, MIEDANER et al. 2010, MAINKA et al. 2007). Especially this tremendous variation generally questions the reliability of the current regulation regarding the upper limit of 1000 mg ergot (C. purpurea) per kg unground cereal grains (=0.1%, see Table 3) as specified by Directive 2002/32/EC for animal health protection. Dietary levels of 0.3% ergot caused increased mortality in broilers while in another experiment ergot levels of 0.7% were without adverse effects supporting the view that variation in EA content of ergot clearly determines its toxic effects (EFSA 2005a). Consequently, EFSA recommended replacing the physical method by chemical analysis.

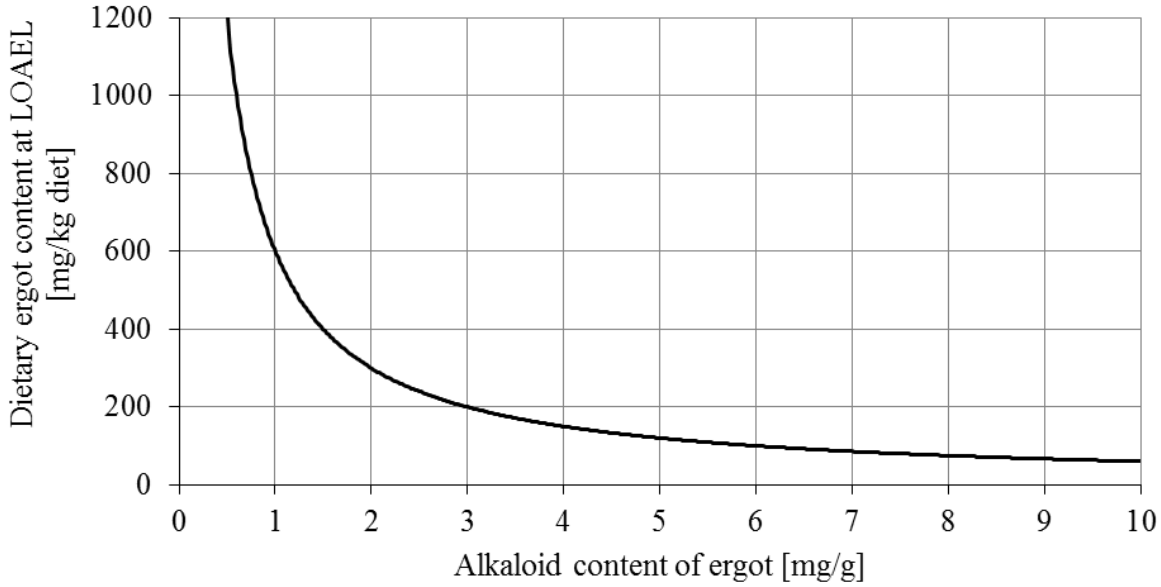

Figure 1. Estimation of the ergot proportion in diets for ducks where the LOAEL of 0.6 mg TA/kg diet is reached in dependence on varying TA contents of ergot.

Especially ducks appear to respond quite sensitive to the presence of EA in feed as the lowest dietary concentrations causing a significant decrease in feed intake and live weight gain (LOAEL) amounted to 0.6 mg/kg (DÄNICKE 2015). This LOAEL further suggests that ducks are not protected by the current EU legislation (see Figure 1).

Table 3. Upper limits for Aflatoxin B1 and for rye ergot (Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed)

Undesirable substance	Products intended for animal feed	Maximum content in mg/kg (ppm) relative to a feedstuff with a moisture content of 12 %
Aflatoxin B1	All feed materials,	0.02
	Complementary and complete feedstuffs	0.01
	excepting:	
	- Complete feedstuffs for dairy cows and calves, dairy sheep and lambs, dairy goats and kids, piglets and young poultry	0.005
	- Complete feedstuffs for cattle (excepting dairy cows and calves), sheep (excepting dairy sheep and lambs), goats (excepting dairy goats and kids), pigs (excepting piglets), and poultry (excepting young poultry)	0.02
Rye ergot *(Claviceps purpurea)*	All feedstuffs containing unground cereals	1000

Interpretation of guidance values for critical mycotoxin concentrations in feedstuffs

The compliance with the guiding values for complete feedstuffs ensures that no adverse effects occur in farm animal feeding under normal production conditions. Deviations from "normality" might include the presence of stress factors such as latent or manifest infections of poultry by pathogens (e.g. DÄNICKE et al. 2011, DÄNICKE et al. 2013), poor keeping conditions (too high stocking density, inadequate nutrition, too high concentration of corrosive gases and others) and can result in a decrease of mycotoxin concentrations in the diet to be considered as critical and thus lower than the guidance values.

It needs to be stressed that the current mycotoxin management is based on the free forms of the toxins in feed which are detectable by routine methods. However, in evaluating the overall toxicity of the free form of mycotoxins it needs to be further considered that mycotoxins often exist in feedstuffs in a modified form (RYCHLIK et al. 2014) which is usually not detectable by routine methods. Digestive processes in general, and species-specific differences in the site of microbial digestion in particular, also influence the sensitivity to mycotoxins.

Pre-ingestively modified forms of mycotoxins (e.g., matrix associated; thermally formed; conjugated by plants) might further be exposed to pre-absorptive (i.e., ingestive) structural alterations, as mediated by gastro-intestinal microbes (e.g., DON-3-O-glucoside → free DON, NAGL et al. 2012) or endogenous digestive enzymes (e.g., fumonisins bound to starch → free fumonisins, HUMPF and VOSS 2004), which can result in a reverted toxicity of the originally free mycotoxin (i.e., DON and fumonisins, respectively) or in a further modification giving rise to an altered toxicity (e.g., DON-3-O-glucoside → DON → de-epoxy-DON, NAGL et al. 2012).

Although de-epoxy-DON seems to be of a generally low toxicity for immune cells, the interspecies differences in pre-absorptive modification need to be considered in evaluating the overall toxicity of DON. While DON is nearly completely modified to de-epoxy-DON in cattle by rumen micro-organisms prior to systemic absorption, this modification occurs also in the hindgut of pigs, but only very small amounts of non-absorbed free DON reach this intestinal segment. Therefore, in DON exposed cattle mostly de-epoxy-DON is detectable in blood while in pigs the majority is in the form of free DON (e.g., DÄNICKE and BREZINA 2013). According to recent findings, modification of DON to de-epoxy-DON appears to play a minor role in the chicken while DON seems to be metabolized to sulfate conjugates to a large extent (WAN et al. 2014). Up to now, the sites of these modifications remain obscure but might be principally managed both at the ingestive and metabolic level.

Conclusions

Compliance with the guiding values for critical mycotoxin concentrations for complete feedstuffs ensures that no adverse effects occur under normal production conditions. Deviations from these assumptions might result in adverse health effects at mycotoxin concentrations lower than the corresponding guidance values. Furthermore, compliance with the guidance values for a particular mycotoxin such as DON assumes that all other modified (non-free) forms of the same toxin as well as other mycotoxins which might be present in the contaminated feedstuff at the same time, but for which no management exists, are controlled at the same time. However, under certain contamination scenarios this assumption might not apply with the result that adverse effects on health are observed at indicator toxin concentrations (e.g. DON) lower than the guidance value for its critical (free) concentration.

These aspects and the occasional exceedance of critical concentrations of mycotoxins in feedstuffs lead to the conclusion that mycotoxins are relevant for health of poultry. Therefore, preventive measures should include both minimization strategies to decrease feedstuff contaminations according to the ALARA (as low as reasonably achievable) principle and all measures to optimize the housing conditions for poultry.

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HOW TO FEED LAYERS UNTIL 100 WEEKS? – A PRACTICAL APPROACH

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Abstract

Due to ongoing genetic improvement in layer type breeders, farmers and nutritionists are facing an increasing persistency and lifetime production period of the hens. Optimal egg shell quality will be the one and only reason to sign off a layer flock with highly extended lifetime production period. Well working calcium metabolism and health status are of highest priority to ensure saleable egg shell quality under the pre-mentioned circumstances. Basic preconditions will be optimal pullet rearing, management during transfer from rearing to layer house and well-adopted nutrition at the start of the production period. The most important nutritional tool supporting calcium metabolism and egg shell quality is high quality limestone with fine and coarse structure – well balanced when the flocks are aging. As the liver functions as a very important organ in general and for egg production as well, liver health is an additional tool to boost even the egg shell quality, which is regularly proven in practise. Additionally the liver provides the protein in the egg shell, which is mainly responsible for a certain elasticity of the egg shell. Feeding and nutrition targeting gut health proof as further tools when it comes to supporting the egg shell quality. Following the varying demand for calcium over the day is another idea to support the egg shell quality, which has mainly been developed in practise and might end up in the modern strategy of split feeding. Control of egg weight throughout extended production cycles of layer flocks is an overall nutritional approach as oversized eggs will always cause downgraded egg shell quality. Last but not least it needs to be mentioned that avoiding heat stress as well as possible will be a basic request to positively influence the egg shell quality in extended lifetime production cycles of layer flocks.

Keywords: persistency**,** long-viability layers, egg shell quality, nutrition layers, calcium supply

Introduction

Modern layer hybrids are a four-line-cross with a long breeding history and show a very high production potential in different housing systems all around the world. This proofs for white egg producing lines and for brown egg producing hens as well. Due to ongoing genetic work the egg industry and farmers realise steadily increasing persistency and longer liability under all housing conditions. The higher performance has been achieved by an increased efficiency too, which means that the daily feed intake has not been increased over the last ten years. In practice this can be shown by a steadily increasing feed conversion ratio. Based on parent stock performance data from the last four years it can already be expected that the performance on commercial level will be further increased. The main increase in laying percentage is based on longer clutch length and improved uniformity of layer flocks. The most popular aim of nowadays' layer geneticists is the 500 egg hen – in one cycle and without molting. Genomic selection will even speed up the process to achieve this aim. Under good conditions this aim has already been reached in quite a lot of flocks all around the world and sometimes farmers start kind of a competition how long they can extend the lifetime production period of layer flocks. By doing this, they realise that the egg shell quality at the end of the production period again deserves much more attention and support in comparison to a one year production cycle (72 weeks of life). Additionally the so called post-peak-dip shows up as an additional challenge at the beginning of the production period. Both topics – a secured start of production together with reaching peak performance and optimal egg shell quality up to 90 weeks (or more) of age of flocks – are highly related to optimal nutrition of nowadays' high prolific layer flocks.

Materials and methods

Importance of rearing and pre-lay phase

Optimal layer nutrition for a long liability and a high number of saleable eggs starts directly after hatch. This means to fulfil the nutritional demand for the enormous growth potential during the first half of the rearing period followed by the decreased growth potential and nutritional consequences in the second half of the rearing period. The feeding programme during the first half of the rearing period needs to focus on an optimal supply of digestible amino acids and even minerals to ensure the basic growth of the inner organs, muscles and even the skeleton in this early stage of life. The second half of the rearing phase is determined by a biologically reduced physiological development of the pullet, which offers the chance of training the pullets for an optimal feed intake capacity respectively feed intake behaviour. This aspect is of enormous importance at the following start of the egg production where all nowadays' layer breeds

tend to have a daily feed intake being way too low. During the second half of the rearing phase a reduced demand for protein and amino acids is offering the chance to include lower dense raw materials with higher content of crude fibre into the diet formulation. If suitable raw materials are available, it can be recommended to reach at least 5.5 % crude fibre in this feeding phase. At the end of the rearing period, just before the start of producing eggs, the bird moves from pullet phase to layer phase, which means that nearly all metabolic processes change to a high extend. Due to hormonal regulation the development of the medullary bones is initiated and all metabolic processes move to the egg production. Nevertheless at the start of the production layer flocks need to show further weight gain. The development of the medullary bones takes place roughly throughout ten days, needs additional calcium supply and can be called the pre-lay period. This physiologically based transition period should be used to carry out a smooth transition between two highly different types of feed – meaning developer feed and layer feed – and encourages layer nutritionists to use a pre-lay feed during this period. A pre-lay feed is mainly determined by a content of calcium of around 2 – 2.5 %; the other nutrients can/should be similar to a layer feed. The major challenge to implement the idea of a pre-lay feed is more a question of feed logistics and correct use on farm side in comparison to a basic nutritional question. It can be suggested to use pre-lay feed for approximately ten days with a maximum amount of maximal 1 kilo per pullet. The biggest mistakes happening regularly in practice on layer farms are too early use of pre-lay feed or too long time of using pre-lay feed. Both practices will always harm layer flocks and might destroy a good peak performance of layer flocks. The advantages of a professional use of pre-lay feed can be summarised as followed: smooth transition between too highly different feed types, adapting the birds to the high calcium-content of layer feed, maintaining daily feed intake during this period, offering protein and amino acids for those birds being behind the average body weight, increasing uniformity of pullet flocks, avoiding decalcification of hens already being ahead of the average flock and producing eggs. As these benefits show up regularly all around the world and under all production systems, it is highly recommended to implement the pre-lay idea into all nutritional programmes of layer flocks.

Support of liver heath

After an optimal start into the laying period the liver will be the most important organ of the bird. The topic of feeding on liver health is not only a veterinarian issue but an important nutritional topic as well. The liver provides nearly all basic nutrients for the development of the yolk and albumen and even supports the development of the egg shell. Additionally the liver provides the protein in the egg shell, which is mainly responsible for a certain elasticity of the egg shell. Feeding layer birds with a certain amount of added fat and oil respectively crude fat in the diets serves as a well-known tool to reduce the incidence of the so called fatty liver syndrome. Furthermore it should be a non questionable standard practice to have a certain amount of added choline chloride in layer diets to support the liver metabolism. Every so called fatty liver supplement will be based on choline chloride together with some important vitamins as there are: K3, E, B12, B1, folic acid. In addition sometimes Methionine and Betaine are used to relieve liver metabolism. A very important aspect in terms of liver health is a possible contamination of layer feeds with mycotoxines. You can never expect a “zero level” of mycotoxines; nevertheless it is well accepted that at a certain level of contamination the liver will be harmed by mycotoxines. It is considered that this level will be reached earlier when causing harm to the liver than veterinarians realise problems with liver health in post-mortem examinations. Recommendations about a more or less harmless contamination with mycotoxines for layer birds vary to a high extend; therefore everybody needs to make his own decision when to use a mycotoxine binder.

Limestone – as a basic tool

Limestone of optimal quality is the most important and cheapest tool to support the egg shell quality in a high prolific layer bird. As a hen’s demand for calcium in the daytime is highly varying, fine and coarse limestone with different solubility should be a standard practice. A further source of calcium for the egg shell development during the night is the complex of medullary bones - already mentioned before. They serve as a fast acting calcium store during the night when the hens normally have no feed intake. To rely on the medullary bones to a too high extend means a loss of phosphorus which needs to be refilled by nutrition. Because of this it should be a feeding strategy to optimise the calcium metabolism by an optimal limestone support from the gut and to reduce the necessity of calcium supply from the medullary bones for egg shell formation as best as possible. The average retention of calcium from limestone in layer breeds varies between 40 - 60% with regard to the amount of calcium being consumed. This means that on average 50% of the limestone being ingested will be excreted by the feces regularly. In addition fine limestone has a higher solubility compared to coarse limestone with an increased particle size. Due to this biologically based pre-condition it is highly recommended to use two sources of limestone in formulating layer diets at all times. Roughly 1/3 should be

added as fine limestone and 2/3 as coarse limestone. This basic idea has proven for decades when layer farmers have been adding oyster shells on top of compound feed in order to improve egg shell quality when the calcium metabolism of layers is aging. In alternative housing systems and non-cage systems practice has shown that the coarse particle size of limestone even has a positive impact on the birds' behaviour, which means that some flocks are more docile and less anxious. The same effect sometimes is being observed when flocks are offered grit which will be small insoluble stones and therefore might even support the gizzard activity as a biologically developed grinder.

Correct calcium intake and timing

Nutritionists nowadays can easily calculate the daily demand for an optimal egg shell development based on standard scientific knowledge. In addition it is urgently needed to know the daily feed intake of an actual specific flock in order to ensure sufficient daily intake of calcium. When targeting on 4.1 gram of calcium intake per hen per day the amount of calcium in the diet needs to be adjusted up to 4.5% with 90 gram of daily feed intake or to 3.4% of calcium with 120 gram of feed per hen per day. As there is a lack of information quite often concerning daily feed intake, a lot of layer flocks are already harmed in an early stage of the laying period due to the situation described before. If layer birds might be observed on feed intake behaviour during the day, one will realise that they consume roughly 1/3 of the daily amount in the first half of the day and 2/3 in the second half of the day. This is particularly the case in order to fulfil the higher demand for calcium during the night when most of the egg shell formation takes place. Due to this situation a top dressing of compound layer feed at the end of the day respectively with the last feeding with 1 - 2% of coarse limestone is being realised on many layer farms. A further development of this idea is the so called split feeding with a morning feed and an afternoon feed, which differ highly in the content of calcium. From a nutritional point of view this idea works very well; the challenge of implementing it into practice is rather related to feed logistics in general and the feeding equipment in the layer house.

Controlling egg size

In order to ensure an optimal egg shell quality in extended production cycles, the egg weight/ size needs to be considered. This is due to the aging calcium metabolism and the decreased percentage of the egg shell in relation to the total egg weight in larger eggs. A big data survey with Lohmann Brown in France from around 30 flocks of Lohmann Brown Classic has shown a variation of plus minus 2.5 gram of egg weight based on all flocks – with having the same genetic background. This proofs as a practical example of the importance of the overall management inclusive nutrition in guiding layer flocks in terms of an optimal egg weight. The basic idea of an optimal egg weight might be an egg weight of 60 gram immediately after the start of the production and 60 - 65 gram at the end of the production. As everybody might know that this will be a theoretical idea to some extend, management and nutrition can serve to reach such an aim. The most important nutritional factors to influence the egg weight are the content of Methionine and sulphur amino acids and crude protein in not well balanced amino acid profile. A sometimes neglected tool to influence the egg weight is added fat and oil respectively crude fat in layer diets and additionally on top of this the content of linoleic acid. The basic nutritional demand of layers for linoleic acid can easily be fulfilled with approximately 1% in practical layer diets. Nevertheless scientific data and field experience from a lot of countries have proven a positive effect on the egg weight if linoleic acid is adjusted to 2%, or even 3% in practical layer diets. Due to this basic knowledge it can be recommended to adjust an increased content of linoleic acid in diets at the beginning of the laying period and a decreased content of linoleic acid at the end of the laying period. It is a common practise to reduce the nutrient density of the feed throughout the laying period in order to control the egg size and to reduce feed costs. As layer birds adjust their feed intake mainly with regard to their energy demand, the reduction of the nutrient density and energy of the formula will be counteracted quite often by an increased feed intake and finally an increased intake of amino acids and crude protein. Therefore it can be recommended to maintain the level of energy throughout the laying period and to reduce amino acids and crude protein in order to achieve kind of a qualitative restriction.

Feeding on gut health

As already mentioned the calcium metabolism of a laying hen is aging and normally the egg size will be increased. The topic feeding on gut health to ensure nutrient and mineral absorption from the gut serves as a further tool to support the overall egg quality. Additionally this topic got a high attraction under the bottom line of a reduced use of feed supplements with antibiotic activity. The feed industry and layer nutritionists are offered a bunch of feed additives to anticipate this idea. Feed enzymes are a well proven tool to support gut health in general. Furthermore: herbs & spices and essential oils, acidifiers and organic acids, probiotics, prebiotics (MOS), medium and short chain fatty acid products, inclusion of non-digestible fibre (Lignin). A further idea of feeding on gut health will be to focus on the biologically given job

of the gizzard – as a grinding machine and start-up of the digestive process. The gizzard basically is a muscle which needs stimulation and needs to be clearly distinguishable from proventriculus. If the gizzard is filled with coarse feed, it will grind and mix feed particles before passing them on into the intestine. If the gizzard is getting too fine feed, it will not work and will not change particle size before passing them on into the intestine. In practise this will result in some kind of diarrhea, wet litter and dirty eggs. As an optimal working gizzard ensures a healthy and stabile digestion, a coarse grinded feed structure is recommended. Larger feed particles have an increased retention time in the crop and the gizzard, which stimulates the ph drop and due to this has a bacteriocide effect. An excess amount of feed particles smaller than 1mm causes a direct outflow through the gizzard without utilisation. As layer birds being fed predominately with mash feed on a worldwide view, the physical structure in terms of coarseness and homogeneity needs to be given more attention because it is the basis for a healthy digestion (dry litter, clean eggs). It even influences the birds' behaviour as the birds are searching for structure during the eating activity. Furthermore a good structure improves the palatability of mash feed and even the intake of the fines in crumbled or pelleted feed.

Recommendations for practice

In order to feed layers until 100 weeks the most important aspects - being outlined in this paper - can be summed up:

- improve overall rearing of pullets
- improve the "conditions" during the transfer period
- emphasize the pre-lay idea
- support daily feed- and nutrient intake at start of production
- emphasize the idea of "feeding on gut health"
- improve overall Ca- metabolism
- take care of and support regularly good liver health
- control egg weight in aging flocks
- never "bad" compromises in feeding of high prolific flocks

Results and discussion

Egg shell quality will be more and more the one and only reason determining the point when to "sign off" the production period of a layer flock. With regard to the topic of this paper, which is "How to feed layers until 100 weeks?" supporting egg shell quality is already - and will be - the major challenge for nutritionists, veterinarians and the management in total to maintain and increase profitability in egg production. When targeting on an extended production cycle with layer hens, optimal nutrition starts just after hatch to develop a pullet on an optimal body weight, uniformity and feed intake capacity. The transition period from pullet to layer phase together with the transfer from rearing to the layer house have an enormous impact on an optimal and healthy start of the egg production period. Good understanding and implementation of the idea of a pre-lay feed serve in this phase already as a support in extended production cycles. Optimal quality of fine and coarse limestone needs to be mentioned as the most important tool for egg shell quality in general. Feed structure together with the idea of feeding on gut health even will support layer hens when aging.

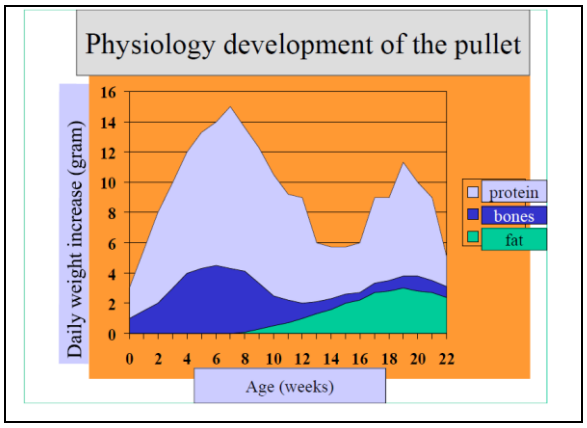
Table 1: The correct use of pre-lay feed

Age of transfer		Feeding program		
		Developer feed	followed by	Pre lay feed
week	days	kg feed	➔	kg feed
15	105	1.0	➔	1.0
16	112	0.5	➔	1.0
17	119	-	➔	1.0
18	126	-	➔	0.5
after 18	after 126	➔ immediately: start lay feed - or layer phase 1		

Table 2: Continuous supply of fine and coarse limestone

Feed type	Fine limestone 0 - 0.5 mm	Coarse limestone 1.5 - 3.5 mm
Layer phase 1	30%	70%
Layer phase 2	25%	75%
Layer phase 3	15%	85%

Figure 1: The physiology development of the pullet (source: Ysilevitz 2007 Israel)



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Available on request

INSECTS AS POULTRY FEED

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Abstract

The consumption of poultry meat and eggs is expected to increase considerably in the nearest future, which increases the demand for poultry feed ingredients to support a sustainable intensive production. Moreover, the constant improvement of the genetic potential of poultry has resulted in an increased density of the diets, which limits the inclusion of low quality feed compounds. Therefore, the feed industry needs new sources of highly digestible protein with a desirable amino acid composition to substitute other valuable limited protein sources of animal origin such as fishmeal. The phylum of insects includes the largest species variety in the world and suitable species providing high protein and high sulphur containing amino acid concentrations have been identified which can be successfully exploited as feed items for poultry. The aim of this paper is to review the state of the art on the use of insect protein in poultry nutrition and the possibilities of insect mass production for the feed industry. Although, legislative barriers for the inclusion of insect meals still exist, there is no doubt that insects have an enormous potential as feed item for poultry.

Introduction

The requirement for valuable protein sources of a continuously growing world population and the simultaneously decreasing areas suitable for agricultural production present a serious global challenge. In this context, the global demand for poultry meat and eggs is expected to increase significantly in the future, which is due to the facts that these products have a very high nutritive value, they are relatively cheap and no religious issues are connected to their consumption. Further, compared to other livestock, the production of poultry is considered to be relatively environmentally friendly having a significantly lower CO_2 footprint. An increasing intensive poultry production requires increasing amounts of protein to cover the amino acid requirements of the bird for maintenance, plumage development, growth and egg production. Currently available vegetable protein sources for poultry are extraction meals of soya beans, rape seed, legumes, and maize gluten. However, the amino acid composition of plant proteins for poultry is inferior to that of animal based proteins, specifically with respect to their content of essential sulphur containing amino acid in particular methionine. Therefore, fish meal is still used to quite an extend in poultry diets. However, due to overfishing, fish meal has become a very limited resource which is reflected by increasing marked prices over the last decades. Alternative protein sources of comparable value are therefore urgently needed in order to make poultry production a sustainable production form in the future. In this connection, the potential of insect protein in poultry diets has attracted much attention. Chickens with access to outdoor area pick up insects at all life stages and eat them with great pleasure, which indicates that they are evolutionary adapted to insects as natural part of their menu. Therefore, it is seems reasonable to consider the inclusion of insect proteins as raw material to be used in commercial feed manufacturing and to develop intensive farming systems for a new six-legged livestock. In order to ensure a cost effective insect based protein production, the ideal insect candidate should have a short reproduction cycle and should be nutritious providing high concentrations of protein and sulphur containing amino acids. In order to guarantee a constant insect supply, the ideal insect candidate should further be easy to rear in intensive rearing sites.

Nutrient composition of insects

Insects at all life stages are rich sources of animal protein. Until now, the main research efforts have focussed on the larvae of the Black soldier fly (*Hermetia illucens*), the maggot and pupae of the housefly (*Musca domestica*), the larvae of the mealworm (*Tenebrio molitor*), and insect families belonging to the order Orthoptera including locusts, grasshoppers, crickets and katylids. However, insects of the order Blattodea, like American, German, and Asian cockroach are also interesting candidates. A comprehensive review on the nutrient content of different insects and insect meals including larvae of the Black soldier fly, meals based on housefly maggot meal and pupae meal, mealworms, as well as meals based on locusts, grasshoppers and crickets and silkworm pupae meal is given by Makkar et al. (2014) and Sánchez-Muros et al. (2014).

As shown in Table 1 and 2, insects are a rich source of protein, essential amino acids and fat. The protein content of insect meals varies considerably from below 40% to over 60% even when the meals are based on the same insect

species (Table 2). The same holds true for the fat content. However, it is important to note that insect meals compared to fishmeal contain lower concentration of methionine, which has to be considered when formulating diets based on insect proteins. Further, the calcium concentration is usually lower than that of fish meal. Larvae of the Black soldier fly do however provide substantially more calcium than other insects (Table 2). The nutrient concentration of insects depends on the life stage of the insect, the rearing conditions and the composition of the growth media used for insect production. For instance, house fly larvae grown on chicken manure had a lower dry matter content, but a higher methionine content per kg dry matter as compared to larvae grown on a medium providing wheat bran, alfalfa, malt and dried yeast (Engberg et al., unpublished). Mealworm larvae prefer protein based diets rather than starch based diets and they appreciate the inclusion of yeast. The feed conversion ratio of mealworms fed 11.9% crude protein was 6.05 kg/kg and improved significantly to 3.04 kg/kg when feed with a crude protein of 32.7%, was fed. No significant change in protein concentration of the mealworm meal was observed (van Broekhoven et al., 2015). However, the fat content of mealworms fed low protein diets was significantly lower as compared to high protein diets (18.9 *vs* 26.3 %). The live insects contain an average of 30% dry matter, therefore the processing parameters of the insect meal are essential to obtain high quality raw material, which can be used at commercial feed milling operations. For today in the available literature, there are no studies describing the effect of processing on insect protein quality. However, crude protein and crude fat are the main nutrients in dried insect meal. Therefore, similarly to other meals of animal origin, oxidation processes and microbial deterioration during storage (Awoniyi et al., 2004) determine the shelf life quality, and should be taken into consideration.

Insect protein in poultry production

For a summary of poultry feeding trials involving diets containing different insect meals, the reader is referred to Makkar et al. (2014) and Sánchez–Muros et al. (2014). Most of the published trials have been carried out with broilers fed housefly larvae meal. The results showed that housefly larvae can be added at approximate dietary levels of 25% (Pretorius, 2011) without any negative effect on weight gain, feed intake and feed efficiency. This suggests that maggot meal can efficiently replace other protein sources, e.g. soy bean meal, fish meal and groundnut cake. With respect to the metabolizable energy (ME) value of maggot meal, only limited data are available, however values of 17.9 MJ/kg DM (Zuidhof et al., 2003) for turkey poults and 14.2 MJ/kg DM (Pretorius, 2011) for broilers have been reported. Due to variations in fat and "fibre"/chitin content of maggot meal, the ME values vary considerably. Both authors observed a high total tract amino acid digestibility (95% and 91%, respectively). Feeding maggot meal at the expense of fish meal at dietary concentrations of 16 % to young pullets in the period from 1- 56 days, did not negatively influence growth and feed intake of the birds (Engberg et al., unpublished).

The feeding of Black soldier fly larvae as a substitute for soymeal resulted in a similar weight gain but a lower feed intake as compared to control indicating an improved feed conversion (Makkar et al. (2014).

High concentrations of chitin present in the exoskeleton of insects influence feed intake negatively and interfere with the use of protein (Longvah et al., 2011). Although chickens have been shown to produce chitinase in the proventriculus and hepatocytes (Suzuki et al., 2002), the digestibility of chitin seems to be limited (Hossain and Blair, 2007), particularly in young birds (Ijaiya and Eko, 2009). Whether chitin may be a substrate for microbial fermentation and therefore may have a positive effect on microbial balance in the gastrointestinal tract similar to a probiotic is at the moment a matter of speculation.

Another interesting aspect of insects considered for poultry feed, is their content of antimicrobial peptides which are highly abundant in several species. In recent studies (Józefiak, unpublished), we used relatively small dietary inclusion levels (up to 0.2%) of low temperature (50^0C) dried full fat meals from *Tenebrio molitor, Hermetia illucens* and *Shelfordella lateralis* and observed an improvement of the body weight gain in broilers, when *Shelfordella lateralis* meal was fed. These results may be explained by antimicrobial effects on bacterial populations in the ileum. In contrast, even low inclusion levels of *Hermetia illucens* full-fat meal impaired broiler performance. It may be speculated that the dietary inclusion of *Shelfordella lateralis* meal rich in antimicrobial peptides (defensin) could have beneficial effects on poultry health and welfare. Although the feeding of fresh larvae to poultry includes potential risks primarily in relation to feed hygiene, in particular when organic waste products or even manure is used as medium for larvae growth, there is no doubt that chicken really enjoy the consumption of fresh larvae. In line with this, the supplementation of living maggots as a supplement on top of a balanced diet for young pullets significantly reduced the fearfulness of the birds (Engberg et al., unpublished).

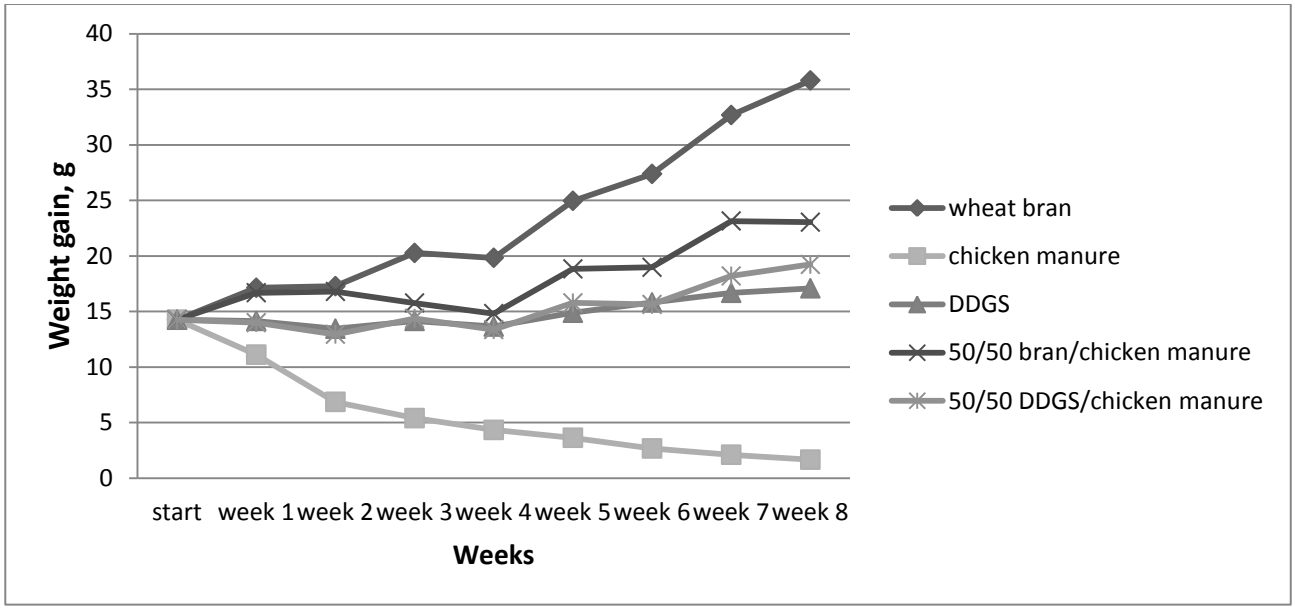
Nutritional requirements of insects

Until now, the main research efforts have focussed on the larvae of the Black soldier fly, the maggot and pupae of the housefly, the larvae of the mealworm and insect families belonging to the order Orthoptera including locusts, grasshoppers, crickets and katylids. However, insects of the order Blattodea, like American, German, and Asian cockroach are also interesting candidates. Of special interest is their robustness and ability to grow under extreme conditions (low oxygen, no lighting, high stocking density) as well as their high nutritive value (Table 1and 2). For instance Orthoptera need relatively high amounts of dietary calcium while larvae of the mealworm grow without any calcium supplementation. For today no nutritional recommendations for the different insects exist, but it is obvious that there is a huge variation in the nutrient requirements of different insect species.

The ability of house fly larvae and Black solder fly larvae to feed on organic waste material Čičkova, et al., 2015) including manure (Newton et al., 2005; Zhu et al., 2015); with high moisture content (60-80%) and convert it to valuable insect protein. This sounds immediately attractive because the production of these larvae can be used as a biological tool to solve environmental problems. Black soldier fly larvae can reduce the accumulation of poultry manure by 50% and more in the process Newton et al., 2005). Moreover, the feeding of larvae reduce the available phosphorous in the manure by 61-70% and nitrogen by 30-50% (Makkar et al., 2014). A further advantage of larvae is their ability to reduce bacterial growth in the manure which consequently results in a reduced odour development and the growth suppression of significant pathogens, e.g. Salmonella (Erickson et al., 2004). As a further co-product, the waste residue of manure can be recycled and used as fertilizer. Even though fly larvae are capable of utilizing animal waste product, e.g. manure and slaughter house offal, it may be doubted whether these feed items will be approved in the diets for a six-legged livestock and may give rise to biosecurity issues. However, fly larvae particularly the Black soldier fly can also easily grow on plant material. In contrast to fly larvae, mealworms and members of the Orthoptera are unable to utilize chicken manure even when dried (FIG.1) and prefer feeds of plant origin.

The great advantage of insect production is that no additional drinking water has to be applied. As compared to other livestock species, insects utilize water very effectively and in most cases the feed is the main source of water. As the feed supplies both nutrients and water, feed optimization is a very important part of insect production and should be addressed in future research. In our laboratory, work is going on to identify different substrates that are preferred by different insects. In a choice feeding test it was clearly shown that mealworms prefer different roughages rather than wheat middlings or DDGS. However, we are only at the beginning to understand the special requirements of different insect species under intensive production conditions and much work has to be done to optimize the diets to support their optimal performance and their nutrient composition.

Figure 1 Weight gain (g) of mealworm larvae fed on different media (88% dry matter)



Intensive insect production

Considering insects as a new six-legged livestock, the prerequisite for any commercial production is knowledge on optimal housing conditions (temperature, humidity and ventilation), and feeding both regarding feed composition and structure. Further, it is important to gain knowledge of insect diseases and biosecurity standards for this production form. In contrast to most of the livestock species (excluding fish), insects can be produced in 3D dimension systems, which allows a very effective usage of the buildings. Taking the life cycle and growth rate of insects into consideration, the most effective are larvae. It is for instance possible to produce more than 180 kg of live weight of Black soldier fly larvae in 42 days from 1m^2 whereas only 30 kg of adult crickets can be produced on the same area. Finally insects are capable to utilize some poor quality raw materials as by-products from food industry for example apple or potato pulps.

The growth rate as well as feed utilization is highly depended on temperature, which for most insects is optimal in the range of 27-30^{0}C. Insects are durable to temperature changes for instance for mealworms optimal rearing temperature is 28^{0}C but they easily survive -15^{0}C for 48h. However under high humidity conditions (>70%) they die very quickly.

Another important issue of insect production is farm biosecurity. Due to size and activity of invertebrates, a very efficient control of the buildings has to be applied. For instance, the usage of plastic nets is effective in the production of Black soldier flies but not in cockroach or cricket rearing. The latter species can easily force most plastic materials. Therefore aluminium or copper nets should be used. Finally intensive insect production should also consider invasive species, which can interfere with the natural environment. This is why insect farming should be considered as “all in all out” system with a separate hatching-brooding area.

Barriers for the inclusion of insect protein in poultry feed

There is no doubt that insect meals from a nutritional perspective are suitable for the feeding of poultry. However, a barrier for the inclusion of insects in feed for livestock is the present EU legislation (Regulation (EC) No. 1069/2009), where insect meals are defined as processed animal protein (PAP). Insects and other invertebrates are classified as Category 3 material (fit but not intended for human food chains). As such, they are suitable as feed for livestock in particular for fish, poultry and pigs. However, despite Regulation (EC) No. 1069/2009, Regulation (EC) No. 999/2001 (“BSE” regulation) prohibits the feeding of farmed animals with PAPs, with the exception of hydrolysed proteins. The feeding of insect meals to aquaculture species is going to be allowed and a re-authorization of these PAPs for pig and poultry feed is expected in the near future.

At the moment, a significant obstacle for the use of insects in animal feed is the limited quantity of produced insects in particular shortcomings in breeding, which does not guarantee a constant supply. The prices for insects and insect meals are presently very high, and cannot compete with other protein sources in this respect. To overcome this problem, the identification of the most suitable insect species giving rise to a cost efficient protein production at an industrial scale is a prerequisite. For mass production, it is necessary to develop automated process technologies for the rearing, harvest and post-harvest procedures, which certainly include the monitoring of product safety and quality (Rumpold and Schlüter, 2013).

The general acceptance of the inclusion of insects in animal feed has been frequently discussed to be a barrier. However, in a recent study from Belgium, cross sectional data were collected among farmers, agriculture sector stakeholders and citizens (Verbeke et al., 2015). The results of this study indicate a broad acceptance. The perceived benefits such as improved sustainability of livestock production, lower dependence on imported protein sources and lower environmental impact, outweighed the perceived risks, such as microbiological contamination, chemical residues in the food chain and lower consumer acceptance of animal products.

At the moment, significant knowledge gaps in the field of insect production exist, particularly in Europe, where insects are not considered as traditional food item (Vantomme et al., 2012, Van der Huis et al., 2013, Veldkamp et al., 2012). However, most signals seem to be set at green for the use of insect proteins in animal feed, so it is all about pulling on the work gloves.

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Table 1. Amino acid composition of different insect and fish meal

Amino acid	Mealworm *(Tenebrio molitor)* Larvae	Tropical house cricket *(Gryllodes silligatus)* Subimago	Field cricket *(Gryllus assimilis)* Imago	Black soldier fly *(Hermetia illucens)* Larvae	Turkish cockroach *(Shelfordella lateralis)* Subimago	Housefly *(Musca domestica)* Larvae	Fish meal
			% of crude protein				
Histidine	2.7	2.2	2.1	2.6	2.5	2.8	2.6
Arginine	4.5	5.7	5.8	4.8	5.6	4.9	5.8
Threonine	3.6	3.5	3.3	3.6	3.3	3.3	4.3
Tyrosine	5.4	4.2	4.5	6.0	5.6	5.1	3.1
Valine	5.9	5.2	5.3	5.6	5.1	4.4	4.8
Methionine	1.2	1.6	1.2	1.4	1.3	2.2	2.9
Cysteine	0.6	0.9	0.5	0.7	0.7	0.4	1.2
Isoleucine	4.0	3.7	3.4	4.0	3.1	3.2	4.0
Leucine	6.9	6.9	6.6	6.6	5.8	5.7	7.4
Phenylalanine	3.2	3.1	2.9	3.8	3.0	5.0	3.6
Lysine	4.9	5.3	5.0	5.6	4.9	6.9	7.8
Tryptophan	1.0	0.9	0.7	1.1	0.8	3.2	1.2
Total	43.9	43.2	41.3	45.8	41.7	47.1	48.7
			% relative to lysine				
Lysine	100	100	100	100	100	100	100
Histidine	55	42	42	46	51	41	33
Arginine	92	108	116	86	114	71	74
Threonine	73	66	66	64	67	48	55
Tyrosine	110	79	90	107	114	74	40
Valine	120	98	106	100	104	64	62
Methionine	24	30	24	25	27	32	37
Cysteine	12	17	10	13	14	6	15
Isoleucine	82	70	68	71	63	46	51
Leucine	141	130	132	118	118	83	95
Phenylalanine	65	58	58	68	61	72	46
Tryptophan	20	17	15	20	17	46	15

Table 2 Nutrient composition of different insect meals

	Field cricket *(Gryllus assimilis)*		Housefly *(Musca domestica)*		Black soldier fly *(Hermetia illucens)*	Mealworm *(Tenebrio molitor)*	Turkestan cockroach *(Blatta lateralis)*
	Imago	Subimago	Pupae	Larvae	Larvae	Larvae	Nymph
Item							
per kg DM							
Gross energy, MJ	21.5	19.3	20.1	20-24	22.1	26.8-27.3	-
Crude fibre, g	70	94	157	16 - 86	70	51-88	86-89
Ash, g	64	54	55-98	31-173	146-284	10-45	46-54
Phosphorus	8.0	8.6	-	9.2-24.0	6.4-15.0	4.4-14.2	0.6-0.7
Calcium	9.9	3.1	-	3.1-8.0	50.0-86.0	0.3-6.2	0.2
Crude protein, g	564	638	630-762	380-604	411-450	451-603	543-734
Crude fat, g	238	168	144-161	90-260	150-350	250-431	176-261
Amino acids per16 g N							
Glycine, g	3.0	2.7	3.9-4.3	3.7-5.1	5.1	3.9-5.6	4.6-4.8
Arginine, g	3.7	3.3	4.2-5.9	3.7-5.8	4.8-8.0	3.8-5.6	3.8-5.6
Threonine, g	2.1	1.9	3.0-3.4	2.0-4.4	1.3-4.8	3.5-4.4	2.5-3.3
Valine, g	3.4	3.0	3.4-4.6	1.3-5.1	5.6-9.1	5.5-6.6	4.4-5.1
Methionine, g	0.8	0.8	1.5-2.6	1.3-4.6	1.4-2.4	1.1-2.0	1.1-1.2
Cysteine, g	-	-	0.4	0.5 - 1.0	0.1	0.8 - 0.9	-
Leucine, g	4.2	3.6	4.9-5.4	4.5-7.8	6.6-8.4	6.7-10.6	4.7-5.8
Lysine, g	3.2	2.9	4.8-6.5	5.0-8.2	5.5-8.0	4.6-6.1	4.0-4.9
Fatty acids per kg fat							
SFAs, g	351	352	476	417	749	229-334	28.7
MUFAs, g	298	261	307	314	155	407-536	46.3-50.2
PUFAs, g	336	369	291	399	74	254-323	138-219
Total Omega 3, g	22	17	-	-	2	2-4	1-11
Total Omega 6, g	314	352	-	-	23	81-93	35-207

IMPRINTING – PRE/POST HATCH AS A NUTRITIONAL TOOL

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Summary

Most traits of economic importance in animal production undergo continuous phenotypic variations due to multi gene changes and environmental factors. Numerous quantitative trait loci that impact agronomic traits have been identified, but generally the specific set of genes responsible, are not defined. Studies using genome-wide associations have shown that genetic variation only partially explains the variability of complex traits and that, it is not only the DNA sequence that affects phenotype, but that epigenetic changes that can be transmitted from one generation to the next are also involved (JABLONGA and RAZ, 2009).

Non DNA mediated changes have been identified in mammals and birds. Nutritional impacts that appear to elicit "epigenetic" changes have been documented. Published results will be discussed on epigenetic effects associated with protein concentration changes in the maternal line changing protein usage in offspring's. Effects seen associated with neonatal nutritional changes impacting the ability of broilers to grow well when fed deficient Ca and P containing diets that decrease growth in non challenged broilers will also be discussed

Introduction

As we look ahead at technologies with the potential to deliver exponential improvements in our ability to produce animal products with lower inputs and at lower production costs as well as with less impact on the environment, application of epigenetic principles in one of them. What the correct term is and how to define it are still in debate. Early on conditioning was used but currently metabolic imprinting, programming and epigenetics are the terms most used.

But what is epigenetics? Numerous and somewhat divergent definitions for epigenetics can be found in the literature and numerous terms are applied to the same "apparent" mechanism. There is no complete agreement on what the term "epigenetics" refers to and this leads to numerous definitions (HOLLIDAY, 2006; BIRD, 2007; JABLONGA and RAZ, 2009; HO and BURGGREN, 2010; FRESARD *et al*., 2013). Epigenetics comes from the words "epi" greek for over or above and genetics. It involves modifications of the phenotype without changes in DNA sequence. These modifications can be transmitted to the next generations (HO and BURGGREN, 2010). BIRD (2007) defined epigenetics as "the structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states". FRESARD *et al*., (2013) used the definition of FEIL and FRAGA (2012) where epigenetics was defined as the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence. Epigenetics allows the same genetic information to be used differently by individuals grown in different environments.

Epigenetic changes can be mediated by DNA methylation, chromatin folding and its attachment to the nuclear matrix, packaging of DNA around nucleosomes, non coding RNA and covalent modifications of histone tails to name a few in the growing list of mediating factors. For the purpose of this review the definition of epigenetic changes will involve both phenotypic as well as changes in gene expression that persist through the life of the animal and/or subsequent generations (mainly F1 generation) and that are changes that are greater than the normal adaptations that, for example, occur during nutrient deficiency states.

There is great potential for these types of changes to be used as a tool to improve productivity in animal production while decreasing the need for costly inputs as well as decreasing environmental impacts. Extensive information on epigenetic changes in wild mammals, humans and wild birds is available. In poultry production, work has been done trying to harness this potential both for poultry management and nutrition. The potential has been demonstrated under controlled conditions but application under commercial conduits lags behind. Full understanding of when these "environmental influences" should occur to maximize positive effects later in life or in future generations has not been fully elucidated. In the area of nutrition, work is just beginning to demonstrate the effects but again full understanding of mechanisms and of how to maximize positive effects is lacking.

Background on Epigenetic Regulation

One of the fundamental assumptions of Mendelian genetics is that a specific allele behaves in the same way no matter from where (which parent) it originates. But as with many assumptions in science this rule does not always hold true. Initial observations in mice looking for the effects of paternal or maternal impacts using genomic translocations indicated that identical chromosomal regions were not equivalent and acted differently in embryo development between maternal and paternal sources (CATTANACH and KIRK, 1985).

The growing incidence of metabolic diseases in humans, such as obesity, diabetes, and cardio-vascular disease has sparked interest and research efforts into both their genetic and environmental (nutritional among others) basis. Fetal programming as it was initially referred to in humans (HALES and BARKER, 1992; BARKER, 2004), encompasses the role of developmental plasticity in response to environmental signals, including nutrition, during early life and its potential adverse consequences (risk of cardiovascular, metabolic and behavioral diseases) in later life. The first studies in this field highlighted an association between poor fetal growth and chronic adult diseases. Adverse long-term effects reflect a mismatch between early (fetal and neonatal) environmental conditions and the conditions that the individual will confront later in life.

Adverse nutritional conditions during fetal development lead to adaptive changes in metabolism that lead to a 'thrifty phenotype' in the offspring (HALES and BARKER, 1992). For example, poor nutrition in early life produces permanent changes in glucose-insulin metabolism, including a reduced capacity for insulin secretion and insulin resistance (HALES and BARKER, 2001). The initiating factor(s) in the case of nutrients for fetal programming may be nutrient(s) interacting directly with genes and their regulatory elements at the cellular level, altering patterns of growth and gene expression. Embryonic and fetal cells have a complex system to integrate nutritional signals from their environment and adapt their development accordingly to ensure survival (FRESARD *et al*., 2013).

In chickens there are reports where conditioning in early-life imparts long-term effects. The first report of this type of response was to thermal stress. High temperatures during the first week of life were reported to modulate the response to thermal stress later in life (YAHAV and MCMURTRY, 2001). By simply increasing the brooding temperature from 30°C to 37.5°C for 24 hr within the first 5 days post-hatch, broiler chickens were able to tolerate an acute exposure to a temperature of 35°C for 6 hr at 42 days of age, while "unconditioned" birds were unable to acclimate. The mechanism for this conditioned response was not elucidated then. More recently studies have shown the gene expression changes occur as a result of a heat stimulus in the neonatal period (YOSSIFOFF *et al*., 2008; KISLIOUK and MERI, 2009; KISLIOUK *et al*., 2010, 2011). These researchers reported that the expression of brain-derived neurotrophic factor, a key regulator of thermo tolerance acquisition in the hypothalamus of the chick, was different between birds grown under normal temperature and those subjected to high temperatures within the first 3 days of life. These changes were associated with increased methylation of CpG sites in the promoter of the brain-derived neurotrophic factor gene.

Although little is known about the underlying molecular mechanisms, there is evidence that feed stress may alter gene expression, in part through epigenetic changes. For example, XU *et al*. (2012) found that when 3-day old chicks were subjected to a 24-hour fasting, there were methylation changes in histone H3 in the anterior hypothalamus, the area in the brain that controls of body temperature and food intake. Feed stressors such as feeding Ca and P deficient diets in the starter (YAN *et al*., 2005) or during the first 90 hr post placement (ASHWELL and ANGEL, 2010) change the ability of broilers to utilize diets deficient in Ca and P later in life. These changes were shown to be greater than those associated with normal adaptations that occur when deficient diets are fed. These changes were partially mediated through increased methylation of the NaP cotransporter (ASHWELL and ANGEL, 2010).

There are other examples of potential epigenetic effects mediated by changes in the embryonic or neonatal period of poultry development. For example, exposure of the embryo to green monochromatic LED light appears to improve growth in broilers and turkeys (HALEVY *et al*., 2006; ZHANG *et al*., 2012). This can be partially explained by the enhancement that was seen in the differentiation and proliferation of myoblasts (HALEVY *et al*., 2006).

Effect of Early Nutrition

Most of our knowledge on the effects of nutrients on gene expression has been acquired in animal models, many examples of which can be found in the overview of the mechanisms by which nutrients interact with their molecular targets to modify gene expression (MULLER and KERSTEN, 2003). In avian species nutrients deposited into the egg by the hen are the only source of nutrients available to the embryo, and this may be the last direct chemical means by which the hen may transfer an epigenetic message to its offspring. Although the digestive capacity begins to

develop after the embryo consumes the amniotic fluid, part of the development occurs post-hatch when the neonatal chick begins consuming feed. Any delay in feed intake initiation will suppress gastro-intestinal development (UNI and FERKET, 2003) and inhibit satellite cell proliferation and muscle growth potential (HALEVY *et al*., 2003) among others changes. Whether these effects are epigenetically driven has not yet been determined.

A means of manipulating the nutrients deposited in the egg, beyond those that can be changed through maternal nutrition, is to inject nutrients into the egg at a time when the embryo is most sensitive to epigenetic programming. This timing has not been fully elucidated and may differ for different system. Injecting an isotonic in ovo feeding (IOF) solution into the embryonic amnion, the embryo can naturally consume supplemental nutrients orally before hatching and thus, nutritional manipulations can be implemented inside the egg. In ovo feeding "jump-starts" the ability of the animal to adjust to external feeding. In addition to the increased body weights typically observed at hatch, the positive effects of in ovo feeding may include increased hatchability (UNI *et al*., 2005); advanced morphometric development of the intestinal tract (TAKO *et al*., 2004) and mucin barrier; enhanced expression of genes for brush boarder enzymes (sucrase-isomaltase, leucine aminopeptidase) and their biological activities, along with enhanced expression of nutrient transporters, SGLT-1, PEPT-1, and NaK ATPase (TAKO *et al*., 2005; FOYE *et al*., 2007); increased liver glycogen status (UNI *et al*., 2005; TAKO et al., 2004) and increased breast muscle size at hatch (UNI *et al*., 2005; Foye *et al*., 2006). In ovo feeding clearly advances the digestive capacity, energy status, and development of critical tissues of the neonate by about 2 days at the time of hatch.

Early Dietary Adaptation

Adaptation to low nutrient diets has been long recognized. Animals respond to nutrient restriction in general by increasing absorption rates and utilization efficiency, which decreases excretion of the restricted nutrients. Although several reports demonstrate the ability to program chicks through early dietary manipulation to improve Ca and P utilization later on in life, there has been little work reported in the literature applying this concept with dietary protein utilization.

A study by RAO *et al*. (2009) where the effect of feeding a low protein diet (100 g/kg) versus a normal breeder diet (150 g/kg) to females of an inbred indigenous Chinese line (Langshan). Chicks from eggs laid by hens fed the low protein diet were lighter (5%) and had less pectoralis muscle weight (16 %) at hatch as compared to chicks from eggs laid by hens fed the normal breeder diet. By 28 days of age these chicks from eggs laid by low protein fed breeders had greater body weight (6%) and pectoralis mayor muscle weight (51%) then those of for birds hatched from eggs laid by hens fed the normal protein diet. These changes in growth rate were associated with changes in patterns of gene expression in the yolk sac membrane, hypothalamus and muscle in the embryo. While these authors did not look at the persistence of these gene expression changes or the mechanisms that caused them, they clearly demonstrated a long term effect of maternal nutrition challenges.

Adaptation to P and Ca restricted diets has been reported in chickens. BLAHOS *et al*. (1987) reported an increase in duodenal and ileal P absorption in broiler chickens fed a low Ca diet for two weeks and a smaller, but still significant increase in duodenal but not ileal P absorption in chicks fed a low P diet. The adaptation to P or Ca restriction has been associated with an increased level of circulating 1,25-(OH)2-D3 (BLAHOS *et al*., 1987) and duodenal calbindin content (MONTECUCCOLI *et al*., 1977). When duodenal calbindin concentrations and changes with age for 1991 and 2001 strains of broilers, BAR *et al*. (2003) concluded that modern broilers exhibit higher adaptation capacity to P or Ca deficiency and this capacity remains high for the whole growth period.

However, the authors could find no published work that evaluate the long-term effects of neonatal P or Ca restriction on growth performance, bone mineralization, and Ca and P absorption in poultry and whether "epigenetic" type of impacts had been documented. Thus, a study was done to determine whether we could elicit a positive epigenetic response to short term neonatal deficiencies in Ca and P. We defined epigenetic responses (phenotypic and in the expression of specific genes) as effects that were significantly above those that occur at the time the animal is exposed to a nutrient deficiency and that persisted through the productive life of the broiler chicken. In the first study, we evaluated the ability of the broiler chicken to adapt to a moderate early life deficiency in P and Ca and characterized this adaptation changes by examining the impact of the previous P and Ca status (starter phase, hatch to 18 days of age) on performance, bone characteristics, and nutrients absorption of broilers in the grower phase (19 to 32 days of age) (YAN *et al*, 2005).

Broilers fed a diet moderately deficient in P and Ca from hatch to 18 days of age demonstrated the ability to adapt to the deficiency, shown in the increased ileal absorption of P and Ca, the increased PP disappearance, greater growth and bone mineralization (tibia ash, tibia and shank bone mineral density and bone mineral content) in a later growth phase (18 to 32 days of age) in broilers fed the deficient diets early on as compared to those fed diets that met requirements (YAN *et al*, 2005). A follow up experiment was done to better define the degree, timing, and length of the early restriction and document gene expression changes of specific genes and further document an increased ability of conditioned broilers to handle P and Ca deficient diets in later growth phases. A main objective was to be able to achieve similar or better productivity with minimal negative impacts on bone mineralization when broilers were fed severely deficient Ca and P diets in the finisher phase.

The second experiment (ANGEL and ASHWELL, 2008) was done to determine if feeding deficient diets for a shorter time after hatch could elicit positive effects on performance and change expression of the chicken intestinal sodium phosphate co-transporter (NaPcoT). Ross 308 male chicks were fed either a prestarter control diet (C) consisting of 11.1 g/kg Ca and 5.0 g/kg non phytate P (nPP) which met or exceeded NRC (1994) recommendations or a deficient or low diet (L) containing 5.9 g/kg Ca and 2.5 g/kg nPP from hatch to 90 hr. Between 90 h and 8 days of age, all birds were fed the prestarter C diet and from 9 to 22 days of age a starter C diet (8 g/kg Ca and 4.5 g/kg nPP). From day 23 to 38 of age, broilers were either maintained on a grower C diet containing 7.0 g/kg Ca and 3.0 g/kg nPP or a grower L diet containing 4.0 g/kg Ca and 1.2 g/kg P. The three treatments, C- C-C, C-C-L, and L-C-L met or exceeded all other nutrient recommendations (NRC,1994) and were formulated to meet average USA industry use for all nutrients except Ca and nPP. Data collected for each phase included body weight and feed intake, and at the end of each phase all birds in preselected pens were sampled and toe and tibias removed to determine bone ash and ileal content for apparent Ca and P digestibility determinations as well as duodenal, jejunal and ileal mucosa for gene expression determinations. Data for performance, bone ash and digestibility are presented in Table 1 and for gene expression in Table 2.

Broilers fed the prestarter L diet from placement to 90 hr were better able to handle a P deficiency in the grower phase (22 to 38 days of age) than those fed the C diet in the first 90 hr (non conditioned birds) and then fed deficient diets (L) in the grower phase Table 1). Broilers fed the L grower diet from hatch to 90 hr were heavier, had better feed conversion, greater toe ash and P digestibility at 38 days of age, as compared to the none conditioned birds when these two groups of birds were fed the same deficient diet in the grower phase. The improved digestibility was greater than that seen simply by feeding a defiant diet in the grower phase. At 38 days of age, P digestibility was lowest in broilers fed the C diets from hatch to 38 days, a significantly greater P digestibility was seen in broilers fed the L diet from 23 to 38 days and that had been fed the C diets prior to 23 days of age, and greatest for the conditions birds (fed the L diet for the first 90 hr after placement) and the L diet from 23 t0 38 d of age. This establishes that there was a “conditioning or imprinting” in these birds meaning that modifications are occurring in these birds that are long term and that allow for improved P utilization when P deficient diets are fed in the grower/finisher phases. Despite this improved ability to handle deficiencies in Ca and P in latter growth phases, these “conditioned” birds were not able to catch up performance wise and in bone ash with those fed the C diets throughout all growth phases.

These data suggest that, in broilers, during the period immediately post hatch there is a phenomenon occurring related to Ca and P digestibilities that allows for changes to be made that can alter the bird’s long term ability to handle Ca and P deficiencies. But are these phenotypic changes associated to long term changes in gene expression. The chicken sodium phosphate co transporter (NaPcoT) gene expression was determined using real-time quantitative PCR. The differences in CT (expression) values for gene expression were determined to be different using a student’s t-test and relative differences in expression caused by the dietary treatment were determined by correcting for the efficiency of the PCR as calculated by the standard curves of dilutions of pooled cDNA across the experiment (ASHWELL and ANGEL, 2008). Expression levels and N-fold expression changes (changes relative to the control) are reported in Table 2. Feeding the prestarter L diet from hatch to 90 hr had a significant effect on the expression of the NaPcoT as seen by the average n-fold increase of 2.8 in the mRNA levels in the small intestine. Nearly identical stimulatory effects were seen across all segments of the intestine with relative expression decreasing from duodenum to ileum.

These apparent epigenetic changes appear to be mediated through changes in methylation. When methylation determinations were done on the small intestine samples obtained at 90 hr, apparent methylation was observed at 29 positions within the 52 predicted CpGs in the PCR products in samples fed the C diets while 19 CpGs were methy-

lated in the DNAs extracted from the programmed or condition birds fed the L diet from hatch to 90 h (ASHWELL and ANGEL, 2008). This 43% reduction in methylation of cytosines in this region may be involved in the increased gene expression observed in the programmed birds relative to controls. Further characterization of the differential methylation patterns is needed both post programming as well as later in life to verify these observations.

This work clearly establishes that "imprinting" or modifications are occurring in the animal that are long term and that allow for improved P utilization when P deficient diets are fed in the grower/finisher phases (Angel and Ashwell, 2007; Ashwell and Angel, 2007). These long term effects on performance and gene expression as a result of nutritional neonatal programming in the chick are strong evidence for the role of epigenetics in the regulation of these phenomena.

The challenge now is how to combine neonatal programming diets while minimizing unwanted negative effects. For example, skeletal growth rate is highest in broilers during the first 8 to 10 days post hatch but neonatal programming Ca and P deficient diet concentrations appears to be required for at least 90 hr post hatch. This feeding of low nutrient concentration diets during the neonatal period goes against the long held "dogma" for prestarter diets where nutrient density tends to be high for nutrients that are in high demand during early post-hatch development. Herein lies the challenge and also the rational for neonatal programming. Research must identify "the window of opportunity" and the extent of the deficiency needed to exert these epigenetic changes where short term early life changes in nutrients fed can elicit long term changes in gene expression while minimizing the deleterious effects of feeding a deficient diet on productivity.

Conclusions

Nutrition is constantly varying and composed of a very large amount of known and unknown bioactive compounds. Furthermore, nutrition touches the core of metabolism by supplying the vast majority of nutrients (both macro- and micronutrients) for maintaining metabolic homeostasis. This homeostasis stretches from gene expression to lipid metabolism and from signaling molecules to enzyme cofactors. Thus, nutrition by its nature *needs* to be studied in an integrated way. So far, most of the tools for this integration have been lacking, thus maintaining an unbridgeable gap between classical nutrition (studying physiology with a focus on biochemical pathways) and biomedical sciences (determination of disease-related molecular mechanisms). In applying Systems Biology to nutritional sciences, these paradoxical extremes are bridged and the complexity of the relationship between nutrition and health can be met in an integrated approach. Many hurdles need to be overcome, before this research area reaches maturity.

The data included in this review along with other preliminary data demonstrating long term effects on performance and gene expression as a result of nutritional manipulation in the chick are evidence for the role of epigenetics in the regulation of these phenomena. Further work must be conducted to elucidate the specifics of neonatal programming in the chicken and the extent of its effects on DNA methylation or other mechanisms that mediate epigenetic changes. Demonstrating these epigenetic effects in the chicken will contribute to the understanding of the impact of the environment during key development in the embryo and neonate on the regulation of the genome and start to elucidate the potential mechanisms that will allow for improving performance and economics of poultry production beyond the incremental improvements that have been seen up to now. Harnessing the potential benefits of these effects for productive purposes will be the challenge.

Nutrition research in the future will increasingly focus on the ways in which genes are affected by what we feed breeders, how these breeders are managed, how eggs are incubated and how we manage and feed the neonatal. If these effects can be better understood and implemented, then dietary "conditioning" offers opportunities for exponential improvements in animal productivity.

Table 1. Impact of early dietary deficiencies of phosphorus (P) and calcium (Ca) on performance and apparent digestibility (AD) of P (ANGEL and ASHWELL, 2008)

Treatment [1]	C-C-C	C-C-L	L-C-L	SEM	P values
Hatch to 90 hour					
Weight gain, g	41.2[2]		36.2[2]	0.775	<0.001
Feed to gain ratio	0.83		0.88	0.016	0.028
Toe ash, g/kg	133.7		111.7	0.57	<0.001
90 hour AD P, g/kg P	576.2		663.9	6.67	<0.001
91 hr to 8 days of age					
Body weight, g (8 days)	150.2		141.8	0.248	<0.001
Weight gain, g	63.5		64.5	1.222	0.557
Feed to gain ratio	1.15		1.12	0.027	0.367
9 to 22 days of age					
22 days Body weight, g	904.9		884.7	6.530	0.025
Weight gain, g	754.2		742.2	6.605	0.179
Feed to gain ratio	1.40		1.40	0.010	0.967
22 day AD P, g/kg P	514.6		529.4	16.12	0.513
23 to 38 days of age[4]					
38 days Body weight	2345.9[a]	2235.4[c]	2285.6[b]	20.158	0.001
Gain, g	1450.9[a]	1333.0[c]	1390.6[b]	15.766	0.03
Feed to gain ratio	1.82[ab]	1.89[a]	1.76[b]	0.033	0.013
38 day Toe ash, g/kg	126.2[a]	105.3[c]	118.4[b]	1.71	<0.001
38 day AD P, g/kg P	453.9[c]	515.4[b]	601.1[a]	1.562	<0.001

[1]Treatments are: Control (C) – C –C (fed in all phases) diets that met or exceeded NRC (1994) nutrient recommendations (including those for Ca and P); C-C- Low (C-C-L) fed the C diets from hatch to 22 d of age (prestarter from hatch to 8 days and starter from 9 to 22 days) and then the grower L diet from 22 to 38 d of age; L-C-L fed a prestarter L diet from hatch to 90 hr, a C diet from 90 hr to 22 d (prestarter C from 90 hr to 8 days and starter from 9 to 22 days) and the grower L diet from 22 to 38 d. The L diet met or exceeded all NRC (1994) nutrient recommendations except for those of Ca and P. From hatch to 90 hr the L diet contained 5.9 g/kg Ca and 2.5 g/kg non phytate P (nPP) and the C diet 11.1 g/kg Ca and 5.0 g/kg nPP. From 22 to 38 d of age the L diet contained 4.0 g/kg Ca and 1.1 g/kg nPP and the C diet 7.0 g/kg and 3.0 g/kg nPP.

[2] Up to 22 days of age, treatment C was replicated 16 times and treatment L eight times. Between 22 and 38 days of age all treatments were replicated 8 times.

[3] Means within row with different superscript letter differ (as specified in the P value column). Where there are only 2 means in a row the P value column shows significance.

Table 2 - Neonatal phosphorus (P) and calcium (Ca) deficiencies on sodium phosphate co-transporter (NaPcoT) gene expression (ASHWELL and ANGEL, 2008)

Dietary treatment	Tissue	Gene expression[1]		n-fold[2]
		18s rRNA	NaPcoT mRNA	change
90 hr				
Control	Duodenum	15.8±0.2a	23.2±0.2a	1.0
	Jejunum	16.1±0.3[a]	24.4±0.3[a]	1.0
	Ileum	16.2±0.3[a]	25.3±0.4[a]	1.0
Low P	Duodenum	16.0±0.3[a]	24.5±0.3[b]	3.1
	Jejunum	16.2±0.3[a]	26.1±0.4[b]	2.9
	Ileum	16.2±0.4[a]	26.9±0.2[b]	2.5
d 38				
Control	Duodenum	16.5±0.4a	24.1±0.5a	1.0
	Jejunum	16.2±0.3[a]	23.7±0.4[a]	1.0
	Ileum	15.8±0.4[a]	24.9±0.4[a]	1.0
Low P	Duodenum	16.6±0.4[a]	25.4±0.4[b]	2.5
	Jejunum	16.1±0.5[a]	25.3±0.6[b]	2.7
	Ileum	16.8±0.5[a]	26.7±0.4[b]	1.8

[1]Expression values are presented as the average ± the Std Dev (n=8).

[2]n-fold change in gene expression was calculated by the 11 11 Ct method of PFAFFL (2001) including the amplification efficiency for both genes. 18s amplified with 94% and Na/Pi IIb amplified with 91% efficiencies respectively as determined by the standard curves of diluted cDNA. Expression levels for the control diet were set as 1.0 for fold change effects within a tissue and within an age.

[ab]Means within a column and age with common superscript letter do not differ (P<0.05).

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IMPROVING BROILER PERFORMANCE THROUGH BREEDER NUTRITIONAL MANAGEMENT

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Abbreviated Title: MATERNAL NUTRITION

Summary

Recent research on the subject of maternal nutrition has focused largely on the effects of vitamins, antioxidants, and most recently the complex interactions of dietary macronutrients (energy and protein) on economically important broiler traits: primarily growth rate and breast meat yield. Vitamin E and organic Se show great promise for improving hatchability and chick health. Recommendations for ideal maternal levels of dietary protein and energy are not as clear. The concept of ideal protein has greatly advanced the discipline of livestock nutrition, and it is likely that a quest for an optimal ratio of dietary energy to ideal protein will simplify our understanding of maternal nutrition. The fit of published research on maternal dietary energy and protein levels, ratios, and intakes are tested against several hypotheses. The most consistent alignment is with regard to the relationship between low maternal protein intake and progeny fatness. Lack of congruency with other hypotheses underscores a need for further research. It is clear that a more strategic approach is needed to improve understanding of this multifaceted question. Rather than studying offspring performance as an afterthought, experimental designs must prioritize discovery of the impact of maternal nutrition on offspring performance. Combined with carefully executed control of breeder experiments to reduce variation in breeder feed allocation, and thoughtful standardization of hen age, egg handling, incubation, hatching, and brooding conditions, carefully considered experiments will add greater value to our knowledge of transgenerational effects of maternal nutrition.

Key words: Maternal nutrition, broiler breeder, offspring, progeny, growth, yield, review

Introduction

Broiler growth rate, yield and efficiency are of key economic importance for chicken meat production. Understandably, most nutrition studies are conducted to understand the direct impact of broiler diet composition on broiler growth, development, and efficiency. Far less information is available on the effects of maternal nutrition on broiler productivity, though minor improvements in broiler productivity achieved through maternal nutrition may have significant economic benefit (Calini and Sirri, 2007). A major challenge with maternal nutrition is that its effects on broiler performance tend to be confounded by a large temporal lag during which many environment factors can influence the results.

Broiler breeder nutrition can directly affect chick health and productivity through egg composition (reviewed by Wilson, 1997; Kidd, 2003). Currently, however, a new and fascinating field of influence is being discovered. There is increasing disparity between the growth potential of broilers and the degree of expression allowed in broiler breeders to maximize reproductive output. In mammals, it is well established that maternal undernutrition can have negative metabolic effects in offspring and even grand offspring. Many of these insights originated from studying the impact of malnutrition on offspring growth, development and health during the 'Dutch famine' at the end of the Second World War (Roseboom et al., 2011). These and subsequent studies with various animal models have led to discovery of epigenetic (non-DNA sequence) moderators of progeny gene expression. The list of potential epigenetic mechanisms is expanding rapidly. Though not well understood, it is probable that aspects of broiler performance may be influenced indirectly by epigenetic mechanisms. Given the severe degree of feed restriction practiced in meat type chicken parent stocks, the importance of maternal nutrition may be increasing.

The objectives of the current paper are 1) to identify maternal nutritional strategies with potential to increase broiler health and performance, 2) to serve as an update on previous reviews, particularly of maternal dietary energy and protein effects on offspring performance, and 3) to outline key considerations for future research aimed at maternal nutrition strategies for optimal broiler productivity.

Mechanisms for maternal nutrition effects

There are two main ways that maternal nutrition can affect broiler performance. The first is through differential incorporation of nutrients into the egg, which *directly* impacts progeny development. The second involves epigenetic effects that *indirectly* affect progeny growth development by changing the regulation of gene expression in the progeny. Epigenetic mechanisms that impact skeletal muscle growth and development, for example, has been reviewed by Saccone

and Puri (2010). For many years, we have known that the phenotype of an animal is the result of its genome and its environment. This has often been referred to as the G x E interaction. The environment dictates the degree of gene expression that influences among other things, the animal's size and composition. There is increasing evidence that the environment can influence gene expression later in the life of an individual, but also trans-generationally through DNA or chromatin modifications (reviewed by Jirtle and Skinner, 2007) or diffusible RNA factors (Daxinger and Whitelaw, 2012). This provides a new framework for understanding the impact of broiler breeder nutrition on broiler performance. Many studies of maternal nutrition in many species have shown an impact on progeny body weight (BW) and carcass composition. Unfortunately, there is often little in the way of an obvious pattern in these effects. However, since many epigenetic mechanisms are influenced by the availability of nutrients to the parents, ever-increasing degrees of feed restriction in broiler breeders suggests direct relevance of epigenetics to the field of broiler maternal nutrition. Though important to note the significance, a review of possible epigenetic mechanisms is beyond the scope of the current analysis.

Challenges of measuring impact of maternal nutrition on broiler performance

It is often difficult to determine patterns in the effects of maternal nutrition on progeny performance. Part of the challenge is the lengthy delay between feeding the pullet and the hen and the resulting progeny performance. There is a time lag of months during which many confounding environmental influences can complicate the interpretation of the effect of maternal nutrition on progeny performance. The following effects summarize some of the important research into maternal nutritional management that may be applied to increase broiler health, livability, growth rate, and yield.

Hatching egg composition, hatchability and chick quality

Vitamins and antioxidants

There is clear evidence that incorporation of vitamins and minerals, many of which are antioxidants, into hatching eggs is highly influenced by maternal intake. Vitamin and mineral deficiencies, or large excesses can harm or kill the embryo, reducing hatchability (reviewed by Wilson, 1997). In reviewing the subject of maternal vitamin and mineral nutrition, Hocking (2007) concluded that organic forms of minerals, potentially in slightly higher than recommended concentrations appear to be beneficial for progeny performance, and since that time further research has provided more evidence for this hypothesis. Surai (2000) reported that supplementation of broiler breeder diets with selenium (200 and 400 µg/kg) and vitamin E (40 to 200 mg/kg α-tocopherol) increased egg concentrations of these antioxidants. The liver antioxidant system in 1- and 5-day-old chicks, but not in 10-day-old chicks was enhanced, suggesting a direct effect via increased antioxidant levels in the egg. Rajashree et al. (2014) fed up to 500 µg/kg of organic Se, and observed both an increase in hatchability of over 4%, and increased antioxidant status of eggs. Urso et al. (2015) found that 120 mg/kg of vitamin E increased 29 week hatchability by 3.2 chicks per 100 eggs set, and high organic Se increased hatchling weight by approximately 0.4 to 0.7 g until 33 weeks of age. In terms of effects on progeny growth, An et al. (2012) found that high maternal vitamin E (100 mg/kg vs. 20 mg/kg) increased FCR to 1.75 vs. 1.68, respectively, but reduced drip loss from 2.60 to 1.85%. *Vitamin E levels of 120 mg/kg and organic selenium at 400 µg/kg are recommended to increase chick quality and hatchability.*

Immune function

Quereshi et al. (1998) exposed broiler breeders to aflatoxin and observed reduced hatchability and compromised antibody response in broiler chicks. Studying maternal supplementation of antioxidants on broiler innate immunity, Johnson (2013) found that maternal dietary replacement of synthetic vitamin E with a natural vitamin E increased *in vitro E. coli* bactericidal capacity of chicks from older (58 wk old) breeders, and oxidative burst in chicks from 46 and 58 wk old breeders. Supplementation with 6 and 12 ppm of canthaxanthin increased plasma antioxidant capacity, while supplementation with 50 mg/kg L-carnitine decreased oxidative burst in 4-day old chicks (Johnson, 2013). Hen and chick age had large effects on immune status of broilers. The degree of variation due to hen and chick age in progeny responses to maternal nutrition were strongly established by Johnson (2013) and Torres (2013). *Inconsistent reports of the effects of maternal nutrition on broiler performance is likely due to lack of consistency in hen age between experiments.*

Omega-3 fatty acids

Supplementation of maternal diets with long-chain omega-3 fatty acids lowered egg weight, and reduced 11- and 28-day BW, and increased both feed conversion ratio and mortality of offspring (Koppenol et al., 2015b). Further, there was little benefit of omega-3 supplementation for the immune response of the progeny (Koppenol et al., 2015a).

Managing maternal nutrient intake

Maternal feed restriction

Waaij et al. (2011) conducted a study to investigate the effects of maternal feed intake on broiler growth, and to specifically to match and mismatch the nutritional environments of hens and chicks. These authors fed 60-week-old broiler breeders at recommended or 35% higher intake, then fed the progeny ad libitum or at 70% of ad libitum. Of hens were fed restricted amounts, their male broiler progeny grew less quickly, while their ad libitum-fed female progeny were 8% fatter. This was a short-term hen feeding study, so it is possible that the nutritional status of the hen caused a shift in egg composition, leading in turn to modified performance of the broilers. However, it raises important questions because notably, they identified outcomes of maternal feed restriction that are undesirable commercially.

Among a suite of complex interactions, Eusebio-Balcazar et al. (2015) observed that maternal nutrient restriction at a rate of 89% of during weeks 20 to 26 followed by rapid growth from 26 to 29 weeks of age worsened the walking ability of corn-fed male broilers, but only when maternal feeder space increased 33% at 22 weeks of age.

Maternal dietary energy and protein

Quantitative restriction of calorie and protein intake is one of the major strategies employed to optimize broiler breeder BW for reproductive success. There is a growing body of information on the subject, of the impact of amino acid or crude protein (**CP**) and metabolizable energy (**ME**) levels on offspring performance, but the approach has not been systematic, and the results often paradoxical. To understand the effects of maternal protein and energy nutrition, the ratio of energy to amino acids, total balanced protein, and other nutrients must be taken into account – not simply dietary level. This is because the partitioning of energy to maintenance, growth, and egg production is dictated by the most limiting nutrient. Growth and reproduction follow the organism's first metabolic priority, maintenance (survival). It is critical to understand maternal maintenance energy and amino acid requirements because the residual energy and amino acids remaining after maintenance is taken care of are used for growth and reproduction. Excess or insufficient energy or essential amino acids precedes complex metabolic decision making that dictates the productivity and health of the hen.

Low ME-high CP diets have generally reduced hatchability (Leeson and Summers, 1991; Pearson and Herron, 1982). These effects on hatchability could be explained by altered composition in the egg, directly affecting the developing embryo. Typically, higher maternal CP intake resulting from higher dietary CP or lower ME levels increases egg and chick weights (Joseph et al., 2000; Enting et al. 2007). Though weakly correlated, this typically translates into larger chicks and larger broilers, but these effects will not be the focus of the current review. The following summarizes much of the recent published literature on the effects of maternal dietary energy and protein levels on progeny performance in the context of some classical works. Because of the complexity of the subject, this section is organized as a series of general hypotheses, specifically supported or contradicted by the research that has been conducted in this area. Factors such as hen age, the timing of the maternal dietary treatment (rearing or laying phases, or both), and the sex of the progeny tested have been considered. Several inconclusive reports have been omitted from this classification scheme.

Hypothesis 1[3]: Low maternal protein intake leads to lipid accumulation in offspring.

Of all the propositions, this one had the clearest support from published studies. Several studies provided evidence supporting, and none contradicted this hypothesis.

- Higher abdominal fat percentage in female broilers from hens fed low CP diets (10% compared 16% CP; Lopez and Leeson, 1995)
- Maternal dietary ME restriction during lay (20, 30, and 50%) increased abdominal fatpad, subcutaneous fat, liver fat, and breast muscle fat content (Li et al., 2014)
- Moraes et al. (2014) predicted a 4.8% larger fatpad in broilers from hens fed lower CP during their rearing period (13.8 vs. 15.5% maternal CP)
- Male broilers from hens with low maternal ME intake during the laying period (450 vs. 385 or 325 kcal/d) had more carcass fat (Spratt and Leeson, 1987)

[3] Corollaries of Hypotheses – since studies almost always tested ratios of energy to protein:

A. High maternal energy intake leads to the same effect
B. High maternal protein intake leads to the opposite effect
C. Low maternal energy intake leads to the opposite effect

Hypothesis 2: Low maternal protein intake leads to lower offspring body weight.

In support of hypothesis 2:

- Female broilers from hens with lower maternal CP intake during rearing had reduced BW (Moraes et al., 2014)
- At a low maternal pullet diet ME level (2,528 kcal/kg), a higher CP level (15.3 vs. 13.7%) increased male broiler BW to 22 day of age (Moraes, 2013)
- Male broilers from hens with higher BW (higher feed intake) had higher BW than those from standard BW hens (Emous et al., 2015)
- Broilers had higher BW when maternal diets contained low ME (ME:CP ratio of 18.0 kcal/g; Peebles et al., 2002)

Because the effect of rearing period nutrition was often more important than the laying phase, these findings point to epigenetic programming. Similarly, after the 'Dutch famine' increased neonatal adiposity was observed in children and grandchildren of women exposed prenatally to nutrient shortage (Painter et al., 2008). Heritable, transgenerational mechanisms for maternal nutrition to influence offspring growth and development are currently being explored (Jirtle and Skinner, 2007; Daxinger and Whitelaw, 2012).

Contradicting hypothesis 2:

- Ciacciariello and Tyler (2013) fed digestible maternal lysine levels ranging from 0.44 to 0.75% during the laying phase and found that progeny of young breeders (not beyond 38 weeks of age) fed the lowest lysine levels grew dramatically heavier and converted feed more efficiently
- Higher maternal pullet phase ME level (2,736 vs. 2,528 kcal/kg; 15.3% CP) decreased CP intake and increased female broiler BW to 22 day of age (Moraes, 2013)

Suggesting the possibility of a nonlinear relationship, an optimal maternal pullet phase ME:CP ratio of 18.25 kcal/g was proposed for maximizing female broiler BW (Moraes et al., 2014). For chick weight, a similar optimal maternal laying phase ME:CP ratio of 18.0 kcal/g can be inferred from Leeson and Summers (1991).

Hypothesis 3: Low maternal CP intake leads to lower breast or total carcass yield.

In support of hypothesis 3:

- Chicks from young broiler breeders (26 weeks of age) fed low digestible lysine (0.6 g/d) had lower BW, carcass weight, and breast weight, and higher drumstick and thigh weights (Mejia et al., 2013). This transient effect was not observed in progeny of 31 and 36 week-of-age broiler breeders.
- Li et al. (2013) suggested that myogenin (MYOG) gene expression was delayed in embryos from hens receiving low feed intake (75% of recommended)

Contradicting hypothesis 3:

- Emous et al. (2015) found that male broilers from pullets fed lower maternal dietary CP (ME:CP = 21.2 vs. 18.4 kcal/g) had higher breast yield than from pullets fed higher maternal dietary CP.
- Feeding higher levels of CP to broiler breeder pullets during the rearing phase reduced breast yield in male and female broilers (Moraes, 2013). Dietary combinations that increased CP intake of broiler breeders during rearing and lay had sex-specific additive effects on broilers: it decreased male broiler breast yield, but increased female breast and carcass yield.
- Moraes (2013) observed that in pullets fed 15.3% CP during rearing, a higher ME level (2,736 vs. 2,528 kcal/kg), which decreased CP intake increased overall broiler carcass yield at 40 d of age.

Zhu et al. (2012) fed Chinese yellow breeders maternal diets containing ME ranging from 2,650 to 2,850 kcal/kg, with 15.5 to 17.5% CP. Hens were fed fixed amounts of feed daily. Moderate ME:CP ratios tended to optimize live performance, whereas yield was highest with low CP intake, and lowest at moderate CP intake. This result also points to a nonlinear relationship between CP intake and carcass yield.

Other notable findings

Several observations did not fit the relatively simple suite of hypotheses, and suggest that more complex nonlinear relationships should be investigated to explain interactions between maternal ME and CP on progeny performance. Leeson and Summers (1991) reported that a laying phase ME to CP ratio of 18 kcal/g maximized chick weight. Enting et al.

(2007) fed normal and 12 or 23% lower maternal ME levels, and reported an increase in 38 d BW in the moderate ME level, which had a pullet phase ME:CP ratio of 16.0 kcal/g, increasing to 16.6 and 17.7 kcal/g during early and late lay periods. Enting et al. (2007) also reported reduced mortality with their lowest ME level. For male broiler breast and carcass yield, optimal maternal ME:CP ratios during rearing and lay were (average of 16.5 and 17.9) and 18.5 kcal/g, respectively (Moraes et al. 2014). For female broiler breast and carcass yield, they reported optimal maternal ME:CP ratios during rearing and lay of 17.2 and 19.4 kcal/g, respectively. Conversely, the worst maternal diet ME:CP for male broiler breast and carcass yield were 19.2 and 18.5 kcal/g, respectively. For female broiler breast and carcass yield, the worst ME:CP ratios during rearing and lay were 17.2 and 18.5 kcal/g, respectively. Interestingly, maternal diets that were optimal for male performance were worst for female broilers. Increasing the dietary ME:CP ratio from 17.2 to 19.4 kcal/kg appeared to be optimal for mixed sex flocks, as the yield performance of male progeny in this treatment was not significantly different from the optimal male scenario. A lower ME:CP ratio during lay should be avoided (Moraes et al., 2014). In other words, *higher (over-?) consumption of CP during the laying period appeared to have the most negative effects on broiler performance.*

Moraes et al. (2014) found that breast yield increased when maternal ME:CP ratio increased during transition from pullet to laying phase diets (17.5 or 19.0 to 19.4; 17.5 to 18.5 kcal/g; Moraes et al., 2014). This is consistent with current recommended practice, where recommended ME levels are maintained and CP levels drop in later dietary phases.

Lopez and Leeson (1995) observed lower feed intake in male broiler offspring of parents fed 10 to 16% dietary CP, but the broilers compensated such that those from hens fed 10 and 12% dietary CP had lower feed conversion rates than those fed 14 or 16%.

Management of broiler breeder feeding

Metabolic stress

Reducing metabolic stress may be key to reproductive success. Zuidhof et al. (2007) demonstrated that radical target BW strategies during the pullet phase can yield similar reproductive outcomes. A key success strategy was adherence to the principle: “Don’t let the bird’s metabolism know you have made a change to the feed allocation”. Stable metabolism in that study was accomplished by weighing birds and determining feed allocations twice per week. Liver weight and composition is an indicator of the metabolic status of birds, and it is a key organ in storage and mobilization of energy. de Beer et al. (2007) reported similar baseline liver glycogen stores, which doubled in skip-a-day fed versus every-day fed broiler breeders. A similar pattern was observed for liver weight. Metabolic stress may also affect progeny. Portha et al. (2011) reviewed the effects of maternal metabolic stress on beta-cell programming. In rats, both undernutrition and obesity decreased the development of beta cells and increased the likelihood of diabetes in offspring and grand offspring. In birds, where there is no physiological contact with the mother during embryo development, epigenetic mechanisms are more likely to be involved in transgenerational inheritance than parental physiological mechanisms. The escalating severity of feed restriction in broiler breeders presents a great model to study maternal metabolic stress on the offspring. However, there are large gaps in the study of transgenerational effects of nutrition in meat-type chickens.

Precision feeding

Given the challenges around metabolic stress in broiler breeders, strategies for achieving more stable metabolism will likely improve breeder performance, and will likely influence progeny performance. A precision feeding system for broiler breeders has been developed at the University of Alberta (Zuidhof, 2014). The system has been used to raise broiler breeder pullets to sexual maturity with a BW CV of less than 3%. The sequential feeding system is a paradigm shift for feeding breeders, as birds can eat multiple small meals per day rather than binging and fasting, which has become the norm for broiler breeder management. Such a system shows promise for accelerating research on maternal nutrition. It is now possible to control previously confounding effects of BW differences and lack of uniformity, and the true effects of nutrition on breeder and progeny performance will become clearer.

Considerations for future maternal nutrition research

Much research is still required to fill the knowledge gaps in this complex field. Better control of confounding sources of variation in studies of the effects of maternal nutrition on progeny growth and development is needed. The following categories should be strategically considered in the study of transgenerational impacts of maternal nutrition. Standardization within and between studies will aid the interpretation of experimental results.

Maternal age

Maternal age has a strong influence on broiler productivity. Effects of maternal nutrition tend to be amplified in progeny from young breeders.

Timing of maternal nutrition

The amount of time prior to egg and chick production that a diet or nutritional management is applied has significant influence on the outcome of many studies. Factorial arrangements of maternal nutrition treatments, including diet changes from the rearing to laying phases will help to identify epigenetic effects.

Genetic factors

The major components of hatching eggs (yolk, albumen and shell) vary in a predictable manner according to age, body composition, and maternal nutrition (Nonis and Gous, 2013), but there are strain- (Johnston and Gous, 2007) and likely hen-specific differences in egg composition that can and should be accounted for. Pedigree information should be used to account for genetic variation in broiler performance. This will allow for more precise estimation of the actual effects of maternal nutrition treatments on progeny performance.

Romero et al. (2009) characterized the efficiency of hens in terms of residual feed intake (RFI) and residual maintenance requirement (RME_m). RME_m was particularly useful for explaining variation in egg and chick weights. Efficient hens (with low RME_m) partitioned more nutrients toward reproduction than inefficient hens (with high RME_m). Broilers from RFI-efficient hens grew at a higher rate from 21 to 28 days, and of age, and had a higher 28- and 38-day BW. Broilers from RME_m-efficient hens grew at a lower rate than less RME_m-efficient hens. In fact, the broilers with the highest BW originated from RFI-efficient and RME_m-inefficient hens. Breast meat yield in broilers from RME_m-efficient hens was lower, whereas no meaningful relationship between RFI and breast muscle yield was detected.

Maternal body weight variation

Body weight differences due to unequal feed distribution is a growing challenge in interpreting the complex effects of maternal nutrition on progeny performance. A more strategic approach to maternal nutrition research must start with rigid BW control and high breeder flock uniformity, achieved through better control of feed distribution. Precision feeding offers promise as a research tool to manage feed intake according to individual birds according to their body weight. Combined with adequate micronutrient supplementation, consistent hatching and brooding management, and better accounting for heritable maternal and paternal contributions to broiler growth, a more clear and consistent pattern of the effects of maternal nutrition on progeny performance will be possible.

Conclusion

The data currently available on the impact of maternal dietary ME and CP levels does not paint a simple picture of how to use maternal nutrition to optimize broiler performance and yield. More strategic experimental design, expert breeder management, control of ancillary sources of variation, and evolution of our understanding of epigenetics and nutritional programming will add resolution to our understanding of this difficult subject.

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NUTRITIONAL SOLUTIONS TO IMPROVE BROILER UNIFORMITY

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Summary

Poor uniformity in a broiler operation reduces revenue and increases waste. Uniformity in body weight at harvest is influenced by variation in genotype, environment and feed composition and form. Variation in broilers within a sex and growing at their potential is relatively small but increases markedly when a poor quality feed is given. In attempting to grow at their potential on a feed low in protein, birds will need to overconsume energy and release excessive amounts of heat to the environment and this ability will be constrained by both feather cover and the ability to fatten. Consequently, as broiler genotypes have become faster growing and leaner there is an increased need to feed higher levels of balanced protein in a cooler environment as a means of improving uniformity. Separating the sexes and reducing the range in day-old body weights will assist in achieving a greater uniformity at harvest.

Introduction

Uniformity is an important measure of performance when optimising the feed and feeding programme of a population of broilers as it relates to the spread of product yield in the processing plant. Poor uniformity reduces revenue and increases waste so broiler producers need to take account of any factors that may influence this. The potential performance of a broiler population depends largely on the inherent genotype of the population and the extent to which this varies between individuals (EMMANS & FISHER, 1986), but many factors conspire to constrain this potential performance resulting in a wider spread of body weights at harvest than would be the case if all birds grew at their potential (GOUS & BERHE, 2006).

The theory of food intake proposed by EMMANS (1981) suggests that a bird will attempt to consume sufficient of a given food to enable it to grow at its potential, thus overconsuming energy if the feed is marginally deficient in an essential nutrient such as an amino acid. It is apparent that strains of broilers and individuals within a strain overconsume energy to different extents when faced with a marginally deficient feed (GOUS *et al*., 1990; CORZO *et al*., 2004; KEMP *et al*., 2005; BERHE & GOUS, 2008). It is likely therefore that such feeds will reduce uniformity, as some birds will be capable of consuming more than others and would thus benefit from the extra feed intake.

But it is not only the variation between individuals in their response to a given feed or environment that contributes to the variation in the response of a population of birds to a feeding programme in a given environment. Variation also exists in the environmental conditions to which the birds are subjected, as well as the composition of the feed used. Each of these sources of variation will be addressed in the following review, but emphasis will be placed on the genotype x environment interactions that result from the feeding of marginally deficient feeds.

Genetic variation

Individuals within a population of broilers vary in many respects, the most important in this context being their potential rates of body and feather protein growth (more specifically their rates of maturing, B), their mature body and feather protein weights (Pm) and their ability to deposit lipid when faced with a nutrient deficiency. EMMANS & FISHER (1986) have given estimates of the variation of these parameters within a population, coefficients of variation (CV) being 0.06 – 0.10 for Pm, 0.02 for the scaled rate parameter B* (=$BPm^{0.27}$), and 0.04 for the rate of lipid deposition (LDR). Differences between males and females in these genetic parameters are considerably greater than the CV's given above, the mean Pm in females being between 0.7 and 0.8 of that of males, the rates of maturing of body and feather protein of females being 0.95 – 0.98 and 1.04 – 1.06 of the male values respectively. The lipid to protein ratio in females is between 1.6 and 2.0 times that of males (HANCOCK *et al*., 1995; GOUS *et al*., 1996; 1999).

Most quantitative biological traits of commercial importance are described by a normal distribution, this being the basis on which genetic selection is made. However, the full expression of these traits is possible only if the environment (including, in this case, the feed offered) is not limiting. In practice the expression of genetic potential in the highest producers is often constrained, and the smallest birds are often culled, thereby limiting the range (CURNOW & TORENBEEK, 2007) and changing the shape of the distribution. This might result in a distribution that is positively or negatively skewed depending on the constraints applied. The trait having the greatest effect in altering the distribution of weights at harvesting is the rate of feathering (FR).

In a sensitivity analysis of the effects of variation in each of the above traits, as well as the initial body weight (W_0), which is not a genetic parameter, on the performance of broilers, GOUS & BERHE (2006) found that food intake, and hence body weight gain, increased almost linearly with B*, Pm and LDR. However, the effect of FR was non-linear, with higher rates of feather growth causing an increased depression of food intake and body growth which was more pronounced in females than in males, and more severe among males fed a low lysine feed in the starter period than those fed the high lysine feed. This genotype x environment interaction would therefore be a particularly important source of variation in fast-growing strains of broiler kept in hot conditions and fed poor quality food (Fig. 1).

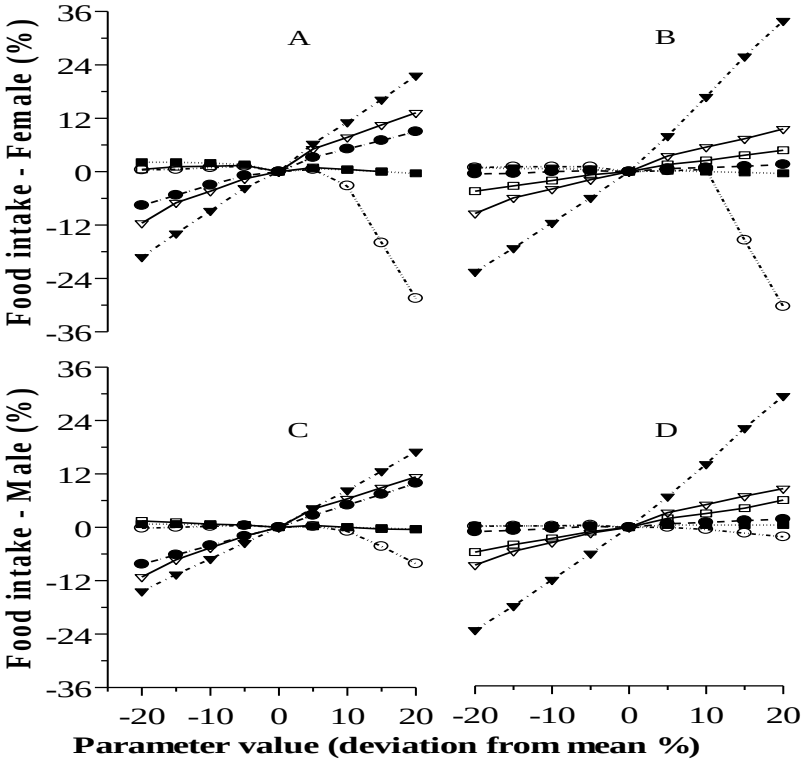

Figure 1. The relative effect on simulated food intake in male and female broilers fed a lysine–limiting feed containing 9 (A, C) or 16g lysine/kg (B, D) from 7 to 21d when the means of each of five genetic parameters were increased or decreased by 0.05, 0.1, 0.15 or 0.20 whilst holding the five remaining genetic parameters constant (B = .-▼.-; FR = ..-○..-; LPRm = ..■..; Pm = __□__; and Wo =__▽__) (from GOUS & BERHE, 2006)

Variation in the environment within a broiler house

Controlling the physical microenvironment in a broiler house is an important element in optimising the production process (REECE & LOTT, 1982; MITCHELL, 1985; PARMAR *et al*., 1992; AERTS *et al*., 2000). However, depending on the system used for such control, variation in the environment within a poultry building may be considerable, impacting significantly on the performance of broilers housed in different areas of the house. Many factors contribute to this variation such as unadjusted inlet openings, unsealed cracks, dirty fan shutters, loose curtains, the amount of time the fans run, the total length of the timer cycle and the position of thermo-sensors (AL HOMIDAN *et al*., 1997; 1998). The behaviour of the broilers themselves will also cause variations in temperature along the length of the house as a result of mass migrations and clustering in some areas (WATHES & CLARK, 1981) which will affect thermoregulation. Such variation in the environment is likely to be positively correlated with the variation in the weight of the end product of the production process, raising the question whether the optimum feeds or feeding programme should be adjusted to take account of this variation.

The vertical temperature profile in a poultry house is affected by many factors, including heat generated by heaters, the flock and solar radiation, microbial fermentation in the litter, heat fluxes between poultry house air and the soil, walls and roofs due to temperature gradients, moisture loss from the litter and natural convection around broilers (VAN BEEK & BEEKING, 1995; BOSHOUWERS *et al*., 1996). However, of greater importance in a tunnel-ventilated house is the variation that exists in the horizontal temperature profile at bird level. Winter and summer conditions alter this profile, with differences in temperature along the length of the house being considerably greater in winter than in summer. In winter, chicks often migrate towards the air inlets, thereby increasing the stocking density in that area, and

the temperature differential in the early stages of growth can be as high as 7 - 9°C (XIN *et al*., 1994) and remains at least 3.5°C cooler at the far end, with 7% higher relative humidity, by the end of the production cycle. In summer, because of the higher ventilation rates used, XIN *et al.* (1994) found little difference in temperature at either end of the house. Many factors interact to influence this gradient, so it would be expected that the temperature and humidity gradient would differ markedly between houses and between seasons.

Poor air quality, due to environmental contaminants such as carbon monoxide, carbon dioxide, ammonia and dust, reduces performance, increases the potential for respiratory disease and may increase mortality (WEAVER & MEIJERHOF, 1991). Conditions at the exhaust end of a tunnel-ventilated broiler house may be far more hostile than at the inlet end, especially in winter, resulting in considerable variation in the performance of the flock.

Because of the range of body weights and potential growth rates in a broiler house at any one time there may be some advantage in providing a range of temperatures in the house so that the fastest growers can move to a cooler area where their potential is more likely to be achieved. However, at high stocking rates it may be impossible for the necessary migration of birds to take place.

The effects of variation in temperature, humidity and air quality in the broiler house on the range of body weights present in the house at the end of the production cycle cannot be estimated empirically because of the expected interactions between these environmental factors and potential body protein growth rate, feathering rate and the chemical and physical composition of the feed, all of which are expected to vary also.

Variation in the nutrient content of the feed

Variation in the nutrient content of feeds offered to broilers in a commercial operation is due mainly to three sources: variation in ingredient composition, mixer inefficiency (including weighing errors), and separation after mixing and during transportation.

Sources of variation in the physical and chemical characteristics of grains used in poultry feeds include variety, seasonal effects, growth sites (METAYER *et al.,* 1993), crop treatment and grain fumigants, post-harvest storage conditions and period of storage and processing (DALE, 1996; HUGHES & CHOCT, 1999), rainfall and environmental temperature patterns during the period of grain maturation, genetic effects, level of fertilizer usage (METAYER *et al.*, 1993; HUGHES & CHOCT, 1999) and inclusion rate (SENKOYLU & DALE, 1999). This type of variation should not be dealt with in the formulation process through the use of a margin of safety as suggested by NOTT & COMBS (1967) but through non-linear stochastic programming (ROUSH *et al*., 1996), but the consequences of variation introduced in this way, and through mixer inefficiency, must be modelled differently from the variation brought about through separation, which is intuitively less random than the first two types of variation.

Performance is adversely affected when variation in nutrient content in feeds is increased (DUNCAN, 1989; MCCOY *et al*., 1994). Some of the more sophisticated feed formulation programs used in the feed industry, which attempt to account for variation in nutrient content in the ingredients used in the feed, generally favour the use of ingredients that exhibit the least amount of variation, thereby potentially reducing the variation in performance of the broilers being fed such feeds. However, whereas natural variation in the composition of a feed has zero cost, a considerable cost is incurred when attempting to reduce variation, as these non-linear programs demonstrate, so there is an economic limit to the extent to which a reduction in variation in nutrient content should be attempted.

FAWCETT *et al*. (1992) attempted to determine this limit by predicting the response of broilers, in margin/m^2, to a range of dietary lysine and ME contents with the use of the 'Poultry Growth Model' of EMMANS (1981), and then integrating the bivariate probability distribution for different degrees of variation in nutrient content over the response surface to determine the value of reducing the variance in the feed. This approach, although interesting, does not simulate the day-to-day variation in feed nutrient content to which broilers are subjected in practice, where nutrient composition varies continuously throughout the rearing period. A more useful technique would be to predict the response of each bird in the population to random variations in feed quality on each day of the growing period, thereby obtaining a more realistic assessment of the effect of variation in feed quality. This would require a stochastic approach to the definition of the composition of the feed offered to the broilers, as well as to the broilers themselves.

Such an approach is a useful tool for determining the upper limit to the value of variance reduction, but does not address the issue of further systematic variation in nutrient content brought about by separation during road, rail, auger, blower or chain transportation. Such variation tends to separate the fine from the coarse particles, particularly when the feed is conveyed in a mash form, or when the quality of pellets is poor, resulting in a high proportion of fines.

This problem does not appear to be addressed in the literature, so the variation introduced in this way is unknown, but could be considerable. This variation is likely to be systematic, in that separation would take place along the length of the feeder lines, with fines being left behind as the coarser particles move to the end of the line. If the fines consisted predominantly of major and minor minerals and vitamins, bone development could be seen to worsen at the far end of a broiler house compared with the end at which the feed is introduced. The effect of such nutrient separation could be modelled in the same way as are the systematic changes that take place in the environment within a broiler house, the birds along the length of the house being subjected to changes in temperature, humidity and air quality. But it is not only the chemical separation of the food that takes place that influences performance along the length of a broiler house; the physical nature of the feed may also be affected.

Pelleted feeds are known to improve feed conversion efficiency when compared with mash feeds (JENSEN *et al*., 1962), this being due to the shorter period of time spent by the broilers consuming pellets, resulting in lower energy expenditure. The physical nature of the feed therefore has an effect on performance and, because of the abrasive nature of some feeding equipment used in broiler houses, the amount of time spent eating, and hence the energy expended in eating, may well increase as the distance of the feed trough from the hopper increases.

Nutritional interventions

As the potential growth rate of broilers has been increased by genetic selection, so the daily amino acid and energy requirements of the bird have also been increased, but these do not increase in the same proportion: the requirement for amino acids increases proportionately faster than does the energy requirement, thus a higher amino acid to energy ratio is required in faster growing strains of broiler. Many nutritionists believe, incorrectly, that broilers will simply increase their intake of an inadequate feed in order to obtain sufficient of the limiting nutrients. Whereas broilers may attempt to do so, it is seldom that they achieve the intake required to enable them to grow at their potential. This is well illustrated by the results of an experiment by MORRIS & NJURU (1990) who fed diets of increasing protein content to broilers and to laying-type cockerels. The maximum gain in weight and in protein content in the laying-type chickens was achieved on diets with considerably lower protein contents than was needed to maximise the gain in the broilers (Table 1). Even on marginally deficient protein levels the broilers were unable to consume sufficient to grow at their potential. The inability to consume sufficient of a marginally deficient feed would be more pronounced with the broiler genotypes available at present, these having been subjected to a further 25 years of selection for feed efficiency since the above publication, which would have further reduced the ability of broilers to fatten (PYM & SOLVYNS, 1979).

Table 1. Liveweight, food intake, feed conversion efficiency (FCE) and protein content of broiler and layer-type chickens at 21 d of age (after MORRIS & NJURU, 1990)

Crude protein in diet g/kg	Liveweight g	Food intake g/chick	FCE g gain/ kg food	Protein content g/chick
Broiler chickens				
167	414	938	400	53
188	618	1193	486	85
209	706	1183	564	100
230	786	1230	607	114
251	836	1320	603	123
Layer chickens				
167	235	683	293	35
188	290	702	362	45
209	295	711	366	46
230	291	650	394	46
251	299	689	384	47

There is strong evidence that uniformity is increased by feeding higher levels of a limiting amino acid or protein (DUNCAN, 1984; LEMME, 2003; BERHE & GOUS, 2008). This is illustrated by the data in Table 2 where the coefficient of variation (CV %) in liveweight at 42 d was reduced by 40 % and breast meat yield by 47 % through the supplementation of DL Methionine to a basal feed (LEMME, 2003). Supplemental vitamins too have been reported to improve uniformity in a broiler flock. MCNAUGHTON (1995) reported continuing economic responses in several characteristics up

to the highest level of vitamins tested. There was little convincing evidence in his trial that the response to vitamin level depended on the level of stress applied, as performance improved with increasing vitamin doses even when no stress was applied.

Table 2 The effect of graded levels of DL-Methionine on liveweight and breast meat yield in broilers (1 – 42 d) (after LEMME, 2003)

	DL-Methionine supplementation (g/kg)			
	0	0.4	0.8	1.2
Liveweight (d 42) g	1831	2452	2646	2836
CV %	16.8	11.1	9.5	6.7
Breast meat (d 42) g	362	543	630	707
CV%	18.5	14.3	11.7	7.8
Breast as % of live weight	19.8	22.1	23.8	24.9

The reason for the improved uniformity is that the requirements of a greater proportion of the population will be met as the concentration of the limiting feed resource is increased. FISHER *et al*. (1973) showed that the response of an individual (laying hen) to an increasing supply of an amino acid differs markedly from that of the population from which the individual is drawn. Whereas the response of each hen can be assumed to be linear, up to a point where output plateaus (the genetic potential), the population response is a continuous, asymptotic curve with no abrupt threshold. This population response curve, the mean response of a group of individuals at a time, is the result of the variation that exists in both body weight (maintenance requirement) and the potential egg output of the population. This so-called Reading Model (FISHER *et al*., 1973) may be used to determine the optimum economic intake of the limiting amino acid for a flock of hens. The increased egg output of those birds with above-average requirements for maintenance and above-average maximum potential egg outputs justifies the cost of increasing the amino acid intake by all birds.

In a population of growing broilers, as with laying hens, at any point in time body weights and potential outputs (growth rates) will be normally distributed about a mean value, but in addition to this, because each bird is growing and food intake is increasing, variation in growth rate within each animal over time also needs to be considered (GOUS & MORRIS, 1985). This is not as critical in the case of laying hens as these birds are in a relatively steady state over time. To understand these interactions a mechanistic model that accurately simulates food intake and growth of an individual broiler over time is useful.

Such a model, based on the theory of food intake and growth of EMMANS (1987) has been developed (EFG SOFTWARE, 2015). This simulates the growth of a single bird, taking account of genetic parameters, diet composition and feeding programme, the environment, stocking density and other factors that may affect the outcome of production decisions in practice. The model works by calculating the desired amount of a given food the bird needs to consume each day to grow at its potential, and will predict the consequences in food intake, growth and carcass composition if this intake is constrained by feed bulk or an environment that is too hot to allow the bird to lose sufficient heat to that environment.

As mentioned above, male and female broilers have different growth characteristics which will impact on the way in which they should be fed and housed during growth. Because males have the potential to grow faster, and to a larger size, than females their amino acid requirements for potential growth would be expected to be higher. When their lysine requirements for maintenance and potential body and feather protein growth are calculated and converted to a feed concentration, these are similar for males and females at day-old, but as the growing period progresses male broilers require considerably more lysine than females, the difference increasing throughout the growing period (Fig. 2).

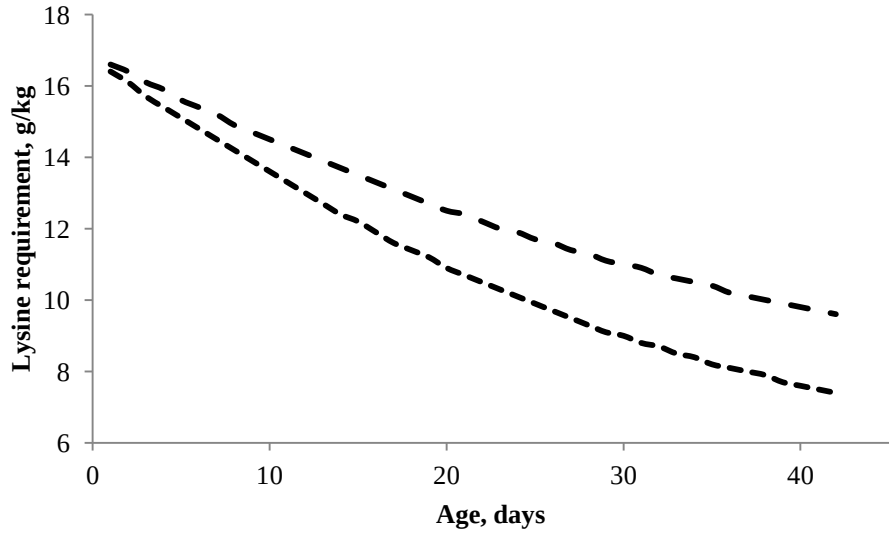

Figure 2. Feed content of lysine (g/kg) required to enable male (dashed line) and female (dotted line) broilers to grow at their potential at a feed energy content of 13 MJ ME/kg, simulated using the EFG broiler growth model (EFG SOFTWARE, 2015)

By growing males and females together the feed composition used is clearly a compromise between that required by each sex. Feeding a fixed protein content during each of the phases of growth is likely to result in male broilers being under-fed amino acids for long periods whereas the opposite would be the case for females. Under certain circumstances birds are capable of partially overcoming the effect of being underfed protein: to do so they would need to be capable of overconsuming energy, and they would need to be housed in cool conditions. The genetic parameter referred to above, which controls the maximum rate of lipid deposition in the short run, reflects the ability of the genotype to overconsume energy and fatten when presented with a feed in which one or more amino acids or other nutrients are marginally limiting.

Birds with a low propensity to fatten, such as turkeys, would be disadvantaged when presented with a marginally limiting feed, as they would be unable to store the excess energy as body lipid, the resultant food intake therefore being lower than desired and weight gain being below the potential. Modern broiler strains are becoming leaner through selection so they are becoming less capable of overcoming marginal dietary deficiencies than were broiler genotypes of the past, and this would explain why uniformity is increased when higher protein and vitamin levels are included in the feed.

The ability to lose heat to the environment is another factor influencing the ability of birds to overcome nutrient deficiencies. For a broiler to be able to grow at its potential it needs to be able to lose all the heat that is produced in growing at that potential, including the heat generated by maintaining itself, in growing and in digesting the feed. Clearly, the density of feather cover will play a major role in determining the extent to which heat can be lost to the environment. Feather growth differs markedly between genotypes and sexes, especially in feather-sexable strains and this variation will impact severely on food intake and hence body weight at harvesting, especially on marginally to severely deficient feeds.

Initial (day-old) body weight has an impact on final body weight, the response being almost linear, as illustrated in Fig. 1. In a simulation exercise using the EFG Broiler Growth Model, for initial body weights varying from 35 to 60 g the simulated 42-d body weights ranged from 3099 to 3373 g, a range of 274 g. This is without taking account of social stresses and difficulties experienced by smaller chicks in reaching the feeders and drinkers, which would have the effect of increasing even further the variation in final weight. Although NETO *et al*. (2013) found no improvement in uniformity at 42 d when grouping day-old chicks according to weight and rearing them in these groups, SINGH & NAGRA (2006) reported that the largest chicks at day-old retained this advantage by 35 d in both male and female broilers. Because broiler chicks of the same genotype have the same potential to grow irrespective of day-old bodyweight, smaller chicks at day-old would benefit from higher dietary protein levels as their ability to consume food is constrained by gut capacity. Broiler producers should strive to reduce the range of day-old chick weights if the objective is to improve uniformity in the end-product.

Conclusions

The spread of product yield in the processing plant is influenced by variation in genotype, environment and feed composition and form. To increase uniformity through nutrition, broiler producers should consider rearing sexes separately, feeding high levels of balanced protein and vitamins, reducing the environmental temperature in the broiler house, minimising the range of day-old body weights, and using good quality pellets.

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NUTRITIONAL REQUIREMENTS AND FEEDING MANAGEMENT OF MEAT TYPE DUCKS

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The introduction presents the main countries producing duck meat and the different species and rearing systems used in production. The review is then divided into four parts. The first three parts describe successively the requirements of meat type ducks only for energy, protein and amino acids and minerals, vitamins and trace-elements. The last part, presents recommendations regarding feeding management (feed presentation, feed restriction, use of enzymes and feedstuffs, fatty acid composition of diets). In the world, the main ducks dedicated to meat production are Pekin, Muscovy and mule ducks. As, the growth rate, the growing performance and the body composition of these different genotypes are different, the specificities of each type of duck are indicated in each part. The review is only focussed on ducks reared under intensive production system.

Keywords: nutritional requirements, growing ducks, energy, proteins, amino acids, minerals

Introduction

From 1993 to 2012, the world duck production largely increased, and meat production rose from 1.72 to 4.34 million tons (Deman, 2014). Asia is the leading production region and this is the area where the industry is expanding most rapidly. Asia accounted for 76 and 83% of duck meat output in 1993 and 2012, respectively (FAO statistics, 2014). In Asia, China's contribution (3 millions of tons) represents 86% of the total region. The next largest producers are Malaysia (128,100 tons), Myanmar (105,200 tons), Vietnam (105,000 tons), Thailand (82,000 tons), the Republic of Korea (68,900 tons) and Taïwan (67,700 tons). Europe represents the second high production region. Annual production grew from 262,736 in 1993 to 480,000 tons in 2013, France being the leading producer with 239,000 tons. Next came Hungary (77,000 tons) followed by Germany with just over 53,000 tons and the UK (30,000 tons). Production in North and Central America is dominated by the USA (54,000 tons in 2012) followed by Mexico (21,000 tons) and Canada (7,600 tons). In Africa, the industry is primarily centred on Egypt and Madagascar, producing 63,300 and 12,000 tons of duck meat, respectively. Less than 4% of duck meat production is exported. In 2011, the main exporters were China (42,616 tons), Hungary (23,670), Germany (20,129 tons) and Netherlands (17,511 tons) and the main importers in Asia and Europe were Japan (4,865 tons in 2011) and Germany (16,232 tons).

The main species used to produce duck meat are Pekin, Muscovy (mostly in France) and mule ducks (hybrid resulting from the cross between males Muscovy duck, Cairina moschata and females Pekin, Anas platyrhynchos, mostly in France for the production of fatty liver and Taïwan). Other duck species such as Jinding and Shao ducks in China, Tsaiya ducks in Taïwan, Khaki Campbell, Indian runner and Desi ducks in India, Vietnam, Cambodia and Indonesia are used mainly for egg production and meat is a by-product (Pingel, 2004).

Different production systems are used in the world. In developed countries, ducks are mainly reared intensively in total-confinement housing or with access to pasture (free-range, organic or "label rouge" systems, mule ducks reared for fatty liver production) and sometimes to water for swimming. In Asia, extensive systems such as fish-duck farming are largely used, ducks being housed above fishponds (Prinsloo et al., 1999; Syed, 2002). To decrease feed costs, ducks can also be reared in rice fields (Men et al., 2002a; 2002b).

As for other poultry species, the industry developed cut and processed duck products. The meat yield of ducks has therefore increased and carcass fatness has decreased owing to genetic selection and to improvements in duck management, particularly in nutrition. This review presents the requirements of meat type ducks only for energy, protein and amino acids and minerals, vitamins and trace-elements. It presents also recommendations regarding feeding management. As, the growth rate, the growing performance and the body composition of Pekin, Muscovy and mule ducks are different, the specificities of each genotype are indicated in each part. The review is only focussed on ducks reared under intensive production system.

Energy

According to Leclercq (1990), the duck energy requirement in the conditions of thermal neutrality (25°C) can be estimated thanks to the following equation:

$E = 105\ BW^{0.75} + 14\ Prot + 11\ Lip$

E is the daily energy requirement (kcal), BW = Body Weight (kg), Prot = amount of daily stored proteins, Lip = amount of daily stored lipids

The duck is able to regulate the amount of ingested energy. It is therefore nonresponsive in the variations of dietary energy level. The growth will be the same with high (3,000 kcal ME/kg) or low (2,500 kcal ME/kg) dietary energy levels but feed consumption and feed conversion ratio will be higher with low dietary energy level than with high dietary level. According to Scott and Dean (1991), the Pekin duck is able to regulate the amount of ingested energy when energy dietary level is comprised between 2,300 and 3,100 kcal ME/kg. For the Muscovy duck, the range of energy level variation is comprised between 2,500 and 3,000 kcal/kg (Leclercq, 1990).

Duck diets are usually formulated using ME values obtained from chickens even if a few studies showed differences between both species for some feedstuffs (for a review, see Elkin, 1987). For Pekin duck, Adeola (2006) recommended diets containing 2825-2875 kcal ME/kg for the starting period (0-2 weeks of age) and 3050-3075 kcal ME/kg for the growing period (2-6 weeks of age). The NRC (1994) recommendations are 2,900 and 3,000 kcal ME/kg for the starting and growing periods, respectively. For Muscovy duck, dietary energy levels are usually comprised between 2800 and 3000 kcal ME/kg (INRA, 1989).

Fan et al. (2008) analysed the effects of dietary energy on growth performance and carcass composition of growing Pekin ducks from 2 to six weeks of age. The dietary energy level varied between 2,600 and 3,100 kcal/kg whereas the crude protein, methionine, cystine and lysine contents were similar for all diets (180, 4.0, 3.2 and 9.0 g/kg, respectively). This study showed that the apparent metabolisable energy (AME) requirements for optimal weight gain and feed: gain ratio were 3,008 and 3,030 kcal/kg, respectively. The dietary energy level had no effect on carcass composition except the proportion of abdominal fat which was lower with diets providing 2,600 and 2,700 kcal/kg by comparison with the other diets.

Xie et al. (2010) determined the AME requirement of Pekin ducklings from hatch to 3 weeks of age. The dietary energy level varied between 2,454 and 3,050 kcal/kg whereas the crude protein, methionine, cystine, lysine, threonine and tryptophan contents were similar for all diets (205, 5.2, 3.5, 11.0, 7.8 and 2.4 g/kg, respectively). This study showed that the AME requirement for optimal feed conversion efficiency was 3,022 kcal/kg. It was not possible to determine the requirement for optimal weight gain. However, the highest body weight at 3 weeks of age was obtained with the diet providing 3,050 kcal/kg (1,319 vs. 1,194 g for diet providing 2,454 kcal/kg) The dietary level did not affect the carcass meat yield but the proportion of abdominal fat increased with the increase in dietary energy level from 0.58 to 0.94% with diets providing 2,454 and 3,050 kcal/kg, respectively.

Several studies have been conducted to determine energy utilization in a variety of grains, meals and by-products for ducks (Adeola, 2006; Hoai et al., 2011; Table 1). The hierarchy of energy values for the grains and meals is the same for ducks and broilers. Of the grains examined thus far, barley, rye and triticale have significantly lower energy values for ducks than corn, sorghum or wheat. It is generally assumed that ME value of individual feedstuffs can be added together to match the required energy supplied by the diet. This assumption was tested and verified in barley-canola meal (complete) diet (Hong et al., 2001) and corn-wheat red dog-soybean meal (complete) diet (Hong et al., 2002a). Zhao et al. (2008) and Wan et al. (2009) showed that neutral detergent fibre (NDF) content could be used as an effective indicator to predict the true metabolisable energy (TME) value of corn and wheat milling by-products, respectively, for ducks.

Protein and amino acids

For Pekin duck, the recommendations for dietary protein and amino acid (AA) contents are indicated in table 2. For most duck diet formulations, methionine, lysine, threonine and tryptophan are likely to be the most limiting AA. Elkin et al. (1986) reported that 1-day-old male Pekin ducklings require between 3.82 and 4.22 g methionine/kg diet during the first 12 days of life. Xie et al. (2004) estimated the optimal methionine and cysteine requirements of Pekin ducklings from hatch to 21 days of age at 4.81 and 3.25 g/kg, respectively. Between 21 and 49 days of age, the optimal methionine requirement was 3.8 g/kg (Xie et al., 2006). For Bons et al. (2002), the dietary lysine concentration should be 11.7

g/kg for the starter period (until 21 days of age) and 10.0 g/kg for the grower period (from 22 days until slaughter). From 7 to 21 days of age, Xie et al. (2009) evaluated the dietary lysine requirement to 9.8 g/kg. With a diet containing 180 g crude protein/kg and 2,990 kcal/kg, Timmler and Rodehutscord (2003) estimated the optimal in dietary valine concentration at 8.0 g/kg for the first three-week post-hatching period. Zhang et al. (2014) estimated the optimal dietary threonine requirement to 8.0 g/kg from 15 to 35 days. Between 1 and 21 days of age, the dietary arginine requirements was 9.5, 11.6 and 9.9 g/kg for optimal weight gain, feed conversion ratio and breast meat yield, respectively (Wang et al., 2013).

For Muscovy ducks, the recommendations for dietary protein and AA contents are indicated in table 3. Leclercq and De Carville (1977) reported that male Muscovy ducklings required 6.0 and 5.5 g total SAA/kg for maximal growth from 3 to 6 and 6 to 10 weeks of age, respectively with diets containing 155 or 130 g CP/kg and 2,986 or 3090 kcal ME/kg, respectively. The 3 to 6 week lysine requirement was estimated to 6.0 g/kg that is 2.2 g lysine per 1,000 kcal ME representing an absolute intake of 21 g lysine over the period (Leclercq and De Carville, 1979). The 6 to 10 week lysine requirement estimation was 1.96 g lysine per 1,000 kcal ME representing an absolute intake of 30-35 g lysine over the period (Leclercq and De Carville, 1979). For threonine, Leclercq (1998) estimated the dietary requirement to 3.97 g/kg between 8 and 12 weeks of age.

For mule ducks, Baéza et al. (2012) showed that 235, 154 and 138 g CP /kg levels could represent optimal levels for growing performance in the starting (0 to 3 weeks of age), growing (3 to 7 weeks of age) and finishing (7 to 10 weeks of age) diets containing 2,900 kcal/kg, respectively. Chen and Shen (1979) reported that for maximal 9 to 21-day weight gain and feed efficiency, the minimal dietary requirements for lysine and arginine were 10.6 and 10.8 g/kg, with diets containing 2,828 kcal and 2,810 ME/kg, respectively. The minimal dietary tryptophan level required for maximal growth and feed efficiency between 8 and 20 days of age was 2.3 g/kg with a diet containing 3,013 kcal/kg and 180 g CP/kg (Wu et al., 1984). The minimal dietary requirements for leucine, isoleucine and valine between 8 to 20 days of age were 12.6, 6.3 and 7.8 g/kg, respectively (Yu and Shen, 1984). Lin and Shen (1994) showed that the minimum requirement of phenylalanine plus tyrosine was 11.1 g/kg between 0 and 3 weeks of age.

For ducks, diets are usually formulated by using the requirements for total AA or the digestibility coefficients of AA determined for broilers even if different studies showed that these coefficients were lower for ducks compared to broilers (for a review, see Helmbrecht, 2012). There are few studies reporting dataset for AA utilisation in feed ingredients for ducks. A comparison of digestibility coefficients for the indispensable AA in corn, barley, bakery meal, canola meal, soybean meal and wheat red dog revealed a considerable variation (Adeola, 2006). In general, true digestibility of the indispensable AA is lower in barley than in corn; and much lower in canola meal than in soybean meal. Digestibility coefficients for most of the indispensable AA in canola or soybean meals are greater than 80%. By comparing diets containing different threonine levels from 5.7 to 8.0 g/kg, Zhang et al. (2014) calculated the apparent ileal threonine digestibility which was comprised between 73 and 83%. The assumption of additivity of individual feed ingredients AA utilization values in complete diets was also tested for barley, canola meal, corn, wheat red dog and soybean meal (Hong et al., 2001; 2002a). While the true digestibilities were additive for most AA, significant exemption was observed for arginine, histidine, lysine, tryptophan and aspartate. Thus, true digestibilities of some AA are not additive, demonstrating some associative effects that vary with the ingredient matrix (Hong et al., 2001; 2002a).

A diet providing 3,012 kcal ME/kg and 182 g CP/kg and supplemented with 10 g L-arginine /kg between 21 and 42 days of age decreased carcass fatness and increased meat yield by comparison with Pekin ducks fed with the same diet not supplemented with L-argninine (Wu et al., 2011). The lipid and protein contents of breast muscle were also increased.

Baéza and Leclercq (1998) tested the possibility to decrease the CP concentration in finishing diets of Muscovy ducks by adding 4 essential AA in order to cover the requirement for these AA. During this period, feed consumption is economically very important as it represents nearly 50% of the total feed intake for the growth of male Muscovy ducks. Protein and AA requirements are lower. Unlike in chicks, feed conversion ratio and fattening of ducks are not modified by decreased dietary CP concentration. Finally, a reduction in dietary CP content should reduce the nitrogen content of excreta and litter, which is a source of environmental pollution. Baéza and Leclercq (1998) showed that it was possible to reduce to 12.4% the CP content of finishing diet supplemented with the 4 essential AA. There was no advantage in supplying more than 4.3 g digestible lysine/kg. When threonine was not added, breast yield decreased significantly (-4.3%), while omitting tryptophan supplementation did not influence growth performance and body composition. It will be interesting to test decreased dietary protein concentration for the starting and growing periods by supplementing diets

with essential and may be not essential AA by using the ideal protein concept developed for broilers according to Baker and Han (1994) and Mack et al. (1999).

Minerals, vitamins and trace-elements

Minerals

For Pekin duck, the recommendations for dietary mineral contents are indicated in table 4. Rodehutscord et al. (2003) showed that Pekin ducks require a dietary nonphytate phosphorus level of 3.4 (1 to 21 days of age) and 2.3 g/kg (21 to 49 days of age) based on phosphorus retention data.

For Muscovy duck, the recommendations for dietary mineral contents are indicated in table 5. Leclercq et al. (1990) showed that the best bone characteristics were obtained with 4.0 g nonphytate phosphorus and 8.4 g calcium/kg between 3 and 8 weeks of age and 2.6 g nonphytate phosphorus and 4.3 g calcium/kg between 8 and 12 weeks of age.

For mule duck, Bernadet et al. (2002a; 2002b) showed that the nonphytate phosphorus requirement was 3.1 and 1.4 g/kg for the starting (0-28 days of age) and growing (28-84 days of age) periods, respectively.

Wendt and Rodehutscord (2004) showed that phosphorus availability for Pekin ducks depended on the inorganic sources and ranged between 77 and 100% which should be considered in diet formulation.

The ducklings require approximately 1.4 g sodium and 1.2 g chloride/kg for maximal weight gain (Dean, 1972).

Vitamins and trace-elements

The recommendations for these nutrients are indicated in NRC (1994), Adeola (2006), Elkin (1987) and INRA (1989). Recently, Wen et al. (2014a; 2014b) revised the choline requirements of Pekin ducks to 810 and 823 mg/kg to optimise weight gain and feed intake during the starting (0-3 weeks) period and to 980 and 950 mg/kg to optimise weight gain and feed intake during the growing (3-6 weeks) period. To prevent an excessive liver lipid deposition during the growing period, the dietary choline concentration should be 1130 mg/kg. For Halle et al. (2011), 20 µg vitamin B12/kg met the requirements of growing ducks. An additional supplementation of cobalt to feed did not have additional advantages for the ducks. The dietary riboflavin requirement from hatch to 21 days of age is around 3.3 mg/kg for Pekin ducks (Tang et al., 2013). Attia et al. (2012; 2013) showed that diet supplementation with 8 mg copper/kg and 30 ppm zinc from inorganic sources were optimal for growth performance of Pekin ducks from hatch to 56 days of age.

Feeding management

Feed presentation

Pelleted diets are utilized more efficiently by ducks than diets in mash form because of reduced wastage (10 to 20%) and ease of consumption (NRC, 1994). Starter diets are usually fed as pellets of 3 mm diameter and 8 mm length and for grower diets the pellets are given in 5 mm diameter and 12 mm length.

Feed restriction

Tan et al. (1999) tested a feed restriction of Pekin ducks to 80, 60 and 40% of ad libitum intake from 7 to 14 days of age. Ducks had the same starter diet (2,900 kcal ME/kg and 230 g CP /kg) from hatch to 21 days of age and the same finisher diet (3,200 kcal ME/kg and 190 g CP/kg) up to 47 days of age. At slaughter age, the feed restriction had no effect on body weight and carcass composition but the feed conversion ratio was reduced by comparison with the ad libitum group. Leclercq and De Carville (1978) tested on male Muscovy ducks a slight (5%) and a severe feed restriction (20%) between 8 and 12 weeks of age. The slight feed restriction had no effect on the slaughter body weight and the carcass composition whereas the severe feed restriction had a negative impact on slaughter body weight and meat yield. It also decreased carcass fatness. The Muscovy duck is able to compensate an earlier feed restriction (25%) realised between 4 and 6 weeks of age with no impact on the growing performance for the whole rearing period and the carcass composition at 12 weeks of age (Baéza, 2000).

Another possibility to restrict poultry is to dilute diets with feedstuffs containing high fibre content. Wu et al. (2012) tested 40 or 60% rice hulls inclusion in the basal diet of Pekin ducks between 8 and 14 days of age. Ducks were fed with the same starter diet (2,902 kcal ME/kg and 210 g CP/kg) from hatch to 14 days of age and the same grower diet (3,002 kcal ME/kg and 180 g CP/kg) between 14 and 42 days of age. The use of 40% of rice hulls had no effect on body weight, feed conversion ratio and meat yield at 42 days of age. The carcass fatness and the lipid content of breast

muscle were decreased whereas the protein contents of carcass and breast muscle were increased. Guo et al. (2013) confirmed that diluting diet with 40% rice hull between 8 to 14 days of age might be a suitable method to improve feed efficiency and to reduce carcass fatness in the production of Pekin ducks.

Use of enzymes

As for broilers, the use of enzymes (xylanase, β-glucanase, amylase, cellulase, protease, phytase) in diets formulated for ducks can easily improve the nutritive values and digestibility of feedstuffs by reducing digesta viscosity (Timmler and Rodehutscord, 2001; Adeola, 2006) and by consequence decrease the nitrogen and phosphorus contents of excreta (Hong et al., 2002b; Rodehutscord, 2006). For ducks, the use of enzymes is more efficient during the starting and growing periods than the finishing period. It allows also the use of feedstuffs rich in phytic phosphorus and non-starch polysaccharides and therefore decreases the use of mineral phosphorus in diets (Baéza et al., 2010). The use of a diet between 4 and 13 weeks of age containing 1.6 g nonphytate phosphorus/kg and supplemented with a phytase at 600 FTU/kg allowed a reduction of phosphorus in excreta reaching 26 g per mule duck (Bernadet et al., 2004).

Use of feedstuffs

In practice, many duck diets closely resemble diets for chickens, in being largely corn-soybean meal type. However, worldwide, several non-traditional nutrient sources are available for formulation into the diets of ducks. In Thailand, broken rice, full fat rice bran and extracted rice bran are available. In Vietnam, duck producers can use full fat rice bran and cassava. In Malaysia, Thailand and Indonesia, palm kernel cake is available and could be used at 10-15% (Creswell, 2012). Distiller's dried grains with solubles (DDGS), imported from USA, are available in all Southeast Asian countries and could be used at 10-15% (Creswell, 2012). Inclusion of corn DDGS in amounts up to 25% in the diet of Pekin ducks between 22 to 56 days of age had no adverse effect on growth performance, carcass composition at slaughter, chemical composition, pH and colour of breast meat (Kowalczyk et al., 2012). Peillod et al. (2010) showed that it was possible to include corn DDGS in amounts up to 24% in growing and finishing diets without negative effect on growth performance of mule ducks. Wheat is available in several Asian countries (imported from Australia) and Europe. Wheat milling by-products (bran, pollard) can also be used. Copra, cottonseed and groundnut meals are suitable ingredients for ducks but testing for aflatoxins is recommended before use (Creswell, 2012). Jiang et al. (2012) showed that dietary alfalfa meal introduced in diet at 3, 6 or 9% between 14 and 49 days of age decreased carcass fatness and increased breast meat yield of Muscovy ducks, without an adverse effect on growth performance. It is possible to use industrial rapeseed meal at 7% for the starting period and 14% for the growing-finishing period without adverse effect on growing performance and carcass fatness of mule ducks (Bernadet and Lessire, 2010). Sunflower meal can be used up to 12% in growing diets without affecting growth performance and carcass composition of Pekin ducks (Attia et al., 1998). El-Deek and Brikaa (2009) showed that red seaweed Polysiphonis spp. washed, dried (72 h at 60°C) and ground can be used in starter (0-35 days of age) and fisnisher (35-63 days of age) diets of Pekin ducks up to 12 and 15%, respectively, without adverse effect on growth performance, carcass composition and sensory quality of meat. The inclusion of sweet lupins at 400 g/kg in the starter and finisher diets had no deleterious effects on growth performance and carcass composition of Pekin ducks (Olver, 1997). The bitter lupins can also be used in duck diets at the same level but it is necessary beforehand to soak and micronized them (Olver and Jonker, 1998).

Fatty acid composition of diets and nutritional quality of meat

As for all monogastric animals, it is easy to modulate the fatty acid (FA) composition of meat duck by using different fat sources in diets. Feeding Muscovy ducks with diets containing fish meal or fish oil enriched adipose and muscle tissues with n-3 FA but reduced the sensory acceptance of meat (El-Deek et al., 1997; Schiavone et al., 2004). Schiavone et al. (2007) enriched breast meat of Muscovy ducks with docosahexaenoic acid (DHA) a long chain polyunsaturated n-3 FA, interesting for health consumers, by supplementing diet with 5 g microalgae meal/kg whereas the protein and lipid contents, colour, pH, oxidative stability and sensory characteristics were not affected. Moreover, it is possible to decrease oxidative susceptibility of duck meat enriched with polyunsaturated FA by supplementing diets with vitamin E. Schiavone et al. (2010) measured 2-fold lower values of thiobarbituric acid reactive substances (an indicator of lipid peroxidation) with breast meat from ducks fed a diet containing 230 mg α-tocopheryl acetate/kg by comparison with breast meat from ducks fed a diet containing 30 mg α-tocopheryl acetate/kg.

Conclusion

The main part of duck nutrition studies only examined the effects of nutrients on growth performance, carcass composition at the end of the experimental period or at usual slaughter age and chemical composition of carcass or cut parts (breast or thigh meat, abdominal fat) which will impact the nutritional quality of meat. However, diet composition and characteristics can also affect the technological (pH, colour, water holding capacity, processing yield, oxidative susceptibility) and sensorial (texture, flavour, juiciness) qualities of meat. In the future, these parameters also should be taken into account. Actually, Berri et al. (2008) showed that an increase in dietary digestible lysine from 8.3 to 9.3 g/kg between 21 and 42 days of age increased growth rate and breast yield, and improved feed conversion ratio of broilers. Growth performance and carcass composition remained unchanged when the concentration of dietary digestible lysine was higher than 9.3 g/kg. However, when the dietary digestible lysine increased from 8.3 up to 10.3 g/kg, the ultimate pH of breast meat increased (6.02 vs. 5.91) and drip loss correlatively decreased (0.85 vs. 1.10%) suggesting modifications of breast muscle energy metabolism, particularly on glycogen storage, improving the technological quality of breast meat. Dietary AA composition can also influence sensory quality of meat. Increasing dietary concentrations of isoleucine and valine, 10 days before slaughter, increased free glutamate (a taste-active component) content and sensory score of overall taste intensity of broiler breast meat (Imanari et al., 2008). In order to determine precisely the AA requirements it will be also necessary to measure digestibility of AA for duck species during the different feeding phases. Apart the enzymes, there are also a large variety of feed additives (probiotics, prebiotics, antioxydants, clays, plant extracts) that can be used also for ducks in relation with their sanitary status, gut microbiota, physiological response faced to environmental stress. These different additives need to be evaluated for duck production. Finally, continued progress in efficient worldwide duck production still requires nutritional evaluation of non-traditional nutrient sources for the determination of optimal inclusion levels in diets formulations in order to attain safe, sustainable and efficient production of duck meat that will contribute to satisfy human nutritional requirements for high-quality protein.

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Table 1. Apparent metabolisable energy (AME), nitrogen-corrected apparent metabolisable energy (AMEn), true metabolisable energy (TME) and nitrogen-corrected true metabolisable energy (TMEn), expressed as kcal/kg of feed ingredients for white Pekin ducks (Hoai et al., 2001; Adeola, 2006)

Ingredients	Number of studies	AME	AMEn	TME	TMEn
Barley	2	2,622-2,753	2,726-2,730	2,973-3,205	2,863-2,960
Corn	12	3,111-3,624	3,100-3,505	3,310-4,014	3,271-3,833
Low-phytin corn	1	3,412	2,391	4,050	3,847
High-oil corn	1	3,563	3,501	4,201	3,959
Oats dehulled	1	3,563	3,482	3,761	3,642
Pearl millet	1	3,392	3,350	3,612	3,484
Rice	2	3,421-3,622	3,452-3,500	3,740-3,653	3,612-3,526
Rye	1	2,633	2,691	2,952	2,851
Sorghum	3	3,021-3,312	3,038-3,260	3,339-3,682	3,310-3,567
Triticale	1	2,800	2,757	3,170	3,065
Wheat	1	3,262	3,144	3,461	3,300
Bakery meal	3	3,731-3,760	3,755-3,796	4,130-4,158	3,896-3,933
Canola meal	1	2,181	2,186	2,764	2,439
Corn gluten meal	1	4,044	3,695	4,367	3,934
Meat and bone meal	12	1,781-3,916	1,772-3,662	nd	nd
Low-phytin soybean meal	1	3,018	2,579	3,539	2,959
Soybean meal	1	2,938	2,691	2,969	2,720
Cassava meal	1	3,301	3,201	3,326	3,227
Fish meal	2	3,132-3,184	2,835-2,852	3,160-3,213	2,861-2,878
Wheat middling	2	2,208-2,478	2,149-2,385	2,711-2,859	2,502-2,673
Wheat red dog	4	2,385-2,571	2,519-2,592	3,117-3,213	2,900-2,948
Rice bran	1	3,079	2,950	3,112	2,978
Wheat bran	1	3,048	2,907	3,093	2,947

nd = not determined

Table 2. Protein and amino acid requirements of white Pekin ducks

	NRC (1994)		Adeola (2006)			
Nutrients (%)	0-2 weeks	2-7 weeks	0-2 weeks	0-2 weeks	2-7 weeks	2-7 weeks
Metabolisable energy (kcal/kg)	2,900	3,000	2,825	2,875	3,050	3,075
Crude protein	22	16	23.0	20.5	17.5	15.0
Arginine	1.1	1.0	1.20	1.04	0.94	0.85
Histidine			0.44	0.37	0.33	0.29
Isoleucine	0.63	0.46	0.84	0.69	0.63	0.56
Leucine	1.26	0.91	1.56	1.28	1.16	1.04
Lysine	0.90	0.63	1.20	0.96	0.86	0.78
Methionine	0.40	0.30	0.60	0.55	0.45	0.30
Methionine + cysteine	0.70	0.55	0.95	0.85	0.75	0.60
Phenylalanine			0.78	0.64	0.58	0.52
Phenylalanine + tyrosine			1.52	1.24	1.12	1.01
Threonine			0.76	0.62	0.56	0.50
Tryptophan	0.23	0.17	0.22	0.18	0.16	0.15
Valine	0.78	0.56	0.93	0.77	0.69	0.62

Table 3. Protein and amino acid requirements of Muscovy ducks (INRA, 1989)

	Starting	Growing	Finishing	
Nutrients (%)			Females	Males
Metabolisable energy (kcal/kg)	3,000	3,000	3,000	3,000
Crude protein	19.0	16.0	13.5	15.0
Arginine	1.07	0.86	0.74	0.82
Histidine	0.40	0.37	0.32	0.36
Isoleucine	0.70	0.56	0.48	0.53
Leucine	1.18	0.96	0.83	0.92
Lysine	0.91	0.76	0.65	0.72
Methionine	0.36	0.33	0.27	0.30
Methionine + cysteine	0.76	0.65	0.56	0.62
Phenylalanine + tyrosine	1.23	1.02	0.87	0.97
Threonine	0.61	0.55	0.47	0.52
Tryptophan	0.17	0.16	0.14	0.15
Valine	0.86	0.70	0.59	0.66

Starting period = 0-3 weeks for females and 0-4 weeks for males

Growing period = 3-6 weeks for females and 4-8 weeks for males

Finishing period = 6-10 weeks for females and 8-12 weeks for males

Table 4. Requirements of white Pekin ducks for minerals, vitamins and trace-elements

	NRC (1994)		Adeola (2006)			
Nutrients	0-2 weeks	2-7 weeks	0-2 weeks	0-2 weeks	2-7 weeks	2-7 weeks
Metabolisable energy (kcal/kg)	2,900	3,000	2,825	2,875	3,050	3,075
Crude protein, %	22	16	23.0	20.5	17.5	15.0
Calcium, %	0.65	0.60	1.2	1.0	0.9	0.8
Chloride, %	0.12	0.12				
Magnesium, mg	500	500				
Nonphytate phosphorus, %	0.40	0.30	0.60	0.55	0.45	0.30
Sodium, %	0.15	0.15	0.22	0.18	0.16	0.15

Table 5. Requirements of Muscovy ducks for minerals, vitamins and trace-elements (INRA, 1989)

	Starting	Growing	Finishing	
Nutrients, %			Females	Males
Metabolisable energy (kcal/kg)	3,000	3,000	3,000	3,000
Crude protein	19.0	16.0	13.5	15.0
Calcium	0.90	0.80	0.64	0.64
Nonphytate phosphorus	0.40	0.35	0.26	0.26
Sodium	0.16	0.16	0.16	0.16
Chloride	0.14	0.14	0.14	0.14

Starting period = 0-3 weeks for females and 0-4 weeks for males

Growing period = 3-6 weeks for females and 4-8 weeks for males

Finishing period = 6-10 weeks for females and 8-12 weeks for males

NUTRITIONAL DETERMINANTS FOR GUT HEALTH AND LITTER CHARACTERISTICS IN TURKEYS

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Summary

Turkey intestinal health is directly related to growth, performance, and productivity. When intestinal health is sub-optimal, we usually think of some disease or parasite as the main reason for the intestinal dis-order. In many cases this is, of course, very true. However, the relationship between nutrition and gut health can be very direct even though the cause and effect may be indirect. Understanding and appreciating underlying relationships between physiology, nutrition, and health are important but many times are over looked, under estimated, or under appreciated. One can make the argument that some intestinal disorders are not first caused by disease but are initiated by intestinal insults caused by suboptimal nutrition, suboptimal environment, or some combination of the two understanding that both of these cover a great many factors. Intestinal disorder can easily, if not usually, lead to loose feces if not diarrhea. This, in turn, can lead to wet litter which provides more direct insult to the animal through a number of pathways including pathogen production in the litter, ammonia production, breast blisters, and food pad dermatitis. The skin disorders can then lead to more insults directed to the bird. Therefore, we can start to appreciate the holistic system affecting turkeys, especially those in confinement, which is greatly dependent upon gut health.

Post Hatch

Turkey poults post hatch are very sensitive to their environment. The first hours and days post hatch are critical to proper intestinal health. The poult's intestinal tract is immature and rapidly developing. The microbiota of bacteria, protozoa, viruses, etc. are in the process of developing and are affected by the environment of both the hatchery and brooding facilities. Avoiding as much stress as practically possible while providing the environmental and nutritional resources for a good start in life are critical to the future health and productivity of any poult. Optimal incubation and hatching profiles yield a short hatch window. Limiting the amount of time in the hatcher while optimizing development produces healthier poults that can transition better to intestinal nutrition and precocial behavior.

Brooding

In order for turkey poults to get off to the best start, avoid stress, and be a good performing flock, the brooding facilities must be prepared and set up properly. When the brooding area is not properly prepared in a timely manner, we introduce unwanted and unnecessary stress to our birds leading to poor growth and performance. If conditions are not optimum during brooding, then initial growth is lost, and either the birds will not be as heavy or efficient at slaughter or days to market age will be increased. Less than optimum brooding can also lead to health problems or disease further slowing growth and increasing cost of production. When birds are placed into suboptimal environments, it is difficult to expect any type of nutritional regime to yield maximum results. Bio-security is absolutely a part of all turkey rearing activities especially brooding. While bio-security is not the topic of this discussion, its importance is fully recognized. Reviews on bio-security are available and their applications are encouraged herein.

Proper brooding conditions are critical to provide. Young poults need proper temperature, feed, water, and ventilation in a disease and pest free environment. Litter and proper litter management are also critical to the poult's welfare. Correct temperatures for our poults are critical. Turkey poults are especially sensitive to improper temperature. Poults seem to have a more difficult time in establishing their body temperature regulation process compared to chicks. They seem to perform better when they can move back and forth and into and out of different temperature zones so that they can regulate and maintain their optimal body temperature. Improper or sub-optimal brooding conditions may result in sub-optimal intestinal development. This, in turn, yields less than optimal results from even the best nutritional regime. During the brooding period, especially the first few days, it is extremely important to maximize poult physiology in order to maximize the provided nutrition.

Stress

Stress is in every life including that of poults. It is unavoidable as only in death is there absence of stress. However, too much stress in a poult's early life can be very counterproductive. Seyle (1973) defined stress as the non-specific response of the body to any demand placed upon it. Each stressor results in specific behavior and physiological

response: cold results in huddling and shivering, hot results in panting and wing drooping, etc. However, in any case of a response to any stressor, there is a general physiological response. This non-specific response has been defined as the "general adaption syndrome (GAS). This general response can be grouped into three categories: alarm, resistance, and exhaustion. The alarm stage includes the classic "fight" or "flight" response and includes immediate physiological actions such as rapid heart rate and redistribution of blood. More long term changes include actions by the glucocorticoid hormones. A cascade of events ensues including the production of corticosterone (the stress hormone). Corticosterone results in both the inhibition of protein synthesis and degradation of proteins. Therefore, in cases of stressful events and environments, we have a difficult time providing a nutritional regime that yields maximum or optimal growth. Usually, in these conditions the requirement of all nutrients is increased and the digestibility of the feed stuffs becomes more critical. Poor digestion of feed and nutrients also leads to more nutrients entering the lower gut which in turn feeds pathogenic bacteria such as some of the clostridia which we want to avoid. In this regard management directly affects the poult's holistic health which, in turn, affects our ability to provide optimal nutrition.

There are numerous reports in the literature describing the effects of stress on turkey physiology and performance (Grimes, 1995). We have conducted several trials on young turkeys simulating the effects of management events on the stress response in turkeys and subsequent performance. We have observed that simple events such as a one-time, over-night feed and water restriction can yield a short term, corticosterone defined stress response with long term reduction in performance. We also observed that this negative response to stress can be mitigated to some degree by dietary fermentation products. Therefore, while the birds were receiving the same nutrition, their response was different because of environmental factors; and, these environmental factors can be mitigated through nutritional changes or supplementations.

Feed

Feed and feed quality are especially critical during the brooding period. There are any number of reasons why young poults might refuse feed, limit their feeding, or have a negative response to their feed. Some of those reasons would include aspects of feed quality such as crumble or pellet size, the amount of fat (or lack of fat) in the feed, the amount of fines in the feed, dustiness of the feed, etc. While this article is not about feed quality per se, it is important for producers to be in tune with their flocks' eating behavior and report any problems, even subtle ones, to the appropriate personnel such as their flock supervisor, veterinarian, nutritionist, or feed mill manager. Numerous disease agents can be associated with intestinal enteritis of poultry. Therefore, any problems with feed quality or abnormal acceptance of feed by the birds should be addressed immediately. The producer should be the first to notice any such change in behavior allowing for a short response time for corrective action. In addition, growers can help their poults get off to a better start by having adequate numbers of supplemental feeders for the first week. Poults should not have to go far to feed. The supplemental feeders or trays should have feed added on a regular basis to stimulate poults to eat.

Poult diets should be simple but highly digestible. Alternative feed ingredients can be challenging to include while maintaining optimal poult performance. It may be worth considering to maintain the highest quality in the first feed even if feed costs are increased. Savings by use of alternative ingredients may be better used when the birds are older with a more mature gut and better able to deal with different feed ingredients.

Even with older birds there are ways to maintain or improve turkey performance with respect to feed ingredients. Using least cost feed formulation, we should consider the final cost of the performance. This final performance may be live weight, breast meat yield, or final product yield and should include the costs of producing this final product. All of these final products may yield, or be based on, differing "least cost" formulas which may or may not be actually least cost diets.

Soybean meal deserves special mention. It is by far the most used protein meal for poultry diets. It is a good source of protein with good amino acid balance except for methionine. When properly processed, the amino acids have good digestibility. However, soybean meal has high levels of indigestible carbohydrates (oligosaccharides) which are difficult for monogastrics to digest. The metabolizable energy level for monogastrics is low compared to corn and other grains. The natural soybean contains high levels of trypsin inhibitors which reduce the protein digestibility. These inhibitors must be heated treated to denature them. When over-heated; however, soybean meal amino acid digestibility is decreased. The phosphorus in soybean meal can also be poorly digested. Therefore, for these and other reasons, high inclusion of soybean meal, above 35%, in turkey diets can lead to increased litter moisture. Many turkey nutritionists utilize other protein sources to limit soybean meal to 35% or less in the diet. These can include animal and animal by-product meals, corn gluten meal, canola meal, limited amounts of DDGS, and other meals. Peanut meal can be good

but can also contain aflatoxin and other mycotoxins. Turkeys are very sensitive to aflatoxin; therefore, peanut meal is usually not used in turkey diets. Diets can be formulated with lower CP levels and increased levels of synthetic amino acids. This helps limit soybean meal inclusion. New varieties of soybeans contain lower levels of trypsin inhibitors, increased protein, and lower levels of oligosaccharides. As these new varieties become more available, there use in turkey diets will increase. Enzymes such as phytases, proteases, and mannanases also help increase the digestibility of soybean meal. (For reviews see Araba and Dale, 1990; Mitchell; Stein et al.; Thakur and Hurburgh, 2007)

Enzymes can be very beneficial in utilizing many types of feed ingredients, some traditional and some considered "alternative". While wheat is a common feed ingredient, it can be variable with respect to nutrient and indigestible fiber content. Research demonstrating variations in bird performance when birds are fed different wheats and the improvements in bird performance when fed enzymes to improve wheat digestibility is well represented in the literature (Crouch et al., 1997). There are various feed ingredient sources that serve as alternative protein ingredients such as shrimp meal, crab meal, low quality poultry meal, feather meal, and others. Protein enzymes are in development to make better use of these chitin based protein meals. These proteinases may also have usefulness in more traditional corn/wheat/soy diets. DDGS from various grains but mostly from corn and wheat are becoming common place in turkey diets. Enzymes, including xylanases and proteases, can be beneficial supplements in such diets. DDGS, however, can be quite variable in their quality and energy (oil) content. Direct and open communication with ingredient suppliers detailing feed ingredient quality requirements is most helpful in avoiding sub-optimal nutrient specifications and poor quality ingredients. Neither of these can be overcome by enzyme supplementation.

Mycotoxins represent a special challenge for rearing turkeys. There are ample reports in the literature on the effects of dietary mycotoxins on turkey physiology, health, and performance. The effects from naturally occurring mycotoxins may be more meaningful than from the effects of feed spiked with cultured mycotoxins. Turkeys are very sensitive to aflatoxins and probably more sensitive than realized to other mycotoxins. In our work we have observed that phyiology, growth, and performance of young turkeys are inhibited by low levels of naturally occurring mycotoxins (Grimes et al., 2010). In addition, we observed little protection was provided by "mycotoxin binders". Mycotoxins in feed will be a continuing challenge for turkey production. A persistent quality control program in which pertinent mycotoxins are continually monitored is time and effort worthy.

Antibiotics

Traditionally and historically, antibiotics have been provided to poults in the feed to help the bird through critical stages, maintain good health, and improve performance. The use of AGP became widespread for rearing livestock and poultry because of the benefits they afford to the producer, the animal, and the environment, as well as the consumer (Gadd, 1997). These feed borne antibiotic growth promoters (AGP) have been fed to livestock for about 50 years as an aid to improve growth performance (Dibner and Richards, 2005). Specific benefits of feeding AGP to poultry by producers include improved feed efficiency and increased body weight gain. Poultry have been fed AGP during the rearing period to protect them from pathogenic organisms, maintain health, improve growth efficiency and improve meat quality and wholesomeness. However, controversy over the feeding of antibiotics as growth promotants in many countries has increased because of the fears concerning the development of antibiotic resistant microbes from the use of AGP in food animals. Therefore, dietary antibiotics have come under increasing scrutiny by some scientists, consumers, activists, politicians, and bureaucrats because of the potential development of antibiotic-resistant bacteria (including pathogenic strains) in livestock and poultry. Poultry companies are now either required or more dedicated to eliminate or reduce dietary AGP depending on their location and/or customer base.

Probiotics

Although the antibiotic debate continues, there is interest and need in developing alternatives to AGP. Two candidates are prebiotocs and probiotics. In addition to the term "probiotic", the Association of American Feed Control Officials (AAFCO, 1999) and the US Food and Drug Administration (FDA, 2003) have recommended the term "direct-fed microbials" (DFM) be used to describe live culture feed additives (Elam et al., 2003). Other types of probiotics that are not live cultures have been referred to as "prebiotics" (Patterson and Burkhold, 2003). Probiotics have been developed to counter the growth depressing effects that certain strains of bacteria elicit in poultry. There are numerous reports and reviews of these supplements being fed to livestock and poultry, including turkeys (Blair et al. 2004; Damron et al. 1981; Gustafson and Bowen 1997; Fuller, 1989; Owings, 1992).

The use of DFM supplements in diets changes and stabilizes the microflora environment of the avian digestive tract (Jin et al., 1998; Knarreborg, et al., 2002; Patterson and Burkholder, 2003; Smirnov et al., 2005). There are numerous reports describing competitive exclusion including the significant reduction of intestinal levels of *Salmonella spp* in turkeys or other livestock by use of DFM (Bailey, 1987; Casas et al. 1998; Juven et al., 1991; Nurmi and Rantala, 1973; Vicente et al., 2005). In addition to colonizing the intestinal tract, the use of *L. reuteri* resulted in shorter, lighter intestines and smaller relative intestinal weight in turkeys; similar results have been observed and reported in other studies with broilers (England et al., 1996).

We have conducted several studies and reported the positive effects of dietary prebiotocs, probiotics, and DFM on poultry including turkeys (Grimes et al., 2008; Parks et al., 2001; Rahimi et al., 2008; Russell and Grimes, 2009). Using these supplements, we have observed improved poult performance, improved performance to market age, and reductions in bacterial intestinal colonizations of birds induced by gavage. One study included both university and field trials. In the field, the water borne probiotic resulted in more weight harvested from the farm representing improved efficiency of production. Therefore, it is apparent that DFM, probiotic organisms, and prebiotics can improve the nutrition of poultry without necessarily changing the nutritional makeup of the feed.

Water

Water is the least appreciated nutrient. Turkeys should be provided with as clean and fresh water supply as possible. Poor quality water laced with bacteria and protozoa can undermine any management and nutritional regimen. This is especially true for the poults during the brooding period when poults are most sensitive to intestinal insults. Cleanliness of water equipment, including water lines, is a major component of providing clean water. Cleaning and sanitation of all water equipment should be accomplished before the poults arrive on the farm. During cold weather it is also important that water lines and extra poult waterers be filled the day before arrival so that the birds do not have to drink cold water. Water samples should be collected periodically to assess the water for contaminants and high mineral levels. Nipple drinkers are becoming popular for brooding turkeys. However, it is advised that only nipple drinkers designed for and tested with turkey poults be used to brood turkeys. Some broiler nipple drinkers will not support proper poult growth and this growth may not be recovered after switching to adult or rearing type drinkers (Russell et al. 2009). In addition, some of the broiler type nipple drinkers may be high flow systems which might lead to wetter litter which is not desirable (Quilumba et al. 2015).

Litter Moisture

As is the case with any poultry flock reared on litter, producers should monitor litter quality closely so that when conditions change, managerial changes can be put into place. At thermo-neutral temperatures, birds consume twice as water by weight as they do feed. Most (80%) of this water leaves the bird and enters the house either through respired air or by feces. Only about 20% is retained in the bird. Furthermore, when the temperature is increased 10 C (20 F), feed intake decreases while water intake can double. Most turkey houses are equipped with water meters – are we paying attention to these meters and the bird's water usage? Even without a meter we can do some math. A flock of 5000 tom turkeys at 20 kg/tom with a FCR of 2.5 equates to 50 kg of feed and 100 kg of water per bird under ideal conditions. This is about 100 L of water per bird or 500,000 L (125,000 gal) of water into the turkeys with 400,000 L (100,000 gal) into the house itself. That is a lot of water! While many turkey producers till their litter, the only way to really handle this water is through forced air ventilation. Some brooder facilities are equipped with forced air systems; however, many, if not most, grow-out houses are not. This places turkey facilities at a disadvantage compared to broiler houses. This is further disadvantaged when turkey houses are cooled using foggers. Much of the water used to cool the air actually ends up in the litter. Wet litter is directly associated with foot pad dermatitis. Most turkeys, especially toms, by market age have foot pad dermatitis to some degree (da Costa et al. 2014; Liesner, 2007). Many of these cases are severe. Litter under turkeys reared in houses equipped with tunnel ventilation with cool cell pads seem to have less foot pad dermatitis. This is advantageous for bird welfare. It also reduces insults to the birds' skin thereby reducing potentially reducing pathogen entry into the bird. Healthier birds have better intestinal health and utilize their nutrition better.

Changes in water usage can be, and usually are, noticed ahead of any feed use changes. We can take advantage of this physiology and begin intervention strategies before the situation deteriorates further. As the poults grow, their air and oxygen requirements grow accordingly, if not exponentially. The birds are producing more manure and the litter can get wetter producing the perfect conditions for increased ammonia production. During cold weather, poult barns may be closed up tight, as they should be. However, just because there is no, or little, ammonia being produced in the litter does not mean there is no need for air exchange. Brooders and poults share one requirement and that is

oxygen. As the brooders and poults use up the oxygen in the house, moisture and carbon dioxide are produced. In some cases, carbon monoxide is also produced, especially with poorly working heaters. None of these are desired in the poult barn. Increased air moisture may lead to increased litter moisture. Increased litter moisture will eventually lead to more ammonia production and foot pad dermatitis. In addition, many turkey veterinarians, turkey health researchers, managers, service people, and producers have noticed and associated increased litter moisture in the turkey brooder barn with increased turkey health issues such as poult enteritis and other diseases. This increase in brooder house litter moisture has been observed in the U.S. especially during spring when weather conditions are changing from cool and dry to warm and humid. As stated above, many turkey poult barns are not equipped with the ventilation systems found in broiler houses and in many cases, even when systems are in place, are not used as optimally as they could be. Most turkey barns are also not equipped with vents, which when present can aid in proper are movement and mixing. It has been mentioned in a number of reports that commercial turkeys are not maximizing their genetic potential. Ventilation systems in turkey facilities, both brooding and growing, should be closely examined to align optimal ventilation with maximum bird performance.

Conclusion

While much is studied and written about turkey nutrition, much of this subject is viewed from the reverse perspective. Of course we want optimal nutritional regimens provided to our birds. However, only through optimal intestinal health can any nutritional package provided to the bird be fully appreciated and utilized. Certainly veterinarian care is critical to our cause. However, turkey intestinal health and overall turkey health and welfare can only be fully maximized when we provide the best environment and daily care for our birds. This the first and most important element of turkey production. Unfortunately, this will not always guarantee success as we have seen lately and historically with severe disease outbreaks. However, this is where we must begin our journey in successful turkey production.

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DIFFERENCES IN AMINO ACID AND PHOSPHORUS DIGESTIBILITY BETWEEN BROILER CHICKENS, TURKEYS AND DUCKS

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Summary

Digestibility of amino acids and phosphorus became highly relevant in feed formulation for poultry. In recent years, digestibility measurements were increasingly done using growing birds and on the precaecal level. More data are available for broiler chickens than turkeys and ducks. Literature evaluated in this contribution does not support the assumption of broiler chicken digestibility data being applicable in feed formulation for turkeys and ducks. Species differences in amino acid digestibility appeared to be dependent on the type of feedstuff investigated. Species differences in phosphorus digestibility were hypothesised to be mainly related to differences in gastrointestinal phytate breakdown. However, conclusions from the comparisons could be drawn only with care because the database is small and differences in assay conditions applied in different institutions affect the studies outcome. Science and industry joint research activities should be initiated to fill the knowledge gap and better understand species-specific metabolism and digestibility. This will support achieving more precise feed formulation through avoidance of safety margins which are needed more when the database of raw material digestibility is less precise.

Introduction

Different raw materials contribute to the energy and nutrient supply of birds to a variable extent. Chemical analysis is the primary step of characterization of raw materials. However, characteristics other than those chemically analysed contribute to the "value" that a raw material has when considered in diet formulation. Determination of digestibility in animal studies thus is a very important tool to characterize the feeding value with the target animal species.

Determination of digestibility is, in its simplest definition, the "balance of the digestive tract". However, in order to make digestibility values comparable in feeding systems and tables, certain standards in the trial protocols need to be agreed on. Such standards must consider the metabolic situation and adaptation of an animal. Therefore, they are specific for individual nutrients or fractions of the feed, meaning that a "digestibility of a feed" does not exist. This leads to considerable effort being necessary before digestibility values can be implemented in practical feed formulation and explains why the consideration of digestibility is primarily aimed for nutrients of high economic or environmental relevance. Next to energy, which will not be considered in this contribution because addressed in another session, amino acids (AA) and phosphorus (P) received most attention in recent years in digestibility studies.

In poultry, the distinct species focus of digestibility studies has been on the domestic fowl using different types of rooster assays and, more recently, broiler chickens. Feed formulation for ducks and turkeys can also benefit from consideration of digestibility values, but data are more scare than for broilers. As differences in anatomic and physiological characteristics of the digestive tract between the species are known, nutritionist are interested in whether data obtained with broiler chickens can be used to fill the gap that exists in duck and turkey nutrition in regard to digestibility values. Hence the objective of this presentation is to compare on the basis of the scientific literature and to draw conclusions for both the scientific community and the feed industry.

Amino acid digestibility

Different assays were used in the past to study AA digestibility. Many studies involved differently standardized rooster assays and excreta collection. Other approaches used broiler chickens as the target category to feed, and more recent studies with broiler chickens in the vast majority had a special focus on precaecal (or ileal) AA digestibility. This technique implies collection of digesta from the ileum rather than excreta collection. Both approaches (rooster-based and broiler-based) found their entry in commercially driven databases and Tahir and Pesti (2012) described differences in the relative ranking of groups of feedstuffs between the two databases. This can be taken as a descriptive example of the relevance that assay conditions have in AA digestibility studies. In broiler studies of precaecal AA digestibility specific assay conditions (section of ileum sampled, procedure of asphyxiation, age of birds, level of feed intake, etc.) and consideration of basal endogenous AA flow in the ileum are also relevant for the determined digestibility values (Kluth and Rodehutscord, 2009). Taking all this into consideration unravels some difficulty when evaluating species differences in AA digestibility based on literature. This is because possibly existing species differences can be masked by

differences in assay conditions used in different studies. In consequence, this contribution will focus on the (few) studies that made comparisons using the same assays conditions for the species considered and will focus on precaecal digestibility studied in growing birds.

COMPARISONS OF TURKEYS AND BROILER CHICKENS

When viewed on a high level of integration of the available literature, the first indication is that precaecal Lys and Met digestibility is very similar in turkeys and broiler chickens (Figure 1) (these AA will be exemplarily focused on in this contribution). However, the range of values was very high. This range was to a large extent caused by the types of feed that were used in the studies. However, against the impression given with Fig. 1, differences between turkeys and broiler chickens were found in some studies and differences were not always in the same direction. Kluth and Rodehutscord (2006) used the regression approach and found that precaecal digestibility of Lys and Met was not different between turkeys and broiler chickens for soybean meal, but lower in turkeys than broiler chickens for rapeseed meal. A similar difference between turkeys and broiler chickens was found by Jia et al. (2012) who used different qualities of rapeseed in studying apparent AA digestibility. In diets that included 70% wheat-DDGS Lys digestibility was around zero and Met digestibility was substantially lower in turkeys than broiler chickens, irrespective of whether a protease supplement was included or not (Adebiyi and Olukosi, 2015b). Extensive comparative work was also done by the colleagues at Purdue University and their collaborators. While Lys digestibility of maize was lower in turkeys than broiler chickens at 5 days of age, Lys digestibility of different types of DDGS, canola meal, and soybean meal was similar or higher in turkeys than broilers (Adedokun et al., 2008). In this study similar trends were seen when turkeys and broilers were 21 days old. When investigating AA digestibility of meat and bone meal, Lys and Met digestibility was either higher or lower in broiler chickens than turkeys, depending on the source of meat and bone meal used (Adedokun et al., 2007b).

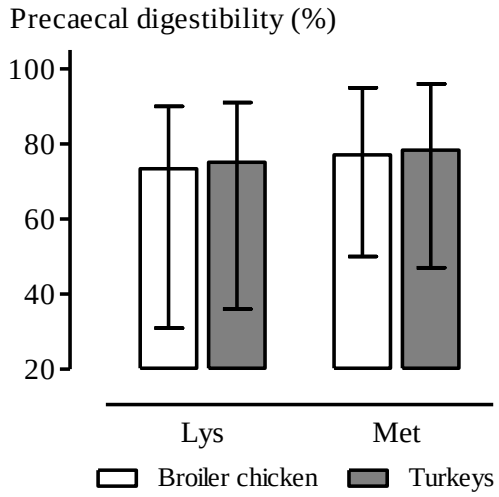

Figure 1. Mean and range of Lys and Met digestibility for different raw materials and diets as investigated in different studies with broiler chickens and turkeys (25 obser-vations) using similar methods (Adebiyi and Olukosi, 2015b, Adedokun et al., 2007b, Adedokun et al., 2008, Jia et al., 2012, Kluth and Rodehutscord, 2006)

It has been debated whether differences in the level of ileal endogenous AA flow between poultry species may be a reason for differences seen in apparent AA digestibility values in some studies. However, species differences remain to persist, at least in part, when digestibility is determined by regression analysis, thus eliminating the effect of basal endogenous AA flow (Kluth and Rodehutscord, 2006).

Knowledge about the ileal flow of endogenous AA becomes relevant when the regression approach is not applied but apparent digestibility values are transferred to standardised values. Some studies systematically compared the ileal flow of endogenous AA between broiler chickens and turkeys. Ileal flow of endogenous AA as studied by using a N free diet and expressed in relation to dry matter intake was higher in turkeys than broilers at the age of 5, 15 and 21 days, the difference being statistically significant only at 5 days of age (Adedokun et al., 2007a) (Table 1). In contrast, ileal flow of endogenous essential AA at the age of 4 weeks was on average one third higher in broilers than turkeys (Adebiyi and Olukosi, 2015b). Adedokun et al. (2008) compared AA digestibility in broilers and turkeys in the age of 5 and 21 days. They also measured endogenous AA flow and concluded that age-specific values for endogenous AA flow should be considered when apparent digestibility values are corrected. Differences in the ileal flow of endogenous AA reported here did not contribute to the range of values shown in Fig. 1 because values were expressed on a standardized basis when possible.

Palander et al. (2006) found significantly lower AA digestibility in 10-wk old turkeys compared to 5-wk old when using diets that contained 50% of peas, faba beans or lupins. In another study of this group using diets containing rapeseed and soybean products, crude protein digestibility was also reduced when turkeys were 12 wk old compared to 4 wk or 8 wk (Palander et al., 2004). The authors hypothesised this age effect to be caused by changes in passage rate and endogenous secretions becoming more relevant with age (Palander et al., 2006). They also suggested, in reference to chicken data reported by Gonzalez and Vinardell (1993) and Gonzalez et al. (1996) that intestinal AA transport systems may become less active with increase in age. The quantitative relevance of such differences seen in processes and mechanisms however still needs to be elucidated before it can be better evaluated whether or not they affect digestibility.

Table 1: Estimates of ileal endogenous amino acid flow based on the use of N free diets (mg/kg of dry matter intake)

	Age (days)							
	5[1)]		15		21		28[2)]	
	Broiler	Turkey	Broiler	Turkey	Broiler	Turkey	Broiler	Turkey
Lysine	522	1168	209	276	182	287	308	216
Methionine	153	391	56	86	44	85	91	121
Reference	Adedokun et al. (2007a)						Adebiyi and Olukosi (2015b)	
	Age (days)							
	19[3)]		26[3)]					
	Broiler	Duck	Broiler	Duck				
Lysine	374	965	382	1402				
Methionine	86	333	100	413				
Reference	Kong and Adeola (2013b)		Kong and Adeola (2013a)					

[1)] Differences between species were statistically significant at 5 days of age.
[2)] Statistical analysis of species difference was not reported.
[3)] Differences between species were statistically significant.

COMPARISON OF DUCKS AND BROILER CHICKENS

Pioneering comparative work on waterfowl and broiler chickens was done by Dorota Jamroz and her colleagues. When feeding mixed diets based on barley, wheat and soybean meal, digestibilities were different between chicken and waterfowl (Jamroz et al., 2001, Jamroz et al., 2002). Mean digestibility for all AA at the age of 14, 28 and 42 days was 70, 73, and 73% in chickens, respectively, and 44, 62 and 60% in ducks (Jamroz et al., 2002). As mentioned before, differences in the level of endogenous AA losses between species may have contributed to these differences. This was a motivation to use the regression approach for further comparative work (Kluth and Rodehutscord, 2006). In their study, the mean AA digestibility of soybean meal and rapeseed meal was investigated in 3-wk old birds and it also was lower in ducks (72% for soybean meal and 68% for rapeseed meal) than broiler chickens (79% for soybean meal and 80% for rapeseed meal). Kluth and Rodehutscord (2006) thus concluded that basal endogenous AA losses were not the primary reason for differences found between ducks and broiler chickens in earlier studies. It is important to mention that in another study on soybean meal and canola meal apparent digestibility values were slightly lower in ducks than broiler chickens at 19 days of age (Kong and Adeola, 2013b). However, following regression analysis of the data thus excluding differences in basal endogenous losses significant species differences no longer existed for most of the AA studied. In both studies (Kluth and Rodehutscord, 2006, Kong and Adeola, 2013b) and irrespective of the level of digestibility the ranking of individual AA digestibility was similar in ducks and broiler chickens.

Estimates of ileal flow of endogenous AA when using a N free diet were much higher in ducks than broilers (on average approximately threefold to fourfold higher) at the age of 19 or 26 days (Kong and Adeola, 2013b, Kong and Adeola, 2013a) (Table 1).

The studies by Jamroz et al. (2004) indicate that differences in the development of the digestive tract might be a partial cause of species differences in digestibility. They found that the relative length of the small intestine (in relation to $BW^{0.67}$) was higher in broiler chickens than in ducks. Similarly, estimates made from Figure 2 and Table 2 of Applegate

et al. (2005) indicate that the length of jejunum + ileum in relation to BW, measured 7 days posthatch, is greater in turkeys than in ducks.

CONCLUSIONS FOR AMINO ACIDS

Based on current scientific evidence, no clear statement can be made regarding species differences. There are indications that precaecal AA digestibility can be lower in turkeys than broiler chickens and again lower in ducks. However, literature is not consistent regarding these differences and likewise the magnitude of difference depends on the feed raw material. However, comparisons between species so far can only be made on a small database because only few studies were performed under *ceteris paribus* conditions. Both the scientific community and industry are encouraged to consider this a challenge. Feeding costs are affected by safety margins needed as long as uncertainties about digestibility values exist. Scientists are encouraged to clarify possible reasons for species differences and to continue in standardising assay conditions. Only on the basis of a standardized methodology all the data that are urgently needed can be generated and used to compile feeding tables.

Phosphorus digestibility

The digestibility of P is affected by different factors related to both the feed and the bird's physiology. In plant-based raw materials, most of the P is contained as phytic acid (*myo*-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate); $InsP_6$) and phytate (any salt of $InsP_6$), but the proportion is variable in different raw materials. Plant seeds also differ in intrinsic phytase activity (Eeckhout and De Paepe, 1994). Mineral feed phosphates and animal by-products differ in their solubility. All these differences affect the availability of P. The animals have physiological mechanisms that allow an adaptation to different levels of P supply and this leads, in contrast to what has been mentioned above for AA, to the need for specification of upper levels of P and optimal Ca:P ratio in diets used in P availability studies. This, together with the specification of other assay details, is considered in the standard protocol for the determination of P availability published by the Working Group 2 -Nutrition- of the European Federation (WPSA, 2013). This protocol defines precaecal P digestibility as the criterion to measure. It was also mentioned that P retention equals precaecal P digestibility under the condition of marginal P supply because in such condition the urinary loss of P is negligibly low (Rodehutscord et al., 2012). The complexity of feed- and animal-related factors influencing P metabolism explains why, as for AA digestibility, a comparison of P digestibility between poultry species can only be made when experimental conditions are well standardised.

When using a low-P basal diet and further diets containing graded levels monobasic calcium phosphate, P utilisation of the basal diet was higher in broiler chickens than turkeys and ducks, but utilisation of supplemented mineral P was highest in ducks, followed by turkeys and broiler chickens, respectively (Rodehutscord and Dieckmann, 2005). In other experiments that investigated the utilisation of different feed phosphates for Pekin ducks values also were very high (Wendt and Rodehutscord, 2004). Adebiyi and Olukosi (2015a) studied precaecal P digestibility and P retention of wheat-DDGS using the regression approach. Estimated digestibility and retention values were very high in broiler chickens (94 and 92%) and were lower in turkeys (76 and 71%).

Differences in $InsP_6$ degradation in the digestive tract and release of phosphate may have contributed to the differences between species. Endogenous mucosal phytase activity was detected in the small intestine of broiler chickens (Huber et al., 2015, Maenz and Classen, 1998) and it cannot be ruled out that this is different in turkeys or ducks, thus leading to differences in phosphate release from $InsP_6$. However, gastrointestinal $InsP_6$ hydrolysis still is a black box in regard to the origin of contributing enzymes and likewise phytase producing microbes resident in the digestive tract are involved (Zeller et al., 2015a). The crop plays some role in microbial enzyme production in broiler chickens and it may be speculated whether the absence of a fully functioning crop in ducks is a reason for lower P utilisation in ducks compared to broiler chickens.

Hydrolysis of $InsP_6$ is also relevant when the digestibility of mineral or animal P sources is determined. This can only be done when the P source under test is supplemented to a low-P basal diet and the basal diet often contains $InsP_6$ if it is not a purified diet. Supplements of Ca and/or P sources, however, can reduce intestinal degradation of $InsP_6$ thus leading to interactions between $InsP_6$ breakdown, mineral supplements and phytase supplements in the digestive tract of broiler chickens (Zeller et al., 2015b, Tamim and Angel, 2003). These interactions can cause a considerable underestimation of digestibility of a mineral P source (Shastak et al., 2014). If $InsP_6$ hydrolysis is lower in ducks than broiler chickens this might mean that the diminishing effects of mineral P supplements on $InsP_6$ hydrolysis are less pronounced in ducks than broiler chickens. Such effects may be suitable to explain the differences in calculated digestibility

of mineral P sources between poultry species. However, the hypotheses presented here still need to be tested in experiments that involve measurements of $InsP_6$ degradation, mucosal phytase activity and microbiota composition in different sections of the gastrointestinal tract.

Conclusions

Differences exist in the digestibility of amino acids and phosphorus between growing broiler chickens, turkeys and ducks. The database for the comparison of species is small because the results of digestibility studies are influenced by assay conditions and methodological details, especially when measured on the precaecal level. In regard to amino acid digestibility, species differences appear to depend on the type of feed studied. In regard to phosphorus digestibility, species differences are probably caused mainly by differences in gastrointestinal $InsP_6$ breakdown. Among the three species, most data are available for broiler chickens and databases including digestibility values already exist for broiler chickens. If these data are used for formulating diets for turkeys and ducks, this should be done with care and using safety margins unless species differences are better understood and quantified. Research is needed in different regard: better standardisation of assay protocols; increasing linkage of quantitative digestibility data with functional studies on tissue, metabolism and microbiota level; poultry species comparisons using *ceteris paribus* experimental conditions. The applied nature and economic relevance of this research should make the industry feel invited contributing to the research questions to be answered.

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SHORT ORAL PRESENTATIONS

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PHYTATE DEGRADATION IN BROILERS AS AFFECTED BY DIETARY PHYTATE AND NON-PHYTATE PHOSPHORUS CONTENT

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Abstract

Broilers have the intrinsic capacity to degrade dietary phytate, thereby increasing precaecal phosphorus digestibility (pcdP). Insight in the extent and mechanism of phytate-P (PP) degradation is necessary to evaluate the contribution to the P-supply of the birds. An experiment was performed to determine the interaction of dietary PP and non-phytate P (nPP) content on pcdP in broilers, using the WPSA (2013) protocol for determination of P availability in poultry. A total of 756 day-old Ross 308 male broilers were obtained from a commercial hatchery and at d14 randomly allocated to 72 floor pens (0.96 m^2). All birds received a commercial starter diet between d0 and 14. Experimental diets were provided from d14 onwards, and ileal digesta were collected on d23 and 24. Phosphorus (P)In total 12 different diets were provided, varying in dietary PP and nPP content. In low PP diets, pcdP of monocalcium phosphate (MCP) was 88% and remained constant over a large range of dietary P contents (2-6 g/kg). In soybean meal (SBM) based high PP diets, pcdP of MCP was 47% over a large range of inclusion levels (total P 3-7 g/kg). In SBM-based high PP diets, PP degradation linearly decreased from 96 to 68% with increasing dietary nPP content. It was concluded that broilers are highly capable to regulate pcdP by increasing PP degradation under conditions of low nPP supply. These findings have to be taken into account in studies that determine pcdP of dietary ingredients.

Keywords: poultry, nutrition, broiler, phosphorus, phytate degradation

Introduction

Among other minerals, phosphorus (P) has been extensively investigated as an essential nutrient in poultry diets, and its major role in chick's growth performance and bone mineralisation cannot be over-emphasized (Underwood and Suttle, 1999). The availability of P in feedstuffs of plant origin is of considerable importance. Poultry diets consist primarily of feedstuffs of plant origin, and a large but variable portion of P in these products is found in phytate (PP) form, which is usually considered to be unavailable for birds (Van Der Klis and Versteegh, 1999). There is, however, some evidence in literature that broilers, when fed P deficient diets, have the intrinsic capacity to partly degrade PP (Marounek et al., 2010), thereby increasing precaecal P digestibility (pcdP). The level of PP degradation depends among others on the dietary available P and the PP content (Van Der Klis and Blok, 1997). The aim of the current study was to investigate the extent and mechanism of PP degradation, which would be of importance for application in protocols for determination of pcdP of feed ingredients.

Material and Methods

An experiment was performed to determine the interaction of dietary PP and non-phytate P (nPP) content on pcdP in broilers using the WPSA (2013) protocol for determination of P availability in poultry. A total of 756 day-old Ross 308 male broilers were obtained from a commercial hatchery and at d14 randomly allocated to 72 floor pens (0.96 m^2). All birds received a commercial starter diet between d0 and 14. Experimental diets were provided from d14 onwards, and ileal digesta was collected on d23 and 24. In total 12 different diets were provided, varying in dietary PP and nPP content. The results of 9 out of the 12 tested diets were included here. To determine pcdP in low PP diets, a semi-synthetic basal diet, low in total P (2.1 g/kg) and PP (0.85 g/kg) was supplemented with 3 graded levels of MCP, resulting in a total P content of 8.6 g/kg in the 4th diet. To determine pcdP and ileal PP degradation in high PP diets, a high PP basal diet containing 40% of soybean meal (SBM) (P = 3.15 g/kg, PP = 1.5 g/kg) was supplemented with 2 levels of MCP, resulting in a total P content of 7.3 g/kg in the 3rd diet. In two more treatments, the high PP basal diet was supplemented with 11% and 22% SBM as PP source, resulting in total P content of 4.7 g/kg and a PP diet of 2.2 g/kg in the 3rd diet. All experimental diets were free of phytase and contained SBM as the only PP source. Titanium oxide was used as indigestible marker and pcdP was determined as the slope of the relationship between total and digestible P content.

At d23 and 24, birds were weighed and thereafter killed with ± 0.5 ml of T61 via the wing artery. A pooled sample of the ileal digesta from the posterior 1/3 (10-15 cm) of the part between Meckel's diverticulum and 2 cm before the junction to the caeca of the birds was collected. The ileal content was collected by flushing the gut segment with distilled water without squeezing the gut. After the collection, the samples were immediately frozen and stored at -20°C until further analysis. Both the experimental diets and the digesta samples were analysed for dry matter, Ca, P, marker (titanium oxide) and PP.

Results

In low PP diets, the pcdP of MCP was constant (88.0% ± 0.05) over a large range of dietary P content (2.1 to 6.0 g P per kg), whereas from 6.0 to 8.6 g/kg, pcdP of MCP was estimated as 15.7% (± 0.07). The pcdP of 88% was in line with the table value (85%) of CVB (2011).

Addition of MCP to a PP rich basal diet resulted over the tested range of 3.2 to 7.3 g P per kg in a constant (46.8% ± 0.02) pcdP of MCP, which was significantly lower than 88% as determined in the low PP diets. These findings indicate an interaction between the dietary PP content diet (0.85 vs. 1.50 g/kg) and the pcdP of MCP, which may be related to the level of PP degradation. PP degradation in the birds fed the basal diet was almost complete (96.9%), but significantly reduced in birds fed the diets containing 5.3 or 7.3 g/kg P by adding MCP (82.6 and 68.2%, respectively). The birds regulated the extend of PP degradation in dependence of the amount of nPP supplemented via MCP. The expected increase in digestible P with increasing MCP levels is partly negated by the decrease in PP degradation, thus reducing the estimate of pcdP.

Adding SBM as PP rich ingredient to the high PP basal diet resulted in a small decrease in PP degradation at the highest SBM inclusion compared to the basal diet (from 96.9 to 91.3%). As a consequence, pcdP of SBM decreased with an increasing SBM inclusion level, indicating an interaction between the dietary P and/or PP content and the level of PP degradation Over the whole test range, in which the dietary SBM inclusion level varied from 40 to 62%, pcdP of SBM was 42.4%, which is in line with the table value (41.5%) of CVB (2011).

PP degradation in the high PP diets, containing at least 40% of SBM as only PP source, showed a wide variation, ranging from 68.2 to 96.9%. As shown in Figure 1. a significant linear relationship was observed between the dietary nPP content and the PP degradation, thereby explaining 84% of the variation in PP degradation. In the tested nPP range in this experiment (1.5 – 5.5 g/kg), the level of PP degradation decreased with 7.4% for each g increase in dietary nPP content. Based on these findings it is suggested that broilers degrade more PP if less easily absorbable nPP is available in gut.

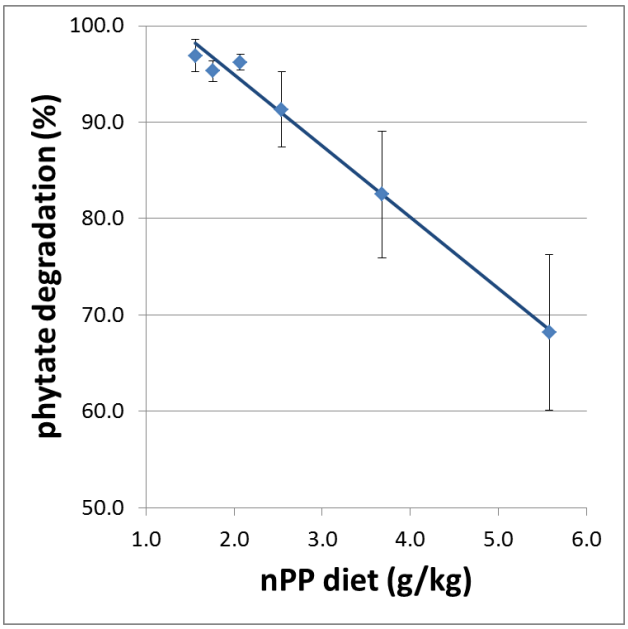

Figure 1 Relationship between the dietary nPP content and the level of PP degradation

Conclusions

In low PP diets, estimated pcdP of MCP was constant at a high level (88.0%) over a large range of dietary P content from MCP. Therefore, the P content of a low PP diet seems not very critical for testing pcdP of feed phosphates. In PP rich diets, pcdP of MCP was constant at a low level (46.8%) over a large range of MCP inclusion level. A significant linear relationship was derived between the dietary nPP content and the level of PP degradation. In the tested nPP range in this experiment (1.5 – 5.5 g/kg), the PP degradation decreased with 7.4% for each g increase in dietary nPP content. Based on the findings of this experiment, it is recommended to use low PP diets to determine P absorbability of feed phosphates, whereas the dietary P content may not be very critical in such studies. Moreover, low nPP diets should be used for testing P absorbability of PP rich ingredients.

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ID 41

GUANIDINOACETIC ACID AS AN ADDITIVE TO BROILER DIETS

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Abstract

Guanidinoacetic acid (GAA) is the biochemical precursor of creatine, which, in its phosphorylated form, plays an important role as a high-energy carrier in the muscle. An experiment was conducted to determine the response of broiler chickens to GAA added to diets with different energy levels during fattening to 35 days of age. Ross 308 male chicks were allotted to 10 treatments, each consisting of six replicates of 140 birds/pen. Five maize-soyabean meal isonitrogenous diets with decreasing AME_n levels (100, 99, 98, 97 and 96 % of requirement) with or without supplements of 0.6 g/kg GAA were formulated. The criteria of response were feed intake, body weight gains, feed conversion ratio and carcass, breast meat, leg meat and abdominal fat yields. Supplementation of broiler diets with 0.06 % GAA resulted in a significant ($P<0.05$) decrease in voluntary feed intake. With decreasing AME_n level, voluntary feed intake and consequently amino acid intakes increased which was manifested by improved growth performance ($P<0.01$). GAA supplements significantly ($P<0.001$) improved feed conversion ratio and efficiency of AME_n utilization and significantly ($P<0.01$) increased breast meat yield. With decreasing AME_n level, the effects of GAA supplementation tended to diminish.

Introduction

Guanidinoacetic acid is formed from the amino acids glycine and arginine in the kidney or absorbed from the gut. It is transformed to creatine in the liver. Phosphocreatine serves as a dynamic reservoir of high energy phosphate. The phosphocreatine/creatine system buffers ATP/ADP ratio for all energy consuming functions of the cell. The degradation of creatine results in creatinine, which is excreted in the urine. Therefore creatine must be continually replaced from dietary sources or synthesized *de novo* from GAA. In contrast to animal proteins, creatine is not found in plant feedstuffs and may thus be deficient in all-vegetable diets. GAA as a creatine source is more stable and less expensive than creatine itself (BAKER, 2009).

The objective of the present experiment was to determine the response of broiler chickens to GAA (trade name CreAMINO®, Evonik Industries) added to all-vegetable diets ranging in AME_n levels from 100 % to 96 % of requirement.

Materials and Methods

The experiment was conducted at the International Poultry Testing Station Ústrašice. A total of 8400 one-day-old male Ross 308 broiler chicks were randomly assigned to 10 dietary treatments. There were six replicates per treatment (140 chicks per pen). On days 10, 24 and 35, the chickens were weighed individually. At the same time, feed consumption per pen was recorded. On the last day of experiment, ten birds of each pen were slaughtered and carcass, breast meat (boneless and without skin), leg meat (without bone, with skin) and abdominal fat yields were determined.

Maize-soyabean meal starter (d 1 to 10), grower (d 11 to 24) and finisher (d 25 to 35) basal diets with different levels of metabolizable energy (100, 99, 98, 97 and 96 % of AME_n requirement as suggested by Ross Nutrition Supplement, 2009) were formulated. To the basal diets serving as controls (Treatments 1 to 5), 0.6 g/kg of CreAMINO® containing a minimum of 96 % GAA was added thus forming five experimental diets (Treatments 6 to 10). The diets for each growing period were formulated to be isonitrogenous. Crumbled starter and pelleted grower and finisher diets were supplied *ad libitum*.

Results and Discussion

The response of broiler chickens to guanidinoacetic acid is summarized in Table 1. As dietary AME_n density decreased, feed intake in both control and GAA-treated chickens increased.

Table 1. Response of broiler chickens to guanidinoacetic acid

Treatment		AME_n level	Feed intake (g/day)	Weight gain (g)	Feed conversion ratio	Energy efficiency (kJ AME_n/g WG)	Breast meat yield (% LW)
Control	T1	100	98.72 [a]	2055 [a]	1.692 [a]	22.31 [a]	19.84 [a]
	T2	99	98.70 [a]	2099 [ab]	1.654 [ab]	21.64 [ab]	20.23 [bc]
	T3	98	100.12 [a]	2125 [bc]	1.658 [ab]	21.46 [bcd]	20.47 [de]
	T4	97	99.25 [a]	2135 [bc]	1.640 [ab]	20.97 [bcde]	20.67 [ef]
	T5	96	99.45 [a]	2129 [bc]	1.652 [ab]	20.86 [cde]	20.73 [ef]
Guanidino-acetic acid	T6	100	96.90 [a]	2088 [ab]	1.630 [c]	21.55 [bc]	20.48 [b]
	T7	99	97.22 [a]	2116 [bc]	1.622 [c]	21.13 [bcde]	20.78 [cd]
	T8	98	98.03 [a]	2132 [bc]	1.618 [c]	20.95 [bcde]	20.61 [de]
	T9	97	99.60 [a]	2157 [c]	1.624 [c]	20.83 [de]	20.93 [ef]
	T10	96	97.27 [a]	2121 [bc]	1.617 [c]	20.48 [e]	20.69 [f]
Pooled SEM			0.890	18.4	0.0120	0.153	0.243
Significance A[1)]			0.012	0.218	<0.001	<0.001	0.003
Significance B[1)]			0.288	0.002	0.122	<0.001	0.002

[a,b] Means within a column not sharing a common superscript were significantly different. Tukey HSD test, P<0.05

[1)]A = GAA vs. control; B = AME_n levels

This response has been frequently observed in *ad libitum*-fed chickens (LEESON *et al.*, 1996). Except for the starting period, the effect of AME_n level on feed intake in the present experiment was rather small and insignificant, possibly due to a narrow range of dietary AME_n concentrations studied. On the other hand, the multiple linear regression analysis relating feed intake (FI, g/d) to AME_n level (MEL, %) and AME_n intake (MEI, kJ/d) for the whole experimental period yielded the following regression equation:

$FI = 98.43 – 1.0038\ MEL + 0.0769\ MEI$; $P<0.001$, $R^2 = 0.999$

All the model parameters were highly significant (P<0.001) and the R-squared statistic indicated that there was a close relationship between the variables. This finding suggests that the chickens were able, at least partly, to adjust their feed intake to meet their AME_n needs. Furthermore, the GAA-treated chickens consumed less feed than their control counterparts, the differences being significant in all growing periods except for the first one. This response might have been due to the better energy utilization in chickens receiving the GAA-supplemented diets or a negative effect of GAA on voluntary feed intake. A similar effect was reported by MOUSAVI *et al.* (2013), even though the differences between GAA-treated and control groups were not significant (P = 0.09) in their study. GAA supplements also reduced feed intake in turkeys (LEMME *et al.,* 2010). In contrast, MICHIELS *et al.* (2012) found slightly higher feed intakes in chickens receiving supplements of 0.6 or 1.2 g/kg GAA as compared with the control birds. No effect of GAA on feed intake of broilers was observed by RINGEL *et al.* (2007).

The effect of GAA supplementation on body weights or weight gains was small and, except for the finishing period, the differences as compared to the control group were not significant. A greater response to GAA was observed at higher AME_n levels. Growth rate increased quadratically with decreasing AME_n level and reached maximum around 97 % of

AME_n requirement. Better growth performance at lower AME_n intakes may be explained by higher feed consumption and consequently by higher intake of protein, amino acids and other nutrients. The response of control chickens to AME_n level tended to be greater than that of GAA-treated chickens, but the difference between the linear slopes fitted to experimental data was not significant (P = 0.35).

Feed conversion ratio was not significantly affected by dietary AME_n level, but it responded positively to GAA supplementation. Significant differences were found in the finishing period and for the whole experiment, but numerical improvements were also observed in the starting and growing periods. This is in line with the report by MICHIELS *et al.* (2012) who concluded that the GAA effect was most apparent in the finisher period, when weight gains were maximized. There are other studies demonstrating a positive effect of GAA on feed conversion (LEMME *et al.,* 2007, RINGEL *et al.,* 2008). Similarly to growth rate, the effect of GAA on FCR diminished with decreasing AME_n level, but the slope of regression line was significantly different from zero only in the control group. In contrast to FCR, efficiency of AME_n conversion was significantly improved by both GAA supplementation and AME_n level. The effect was most apparent in the finishing period and at the highest AME_n intake (Figure 1). In both groups, energy utilization significantly improved with decreasing AME_n level (and consequently increasing feed and amino acid intakes). These results suggest that the ratio of amino acids to AME_n in the present diets might have been underestimated.

Of the carcass analysis parameters, only breast meat yield was significantly affected by GAA supplementation. However, the magnitude of response was small. On average, the difference between control and GAA-treated groups was 0.4 % (20.4 vs. 20.7 %). RINGEL *et al.* (2007) and MICHIELS *et al.* (2012) reported a more pronounced effect of GAA on breast meat yield, which tended to increase with increasing dietary GAA level (RINGEL *et al*., 2007). In contrast, no effect of GAA on carcass parameters was found in the study by MOUSAVI *et al.* (2013). As dietary concentration of AME_n in the present experiment decreased, the percentage of breast meat increased, particularly in the control birds. The response pattern coincided with that of body weight which corroborates the well-known fact that breast meat yield is closely related to body or carcass weight (Ross 308 Broiler Performance Objectives, 2012).

The mechanism by which GAA affects broiler performance is not fully understood. Guanidinoacetic acid is a natural precursor of creatine, which is involved in cell energy metabolism, particularly in tissues with high and varying energy demand such as skeletal muscle (MICHIELS *et al.,* 2012). In fast-growing animals fed all-vegetable diets lacking creatine, GAA may restore creatine reserves, thus improving tissue energy metabolism and body growth. Another mode of action of GAA may be associated with amino acid metabolism. *In vivo,* GAA is synthesized from glycine and arginine and GAA supplementation may thus spare arginine, one of the potentially limiting amino acids in low-protein broiler diets (BAKER, 2009). Indeed, DILGER *et al.* (2013) showed that GAA supplementation markedly improved growth of chickens fed arginine-deficient casein-based diets.

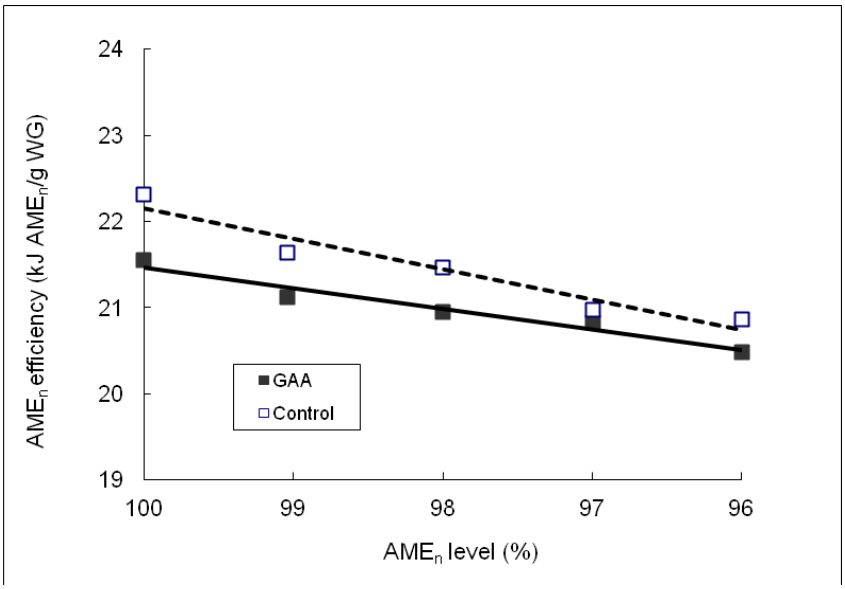

Figure 1. Effect of GAA supplementation on AME_n efficiency in chickens at five dietary AME_n levels. Plotted from the equations y = –14.82 + 0.370x, R^2 = 0.62 (Control) and y = –3.878 + 0.253x, R^2 = 0.53 (GAA)

In practical-type diets with moderate arginine deficiency, there was no growth response to GAA, but feed conversion was significantly improved. In accordance with the present study, these results suggest that feed conversion ratio is a more sensitive indicator of nutrient adequacy than growth rate in broilers.

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ID 151

EFFECTS OF DIFFERENT FEEDING STRATEGIES DURING REAR AND LAY ON INCUBATION TRAITS IN BROILER BREEDER FEMALES

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Summary

An experiment was conducted to investigate the effects of different dietary protein levels during rearing and dietary energy levels during lay on incubation traits of broiler breeders (BB). A 2 × 3 × 2 factorial design was used with 2 crude protein (CP) levels (high = CPh; low = CPl) during rearing; high, standard and low dietary energy levels (3,000 = MEh1; 2,800 = MEs1; 2,600 = MEl1) during the first phase of lay; and standard and high dietary energy levels (2,800 = MEs2; 3,000 = MEh2) during the second phase of lay. A total of 2,880 one-day old Ross 308 BB female chicks (36 pens) were followed until 60 wk of age. Starting at 28 wk of age, at a 5-wk interval, 150 eggs per pen were collected to determine incubation traits. Hatchability of set and fertile eggs (first phase) from CPl birds was increased compared to CPh birds due to a decreased embryonic mortality. No effects of energy level on incubation traits were found for the treatments during the first phase of lay. Birds fed the MEh2 diet showed, during the second phase of lay, an increased hatchability of fertile eggs, a decreased embryonic mortality, and a lower portion of second grade chicks compared to birds fed the MEs2 diet. It was concluded that a low CP diet during rearing positively affected incubation traits during the first phase of lay. An altered energy level during the first phase did not affect, whereas a higher energy diet during the second phase of lay showed positive effects on incubation traits.

Keywords: broiler breeder, rearing, laying, feeding strategies, incubation traits

Introduction

An important factor for profitability in broiler breeder production is fertility and hatchability of hatching eggs. Fertility of hatching eggs declined from 88.8% in 2000 to 84.7% in 2005 (VAN EMOUS, 2010). As reviewed by YASSIN et al. (2008), this decrease in fertility may be caused by a wide range of factors such as strain, health status of the flock, egg size, egg weight, egg quality, egg storage duration and conditions, egg sanitation, season of the year, and age of the breeders. Besides these factors, nutrition plays a very important role on fertility and hatchability. A negative effect of a high daily crude protein intake (> 25 g/day) during the laying period on fertility or hatchability of eggs has been reported (PEARSON and HERRON, 1982; WHITEHEAD et al., 1985; LOPEZ and LEESON, 1995). A decreased hatchability of fertile eggs could be explained by an increased embryonic mortality (PEARSON and HERRON, 1982; WHITEHEAD et al., 1985). The objective of this study was to evaluate the effects of different dietary protein levels during rear and different dietary energy levels during lay on incubation traits of Ross 308 broiler breeder hens.

Materials and methods

The effects of different dietary protein levels during rearing and different dietary energy levels during lay on body composition and reproduction were studied using a 2 × 3 × 2 factorial design with 2 CP levels (CPh = high protein; or CPl = low protein) during rearing between 2 and 22 wk of age; high, standard and low dietary energy levels during the first phase of lay between 22 and 45 wk of age (3,000, MEh1; 2,800, MEs1; and 2,600, MEl1, kcal/kg AME_n, respectively); and standard and high dietary energy levels during the second phase of lay between 45 and 60 wk of age (2,800, MEs2; and 3,000, MEh2, kcal/kg AME_n, respectively).

A flock of 3,000 Ross 308 female broiler breeder day-old chicks was allotted to 36 floor pens (4.5 × 2.5 m) in two identical climate controlled rooms. The experiment started at d 14 with 80 pullets per pen. Due to mortality, sex errors and culling (removing the smallest), the number of pullets was gradually reduced to 70 at 22 wk of age. Males were reared elsewhere and introduced into the pens at 22 wk of age. Starting at 28 wk of age, at a 5-wk interval, 150 eggs per pen from a 3 to 4 d production period were transported and set, after a 5 d storage period, in an incubator at a commercial hatchery. Eggs were candled on d 10 of incubation and clear eggs and dead in shell were opened to determine infertility

and the timing of embryonic mortality by visual inspection (categorized in d 1 to 2 and d 3 to 9). At d 18, eggs were transferred to hatcher baskets and placed in a hatcher. At d 21 of incubation, unhatched eggs were calculated and scored as d 10 to 21 embryonic mortality. All chicks were graded as first or second grade chicks. Fertility was calculated as the percentage of fertile eggs of the set eggs. Hatchability of set and fertile eggs was calculated as the percentage of first grade chicks hatched of set and fertile eggs, respectively. Embryonic mortality was calculated as a percentage of fertile eggs. Second grade chicks were calculated as a percentage of total hatched chicks.

Results and discussion

Fertility during the first phase of lay was not affected by the CP diets during rearing (Table 1). However, hatchability of set eggs and fertile eggs from CPl birds were increased compared to CPh birds due to a decreased embryonic mortality between d 10 to 21 of incubation. The portion of second grade chicks during the first phase of lay was not affected by the dietary protein level during rearing.

Table 1. Incubation traits during the first phase of lay (22 to 45 wk of age) as affected by dietary protein level during rear and dietary energy level during the first phase of lay.

Source	Fertility (%)	Hatch of set eggs (%)	Hatch of fertile eggs (%)	Embryonic mortality (%)			2nd grade chicks (%)
				1 to 2 d	3 to 9 d	10 to 21 d	
Protein rear							
CPh	96.5	87.7[b]	91.1[b]	1.1	2.2	5.1[a]	0.5
CPl	96.7	89.0[a]	92.2[a]	1.1	2.0	4.2[b]	0.7
SEM	0.3	0.5	0.3	0.1	0.2	0.2	0.1
Energy lay 1							
MEh1	96.9	88.7	91.7	1.1	2.0	4.7	0.6
MEs1	96.6	88.4	91.6	1.1	2.2	4.4	0.8
MEl1	96.3	88.0	91.6	1.1	2.0	5.0	0.5
SEM	0.4	0.6	0.4	0.1	0.2	0.3	0.1
P-value							
CP	0.54	0.05	0.02	0.82	0.57	0.01	0.37
ME1	0.56	0.62	0.93	0.94	0.83	0.38	0.34
CP x ME1	0.12	0.10	0.54	0.08	0.31	0.09	0.61

[a-b] Means within a column and within a source without a common superscript differ significantly ($P < 0.05$)

It was hypothesised that the positive effects on incubation traits were caused by the different body compositions at the end of the rearing period. The increased abdominal fat pad (1.3 vs. 0.7%) and decreased breast muscle (17.4 vs. 20.2%) of the pullets at 22 wk of age fed the CPl diet decreased embryonic mortality of the offspring between d 10 and 21 of incubation of 0.9% during the first phase of lay. HOCKING et al. (2002) and VAN EMOUS et al. (2013) did not find any effects on incubation traits when pullets received different dietary protein levels during rearing. The explanation of the positive effects of changed body composition (more abdominal fat and less breast muscle) of the CPl birds on lower embryonic mortality in the current study is unclear.

No effects of different dietary energy levels during the first phase of lay were found on incubation traits during the first phase of lay (Table 1).

During the second phase of lay, birds fed the MEh2 diet showed an increased hatchability of fertile eggs (90.0 vs. 88.5%), a decreased embryonic mortality (8.4 vs. 9.7%) and a lower portion of second grade chicks (1.7 vs. 2.3%) compared to birds fed the MEs2 diet. No effects on fertility and hatchability of set eggs were found for the treatments during the second phase of lay. A high energy diet (and as a consequence a 10% lower daily CP/AA intake) resulted in no effects on fertility but improved hatchability of fertile eggs, which in turn was caused by decreased embryonic mortality between d 3 to 21 of incubation. This is in agreement with the studies of PEARSON and HERRON (1982) and WHITEHEAD et al. (1985). LOPEZ and LEESON (1995) observed an increased fertility and hatchability of set eggs and

lower embryonic mortality when birds received a lower daily CP intake during the entire laying period. The improved incubation traits in the current study probably also positively affected the quality of the embryos resulting in a lower number of second grade chicks. This was previously underlined by PEARSON and HERRON (1982) who found that the portion of dead and malpositioned embryos could be reduced if daily protein intake was decreased from 27.0 to 21.3 g protein per animal per day.

Conclusions

A low-protein diet fed to breeder pullets during the rearing period resulted in positive effects on incubation traits during the first phase of lay. Changing the dietary energy level compared to the standard (2,800 Kcal/kg) during the first phase of did not affect incubation traits whereas a high-energy diet (3,000 vs. 2,800 Kcal/kg) during the second phase of lay resulted in positive effects on incubation traits.

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ID 60

SOLID-STATE FERMENTED CEREALS PROMISES A GROWTH PROMOTING EFFECT IN BROILER CHICKEN

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Abstract

Effects of 40-50% dried wheat, barley and oats grains fermented with liquid whey and citrus pomace at 35 °C and 60-70% moisture for 8 h were tested from day 1 to day 42 on bird's performance, carcass yield, digestive organ development, intestinal viscosity and pH. In comparison to the control diets, feed intake (FI) increased with all fermented grain-based diets by 0.3%, weight gain (WG) by 5.6% and feed conversion ratio (FCR) by 4.7%. Significant improvements in WG (9%) and FCR (8.2%) were obtained from fermented barley based diet. Carcass yield was significantly higher in fermented wheat and oats diets than their control diets. Marked reductions in both digesta viscosity and in number of birds with sticky droppings were seen with all fermented grain based diets; this effect was more pronounced for the fermented barley. Birds fed on the diets with fermented feeds had lighter and shorter digestive tract than those on the unfermented feeds. Duodenum length significantly reduced, and liver weight increased by fermented feeds. In short, this fermentation method improved nutritive value of cereals for broiler chicken. The improved FCR and WG as well as marked reduction in viscosity by fermentation indicate a growth stimulating effect in broilers.

Keywords: broilers, fermented cereals, solid-state, whey, citrus pomace

Introduction

Performance impairments are inevitable in poultry species fed with the diets of high levels of cereal grains (YASAR and FORBES, 1999). Wetting (YASAR and FORBES 1999) or fermenting (ENGBERG et al., 2009; UCHEWA and ONU, 2012) cereal grains or their diets improved FCR, increased WG, reduced viscosity, degraded non-starch polysaccharides (NSP), increased concentration of DL-lactic acid and lactic acid bacteria counts in the gut, increased digestibility of nutrients, improved activities of exogenous NSP-degrading enzymes. Feeding moisturised diets after several hours of fermentation with or without microbial inoculant or simply soaking in water are not considered as practical feeding tool in commercial poultry production. A suitable (cost-effective) drying process is required after fermentation in order to yield a sustainable beneficial effect in poultry nutrition. Furthermore, an appropriate (easy-made) and suitable (cost-effective) fermentation method needs to be developed. Therefore, a short period of fermentation (maximum 8 hours) with the use of non-microbial inoculants and some agricultural wastes (whey and citrus pomace), followed by a drying process below 30 °C were tested in this study.

Material and Methods

An in-house solid state fermentation (SSF) system was used to produce the fermented grains. 1 part of ground (3 mm) wheat, barley and oats grains were soaked in 1.1, 1.1 and 1.2 parts of fresh liquid whey, respectively; to provide a 60 to 70% of moisture. Temperature was set at 35 °C, and pH of material was set under a pH of 5.0 by addition of citrus pomace (inhibition of pathogens). A pH of 4.2 for wheat grain and 4.5 for both oats and barley grains were achieved by the addition of 1 (w/w) and 2.3 % citrus pomace, respectively. The final product at the end of 8 h was dried out at room temperature for 2 days and ground (5 mm). Isocaloric and isonitrogenous diets were formulated with 45-50% of unfermented and fermented grains. A 378 of 1 d-old Ross chicks were equally distributed to 18 floor pens. Each of the 6 diets was offered to the chicks of 3 pens for 42 days. FI, BW (body weight), WG and FCR were recorded weekly. Three birds from each pen at 21 d and all birds at 42 d were killed to collect the data for digestive organ developments, digesta viscosity and carcass quality. A factorial model of analysis of variance, 3x2 (grains by fermentation), was used to statistically analyse all the data. Significant differences between the means of groups were properly separated.

Results

Inclusion of high level of unfermented wheat in broiler diet increased FI and caused a high FCR as compared to the diet with fermented wheat with which significantly reduced FI and improved FCR with the same final BW were achieved (Table 1).

Table 1. Performance of broiler chickens at 42 days on the diets based on fermented (F) and unfermented (U) wheat, barley and oats grain (means with SEM).

Diet	Wheat		Barley		Oats		
	U	F	U	F	U	F	SEM
BW (day 1) 1d, g/b	56.0	55.9	55.9	56.6	56.6	55.2	0.50
BW (day 42) 42d, g/b	2856.0a	2884.0a	2675.0b	2916.6a	2274.5c	2435.0d	52.0
FI (day 42), g/b	4715.5a	4446.2b	4481.9b	4485.3b	3856.2c	4140.4d	75.0
FCR (day 42)	1.68a	1.58b	1.71a	1.57b	1.74a	1.74a	0.03
Carcass yield, %	70.1a	72.5b	70.3a	69.6ad	66.0c	68.5d	0.5

The use of fermented barley did not influence FI as compared to the unfermented barley. But, a significant increase in BW and improvement in FCR was obtained from the diet of fermented barley. The use of unfermented oats significantly reduced FI, BW and FCR, whereas the fermented oats increased FI and BW with no change in FCR. Carcass yield was significantly high with the diet of fermented wheat and worse with the diet of unfermented oats.

A viscous gel-forming constitute is present in the intentine of young broiler chicken when fed with high levels of unfermented cereal grains (Figure 1). A marked reduction both in viscosity of ileal contents and in the number of birds with sticky droppings was observed with the fermented grain-based diets both at 21 and 42 days (Figure 1). Reduction in viscosity was better with fermented barley. Weight (227 *vs* 193 g/b) and length (200 *vs* 187 cm/b) of digestive tract were reduced by the use of fermented cereal grains. Liver weight was high with the use of fermented barley and oats based diet. All changes were well correlated with the WG and FCR values under the effect of fermentation.

Conclusions

The results indicated that a SSF process (35°C of substrate temperature, pH of between 4.0 and 5.0, with the use of whey and citrus pomace and no microbial inoculants) of cereal grains followed by drying at room temperature provided an effectual functional property. In a similar study, CARLSON and POULSEN (1999) reported significant degradation of phytic acid, increased enzyme activities, nutrients enrichment (crude protein), increased lactic acid bacteria and lactic acids with fermentation. Reduced digesta viscosity and an efficient gut function by fermentation of cereals were parallel to improvements in FCR and WG in broilers in the present case. All these were good indicators of effectual functional properties gained under a SSF.

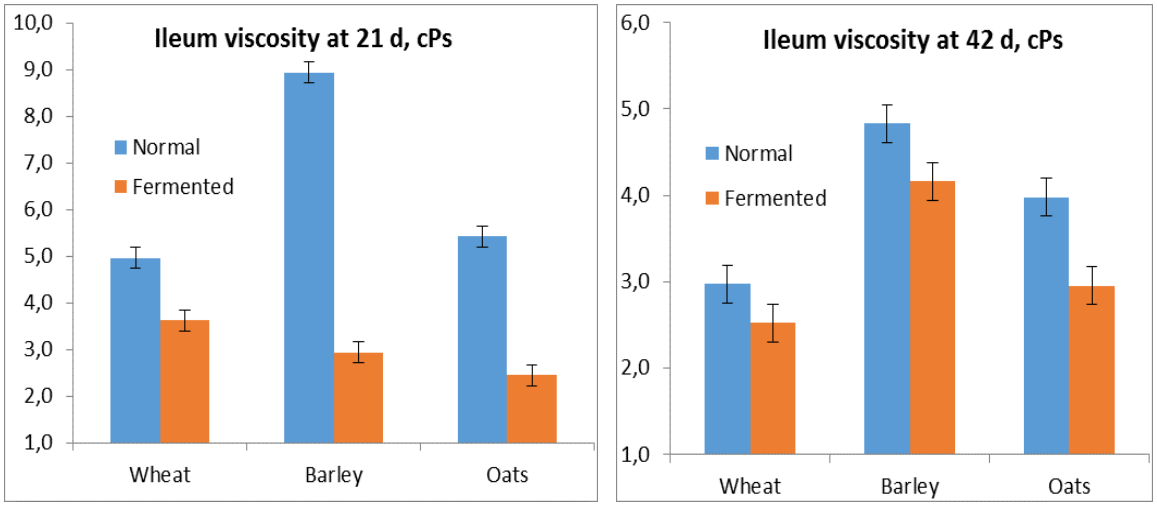

Figure 1. Changes in viscosity (cPs) of ileal contents in broilers at 21 and 42 d old.

Using whey, instead of water, during SSF in the presence of citrus pomace and in the absence of any microbial inoculants had an added value for a valuble end product (FORBES and SHARIATMADARI, 2005; ALBORES et al., 2009; YASAR and GOK, 2014). Due to a suitable drying process (room temperature) it can be speculated that the end product may still have an appreciable amount of living microbes beneficial to the animals, as several other researchers reported such enrichment.

In short, this SSF process is recommended to be easily installed into any feed production line. Significantly important improvements both in growth rate and in gut function of broiler chickens fed with the diets of fermented wheat and barley products promise a growth promoting effect in broilers. But this is not the case with oats, probably due to high level of crude fibre per se, limiting the regulation of feed intake to provide sufficiently enough amounts of nutrients available for intestinal uptake.

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ID 5

INFLUENCE OF COMBINED EFFECT OF TEMPERATURE STIMULATION DURING THE LAST DAYS OF INCUBATION AND OF DIFFERENT PROTEIN AND ENERGY CONCENTRATIONS IN THE FEED ON GROWING PERFORMANCE OF COCKERELS OF DIFFERENT STRAINS

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Abstract

Laying-type cockerels or spring chicken cannot be reared economically. Lohmann Tierzucht bred the dual-purpose chicken (producing eggs and meat) in response to growing criticism of conventional practices in modern egg production. Recent research with birds shows that incubation climate may have a long-lasting influence on poultry performance up to the age of slaughter. Therefore the following study was carried out, to investigate whether short-term variation in incubation temperature during the last days of incubation have a long-lasting effect on performance, also in laying-type cockerels. 2880 eggs (Lohmann Brown-LB/Lohmann Dual-LD) were incubated from days 1 to 17 under common incubation temperature (37^{0}C). From day 18 until hatching the eggs were sorted in hatch incubators with different temperature programs. The male one-day-old chicks were randomly distributed in 8 treatment groups during a study time of 70 days: two origins of chicks (LB, LD), two hatch incubators and twofold protein/energy (200g crude protein/11 MJ AME_N/kg; 215g/12 MJ) levels in the diet. Growing performance of LD cockerels was significantly better compared to LB males. Final body weight of LD birds was 1000 g higher and feed to gain ratio 10% lower. Short-term temperature stimulation during the end of incubation resulted in a 3.5% higher final body weight by LD cockerels.

Keywords: cockerels, incubation, temperature stimulation, growth

Introduction

Laying-type cockerels or spring chicken cannot be reared economically (Damme and Ristic, 2003; Schäublin *et al*., 2005; König *et al*., 2010; Halle *et al*., 2012b). But routine culling of these day-old male chicks is more and more an ethical problem and to find alternative solutions is a great challenge. Lohmann Tierzucht bred the dual-purpose chicken (producing eggs and meat) in response to growing criticism of conventional practices in modern egg production. Recent research shows that incubation climate may have a long-lasting influence on poultry performance (Collin *et al*., 2007; Hulet *et al*., 2007; Pistun *et al*., 2008; Tzschentke and Halle, 2009; Halle and Tzschentke, 2011; Shinder *et al*., 2011; Halle *et al*., 2012a). The most important climatic incubation factor is the incubation temperature (Decuypere and Michels, 1992). In broiler chickens, for instance, short-term temperature training during the last 4 days of embryonic development, improved hatching results and later robustness and performance (Tzschentke and Halle, 2009). Therefore the following study was carried out, to investigate whether short-term variation in incubation temperature during the last days of incubation have a long-lasting effect on performance, also in laying-type cockerels.

Material and Methods

In the trial 2880 eggs (Lohmann Brown-LB/Lohmann Dual-LD) were incubated from days 1 to 17 under common incubation temperature (37°C). From day 18 until hatching the eggs were sorted in hatch incubators with different temperature programs: 37°C (control) and 1^{0}C over standard for 2 hours daily (38°C: short-term warm stimulation). The one-day-old chicks were sorted by sex and only the male laying-type cockerels were randomly distributed in 8 treatments (6 pens/group) resulted from the two origin of chicks (LB, LD), from two hatch incubators and twofold protein/energy (200 g crude protein /11 MJ AME_N/kg - low; 215 g /12 MJ - high) levels in the diet (Table 1; 2). The duration of the trial was 70 days. Data from the trial were analyzed by a three-way ANOVA (SAS) ($P \leq 0.05$).

Table 1. Study design

Group	Cockerels origin	Temperature stimulation	Feed level
1 / 5	LB / LD	Control	Low
2 / 6		Control	High
3 / 7		Short-term warm stimulation	Low
4 / 8		Short-term warm stimulation	High

Table 2. Ingredient composition and nutrients of the diets (g/kg) (Dry matter 885 g/kg)

Ingredient	Feed level	
	low	high
Wheat/Corn/Bran	695	594
Soya bean meal/Oil	267	365
Vitamin and minerals	38	41
Crude protein	185	205
ME, MJ/kg	11.2	12.4
Lysine/Methionine+Cystine	10.0/8.5	11.5/9.8

Results and conclusion

Table 3. Performance of laying type cockerels

Genetic line	Temperature stimulation	Feed level	Feed intake g/bird/day	Final body weight, g/bird	kg feed/kg weight gain
LB	no	Low	47.6	1336	2.568
	no	High	43.3	1360	2.293
	yes	Low	47.1	1336	2.544
	yes	High	44.1	1374	2.309
LD	no	Low	78.6	2432	2.299
	no	High	71.4	2482	2.049
	yes	Low	81.9	2558	2.275
	yes	High	73.9	2528	2.070
ANOVA, P-value					
Cockerels origin			<0.001	<0.001	<0.001
Temperature stimulation			0.14	0.09	0.96
Feed level			<0.001	0.45	<0.001
Temperature x Feed			0.9	0.5	0.2
Temperature x Origin			0.2	0.2	0.8
Origin x Feed			0.07	0.7	0.4
Origin x Temperature x Feed			0.6	0.4	0.9

Growing performance of LD cockerels was significantly better compared to LB males. Final body weight of LD birds was 1000 g higher and feed to gain ratio 10% lower. Short-term temperature stimulation during the end of incubation resulted in a 3.5% higher final body weight by LD cockerels. The daily feed intake and the feed to gain ratio was significantly improved through the increased protein/energy concentration of the “high” feed.

The results allow the conclusions that an incubation temperature profile, which includes short-term warm stimulation of +1^{0}C for 2 h daily during final incubation, had a long-lasting effect on performance in male laying-type cockerels of origin Lohmann Dual. The most important factors on growing performance of the Lohmann Brown laying type cockerels were the protein-energy concentration in feed.

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ID 76

EFFECT OF DIFFERENT DOSAGES OF COATED BUTYRIC ACID ON GROWTH PERFORMANCE AND ENERGY UTILIZATION IN BROILERS

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Abstract

Four dietary treatments were used in experiment I and II to investigate the effect of protected butyrate, on growth performance and nutrient digestibility in broiler chickens. One-day-old male Ross 308 chicks (total 960, 480 per trial) were randomly assigned to four dietary treatments. In experiment I, the basal diets were fed without or with protected calcium butyrate (B), inclusion (0.2, 0.3, or 0.4 g/kg). In experiment II, the basal diets were fed with or without B (0.3 g/kg), or with Avilamycin (A), or in combination of both. In experiment I, the use of B improved FCR (P<0.05) irrespective of the dose. Diets with B were characterized by higher AMEn value than the control treatment (P<0.05). Histomorphological evaluations showed that microencapsulated B had a positive influence (P<0.05) on villi height In experiment II, there was an effect (P<0.05) in improved BWG after B or A supplementation separately or in combination. The use of avilamycin improved (P<0.05) FCR in comparison with the control treatment. Ileal digestibility of crude protein and crude fat increased after B, A or the combination of both (P<0.05). The use (14, 35 and 42d) of B and A in combination improved AME content in comparison with the control treatment (P<0.05). The use of A, B or AB increased ileal digestibility of Thr, Ser and Pro (P<0.05). Valine, Ile, Leu, Tyr and His ileal digestibility was improved in comparison with the control after A addition (P<0.05).

Keywords: broiler, villi, amino acid, digestibility

Introduction

It is certain that butyric acid is a very complex and dynamic molecule with different modes of action. After B supplementation, an increase in villi height and crypt depth has been observed in pigs and poultry (GALFI and BOKORI, 1990, LEESON et al., 2005). The growth in villi height increases the absorptive surface of the small intestine leading to better nutrient utilization, which is why recent studies have shown that when B is orally ingested it can have beneficial effects on animal performance (MAZZONI et al., 2008, CZERWIŃSKI et al., 2012). However, there is limited information on the effect of protected B on the AME of diets in broiler chickens. The limited literature data have shown that B may improve total tract digestibility, but not ileal nutrient digestibility in pigs (LE GALL et al., 2009). In contrast, MANZANILLA et al. (2006) reported that B reduced ileal digestibility of starch in weaned pigs.

The aim of this study was to investigate the effect that a protected butyrate can have on diet digestibility, AME, birds' performance and ileum histomorphology. This study consists of the results of two trials.

Material and Methods

Ross 308 broiler chicks (total 960, 480 per trial) were obtained from a local hatchery. The birds were individually weighed and allocated to 10 weight classes. Groups of 10 birds were then randomly assigned to pens in such a way that the average initial bodyweight of birds was similar across pens. Ten replicate pens of 10 birds each were randomly assigned to four dietary treatments. All diets were fed in pelleted form throughout the 6-week experimental period.

The birds were held in pens and had *ad libitum* access to water and feed. On day 35 (Experiment I) and on days 14, 35 and 42 (Experiment II), excreta samples from each pen were collected over a 3-h period and subsequently frozen, freeze-dried, and finely ground. Collection trays were installed in floor pens to allow for excreta collection; ten excreta samples per treatment were collected. On day 35 (Experiments I and II), 10 birds from each treatment were randomly selected and killed via cervical dislocation. The contents of the terminal ileum (15 cm, adjacent to the ileo-cecal junction) were collected. Additionally, samples (n = 8) of the whole intestinal tract were removed, and segments of approximately 2 cm were collected. In experiment I, the basal diets were fed with or without protected calcium butyrate (B) (ButiPEARL™, Kemin) inclusion (0.2, 0.3, or 0.4 g/kg). In experiment II, the basal diets were fed with or without pro-

tected calcium butyrate (0.3 g/kg), (ButiPEARL, Kemin) or with avilamycin (6 mg/kg, active substance), (Maxus, Elanco) or with a combination of both.

Results

In experiment I, considering the entire study period, the use of B improved FCR (P<0.05) irrespective of the dose. Apparent total tract crude fat digestibility was improved after B supplementation (P<0.05). Diets with B were characterized by higher AMEn values than the control treatment (P<0.05). Birds fed diets supplemented with B were characterized by higher VH than that of unsupplemented birds (P<0.05). At the lowest dose (0.2 g/kg), the difference was over 150 µm compared to the control treatment (P<0.05). Results from experiment II showed that avilamycin alone or in combination with B improved (P<0.05) BWG compared to the control treatment. A tendency (P<0.1) for improved BWG (14–42d) was observed after B or A supplementation, either separately or in combination. Similar differences (P<0.05) between A and B were observed through the whole trial period (0–42d). The use of avilamycin improved (P<0.05) FCR compared to the control treatment. To a similar extent, A, B and the combination of both improved (P<0.05) FCR compared to the control treatment. Birds from the control treatment were characterized by having the thickest mucosa (P<0.05). The thinnest mucosa was observed when birds were fed the control diet supplemented with the combination of B and A (P<0.05). Villous height was not affected when B or A were used separately; however, animals fed B showed numerically higher villi than the controls. Birds given the mixture of B and A consistently showed lower villi compared to the control treatment (P<0.05). Crypt depth decreased in the ileum for all treated animals compared to the control birds (P<0.05). The ileal digestibility of crude protein increased after B, A or the combination of both (P<0.05). Similar differences were observed in ileal crude fat digestibility, but significant differences (P<0.05) were noted only between the control and avilamycin treated animals. On day 14, the use of B and A in combination improved AME content compared to the control treatment (P<0.05) and the difference was over 150 kcal/kg. Considering days 35 and 42 of the experiment, determined AME increased after A, B or A and B combination supplementation compared to the control treatment (P<0.05). B, A or AB treated birds were characterized by higher total tract crude fat digestibility on days 14 and 35. On day 42, A and AB treated birds were different from the control (P<0.05). The most pronounced improvement was noted after A supplementation.

A or AB treated birds were characterized by higher Asp, Glu, Cys, Gly and Ala ileal digestibility than the control animals (P<0.05). The use of A, B or AB increased the ileal digestibility of Thr, Ser and Pro (P<0.05). Valine, Ile, Leu, Tyr and His ileal digestibility was improved compared to the control after A addition (P<0.05).

Conclusions

The results of our studies indicate positive effects of feeding of up to 0.3 g/kg protected butyrate throughout the 42-d broiler growth period when the compound was used either alone or in combination with avilamycin. There is an indication that butyrate helped maintain intestinal integrity and, consequently, improve the digestion and absorptive processes. Future studies are warranted to investigate the dose response to protected butyric acid on enzyme secretion and starch, as well as other nutrient digestibility in birds.

Table 3. Broiler chicken performance (BWG, FI [g] and FCR [g:g]), ATTD, AID, AMEn and mucosa thickness – experiment I and II.

Experiment I

B dose [g/kg]	BWG		FI		FCR		ATTD of CF	AMEn
	0–14d	0–42d	0–14d	0–42d	0–14d	0–42d		
0	344	2616	512	4336	1.48	1.66	82.8	3,136
0.2	363	2696	511	4409	1.41	1.64*	86.9*	3,358*
0.3	354	2741	475	4348	1.31*	1.59*	85.5*	3,373*
0.4	357	2699	467*	4207	1.31*	1.58*	86.9*	3,263*

Experiment II

	BWG 0-42d	FCR 0-42d	Mucosa thickness	AID of CP (14d)	ATTD of CF (d14)	AMEn at day: 14	35	42
C	2123	1.59	1210	69.6	92.5	3088	3105	3241
C+B	2323*	1.49*	981*	78.1*	95.4*	3154	3235*	3305
C+A	2356*	1.50*	895*	78.8*	95.6*	3148	3320*	3348*
C+B+A	2361*	1.48*	837*	78.2*	94.4*	3243*	3247*	3333*

CF – crude fat, AID – apparent ileal digestibility, CP – crude protein, ATTD – apparent total tract digestibility, C – Control treatment, C+B – control + ButiPEARL, C+A – control + avilamycin, C+B+A – control + ButiPEARL + avilamycin.

* – Means significantly different from control treatment ($P < 0.05$).

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ID 164

EFFECT OF ISO-ENERGETIC EXCHANGE OF DIETARY FAT AND STARCH ON THE GROWTH PERFORMANCE AND BODY COMPOSITION OF BROILERS

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Abstract

Dietary factors such as the concentrations of protein/amino acids, fat, and carbohydrates and their ratio, may affect the post-absorptive metabolism of energy and protein and energy deposition in the body. In a 2x2 factorial design, the effects of two dietary crude protein (CP; high protein (HP) vs. low protein (LP) concentrations; 200/190 vs. 170/160 g/kg in grower and finisher phase and two dietary fat/starch concentrations; high fat (HF; fat and starch 105 and 340 g/kg, respectively) and low fat (LF; fat and starch 65 and 420 g/kg, respectively) on growth performance and body composition of Ross 308 broilers were studied (9 to 35 d). Concentrations of apparent faecal digestible essential amino acids were similar in HP and LP diets. Feed intake (FI) of broilers fed HP diets tended (P=0.06) to be lower than in broilers fed LP diets. Body weight gain (BWG) of broilers fed HP diets was significantly higher than BWG of broilers fed LP diets (P<0.001) resulting in a significantly lower feed conversion ratio (FCR) of broilers fed HP diets (P<0.001). Exchange of dietary fat by starch resulted in a significantly lower FI and FCR, while BWG was not affected. The FCR was improved by substituting dietary fat by starch, the effect being more pronounced in LP than in HP diets (P=0.012). On d 35, the body fat content of birds fed LF diets was higher than in birds fed the HF diets (P=0.03). It can be concluded that dietary energy source and protein level in iso-energetic diets balanced for first limiting essential amino acids influence growth performance and body composition of broilers.

Keywords: broiler chicken, diet, energy, fat, starch, protein

Introduction

Selection for improvements in body weight gain and breast meat yield affects protein and fat deposition in broilers. Energy deposition is the result of dietary energy intake and the efficiency of utilisation of digested energy for maintenance and deposition of protein and fat in the body. Energy distribution is not only affected by genetic factors but also factors such as climate and nutrition (quantitative feed intake and diet composition). In general, diets with high metabolisable energy or high energy to protein ratio result in a higher fat deposition (Swennen et al., 2007) and diets with amino acids content beyond requirements will result lower fat deposition but with poorer efficiency because elimination of excess nitrogen is an energy-consuming process. Reducing the dietary protein content to sub-optimal levels will result in a higher fat deposition (Buyse et al., 1992). Most studies in literature are focussed on the effect of dietary protein content (apparent faecal digestible amino acids) and less research is conducted on the effect of dietary energy source (carbohydrates and fat) on the post-absorptive metabolism of energy. Literature on effects of iso-energetic exchange of dietary fat and starch in broiler diets on growth performance and body composition is scarce and not consistent. In the present study, the effect of iso-energetic exchange of dietary fat and starch on the growth performance and body composition of broilers was studied.

Material and Methods

Day-old male and female broiler chicks (Ross 308) were obtained from a commercial hatchery and divided over 72 floor pens with wood shavings (36 floor pens with males and 36 floor pens with females) in an environmentally controlled experimental broiler house. In each floor pen (1.00 x 0.75 m) 14 broilers were placed. Feed and water were provided *ad libitum*, and temperature was set initially at 34 °C and gradually reduced until 22 °C was reached at 25 days of age. From day 1 to 9, all broilers received a starter diet with 21% CP and 2,840 kcal ME/kg. The four experimental diets were randomly assigned to the floor pens (per gender 9 pens per diet). From 10 days of age, broilers in each pen received one of the four iso-energetic experimental diets. The diets contained the same ingredients, but different concentrations of particular ingredients to realise the experimental treatments. The levels of wheat, wheat middlings, cellulose, soybean meal, potato protein, corn gluten meal, corn starch and soybean oil were adjusted to create contrasts in macronutrient levels and to keep metabolisable energy content of all diets similar. Metabolisable energy content in the

grower (10-28 d) and finisher (29-35 d) diets was 12.24 MJ/kg. The high-protein (HP) and low-protein (LP) grower diets contained 200 and 170 g crude protein per kg diet, and the HP and LP finisher diets contained 190 and 160 g crude protein per kg diet, respectively. Free amino acids (lysine, methionine, threonine, valine, arginine, isoleucine and tryptophan) were included in the diets to meet the apparent faecal digestible amino acid requirements (CVB, 1996). The high-fat (HF) and low-fat (LF) grower and finisher diets contained per kg 105 and 65 g crude fat and 380 and 460 g starch + sugars, respectively. The same batches of ingredients were used to formulate the four experimental diets. All birds of each pen were weighed at 9, 18, 28 and 35 days of age and feed consumption was recorded per pen to determine feed intake (FI), body weight (BW), body weight gain (BWG) and feed conversion ratio (FCR). After weighing, two birds corresponding to the mean weight of each pen were selected and euthanized. Pooled in one sample, these birds were autoclaved, grinded, subsampled and analysed to determine dry matter (DM), protein, fat and ash concentration in the body.

Results and Discussion

Results for the growth performance over the period of 0 to 35 days of age are presented in Table 1. Feed intake of the birds fed HP diets tended (P=0.06) to be lower than FI of broilers fed LP diets and FI of birds fed HF diets was significantly higher than FI of birds fed LF diets (101.6 vs. 99.2 g/d; P=0.01). Body weight gain of broilers fed HP diets was significantly higher than BWG of broilers fed LP diets (63.5 vs. 61.5 g; P=0.001). Body weight gain of broilers was not affected by the dietary energy source. Feed conversion ratio of birds fed HP diets was significantly lower than FCR of broilers fed LP diets (1.568 vs. 1.646; P<0.001). Feed conversion ratio of broilers fed HF diets was significantly higher than FCR of broilers fed LF diets (1.625 vs. 1.590; P<0.001). This effect was more pronounced in LP diets than in HP diets (Protein x Energy interaction; P=0.012). Despite inclusion of free essential amino acids in LP diets in order to meet (CVB, 1996) amino acid requirement values, BWG of birds fed LP diets was lower than on HP diets. The concentration of non-essential amino acids in LP diets may have been limiting BWG related to the 30 g/kg lower crude protein content in LP diets compared to HP diets. Body composition at 35 days of age of broilers fed HP diets showed a lower DM content, a higher protein content and a lower fat content compared to broilers fed LP diets. Body DM, ash and fat content in broilers fed HF diets was lower than in broilers fed LF diets. However, differences in fat content were rather small and the lower fat content in broilers fed HF diets was not expected. Body protein content was not affected by dietary energy source. No interaction effect with gender has been observed, neither for growth performance nor for body composition.

Conclusions

Protein level and dietary energy source in iso-energetic diets balanced for most limiting essential amino acids influence growth performance and body composition of broilers. Lowering dietary crude protein content with 30 g/kg, however, in the grower and finisher period adversely affected growth performance of broilers. Partly substitution of fat by starch and sugars as dietary energy source improved growth performance. Low fat diets (higher in starch + sugars) resulted in a higher body fat content in birds at 35 days. The observed effects are related to differences in the post-absorptive utilisation of amino acids, carbohydrates (glucose and other sugars) and fatty acids and retention in the body.

Table 1. Effects of diet composition on the growth performance of broilers over the period of 0 to 35 days of age.

Treatment		FI (g/d)	GW (g)	BWG (g/d)	FCR
HP		99.5	2264[b]	63.5[b]	1.568[a]
LP		101.3	2196[a]	61.5[a]	1.646[b]
HF		101.6[b]	2233	62.6	1.625[b]
LF		99.2[a]	2227	62.4	1.590[a]
HP	HF	100.6	2277	63.9	1.576[a]
HP	LF	98.5	2251	63.1	1.559[a]
LP	HF	102.6	2189	61.4	1.673[c]
LP	LF	99.9	2202	61.7	1.620[b]
P-values					
Protein		0.060	0.001	0.001	<0.001
Energy		0.010	0.760	0.760	<0.001
Protein x Energy		0.757	0.342	0.342	0.012

Table 2. Effects of diet composition on the body composition of broilers at 35 days of age.

Treatment		DM (g/kg)	Ash (g/kg)	Protein (g/kg)	Fat (g/kg)
HP		323.2[a]	24.2	180.3[b]	122.3[a]
LP		335.7[b]	24.7	176.7[a]	137.9[b]
HF		325.4[a]	24.0[a]	178.8	127.0[a]
LF		333.5[b]	24.9[b]	178.2	133.2[b]
HP	HF	319.4	23.6	180.5	119.5
HP	LF	327.0	24.9	180.2	125.0
LP	HF	331.4	24.3	177.2	134.4
LP	LF	340.0	25.0	176.2	141.3
P-values					
Protein		<0.001	0.298	0.002	<0.001
Energy		0.013	0.031	0.552	0.029
Protein x Energy		0.878	0.485	0.748	0.790

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ID 231

STARCH DIGESTION IN XYLANASE SUPPLEMENTED MAIZE-SOY DIETS

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Abstract

Emerging evidence suggests a beneficial effect of xylanase supplementation in maize-based diets through improved nutrient digestibility. Previous work has revealed that when xylanase is added to a maize diet and fed instantly there is no significant effect on starch digestion in broilers. This would suggest that the amount of time that xylanase is present in the digestive tract is a limiting factor for enzyme efficacy and would support the idea of an indirect mechanism of xylanase action. To test this hypothesis, the current study was designed to investigate the effect of adding xylanase to a maize-soy diet over different time scales. Ross 308 broilers (60) were divided between 6 treatment groups; a control group without xylanase, and groups supplemented with xylanase from 1, 2, 3, 4 or 5 weeks before slaughter. At day 35 and 36, digesta was collected from the gizzard, upper and lower small intestine, and both caeca. Scanning electron microscope (SEM) images revealed starch granules entering the caeca along with fractured plant material for all groups, signifying incomplete starch digestion. Greater cell wall degradation was not evident with xylanase supplementation. However, this may be explained by the *ad libitum* feeding of birds which would lead to the existence of feed particles at all stages of digestion in the tract. Understanding the effect that xylanase supplementation has on starch digestion is essential for improving bird performance while lowering feed costs.

Keywords: Xylanase, Maize, Starch, Digestion

Introduction

For many countries, maize is the predominant source of starch and available energy in broiler diets. Maize-based diets are generally considered to be highly digestible, with a relatively high starch content and low concentrations of anti-nutritional factors, such as non-starch polysaccharides (NSP) when compared to other cereals such as wheat and rye (CHOCT, 1997). However, with increasing demand from the biofuels industry for cereals such as maize to be used for ethanol production, the cost of such feed in recent years has risen. Consequently, considerable attention has been given to improve nutrient utilisation of these diets through the use of exogenous enzymes, including xylanases, to release more energy from the diet. Traditionally, xylanases have been added to more viscosity-promoting diets, such as those based on wheat, to reduce digesta viscosity and improve nutrient digestion. However, there is also a beneficial effect of xylanase inclusion in maize-based diets through improved broiler performance (COWIESON *et al*., 2010), indicating that additional mechanisms other than reduction in viscosity are important. In previous work, adding xylanase to a maize diet and feeding on a single occasion did not significantly affect starch digestion in broilers. This suggested that the amount of time that xylanase was fed for may be a limiting factor for enzyme efficacy and would support the idea of an indirect mechanism of xylanase action. Therefore, the purpose of this study was to investigate the effect of feeding xylanase-supplemented maize-soy diets over different time periods before slaughter on starch digestion in broilers.

Materials and methods

Day-old male Ross 308 broiler chicks were supplied from a commercial hatchery and raised on a maize-soy diet (Target Feeds, UK) with or without xylanase inclusion (Econase® XT 25; 100g/t; supplied by ABVista). A total of 60 birds were used in this study with 10 birds per treatment group; birds were group-housed in separate pens for a specific treatment group. Treatment groups (6) included a control group fed a diet without xylanase, and groups fed the diet supplemented with xylanase from 1, 2, 3, 4 or 5 weeks before slaughter. Diets and water were provided *ad libitum* throughout the trial. On days 35 and 36, birds were euthanised by intravenous injection of pentobarbital before dissection was carried out. Digesta was collected from the gizzard, upper and lower small intestine, and both caeca. The morphology of starch granules from the digesta was examined using a scanning electron microscope (SEM; Jeol JSM-6490LV). The total starch assay (Megazyme International Ireland Ltd) was performed for the determination of starch in digesta samples. Digesta samples were washed twice in 80 % ethanol and the precipitate digested with thermostable α-amylase (300 U)

and amyloglucosidase (20 U). Glucose release was measured using a glucose analyser (Analox GM9 Analyser). Both ethanol washes, assumed to contain soluble glucose, were collected and the ethanol evaporated. Glucose concentration in this fraction was measured using the glucose analyser.

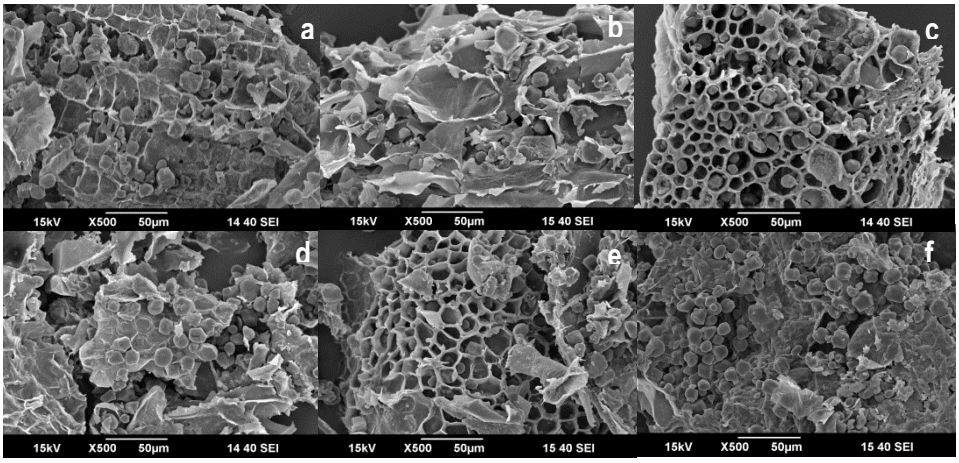

Figure 1. Starch granules in gizzard digesta taken from control birds (a) and birds fed diets supplemented with xylanase 1 (b), 2 (c), 3 (d), 4 (e) and 5 (f) weeks before slaughter.

Results

Birds were fed *ad libitum* before slaughter and therefore samples from the digestive tract will show feed particles that had been ingested for different periods of time. All gizzard samples showed cell wall fractionation (Fig.1), however greater cell wall destruction with xylanase treatment was not evident. Gizzard digesta from all treatment groups revealed a large number of trapped starch granules. The reasoning may not be solely down to cell wall materials, but also protein matrices that can form a continuous network that encapsulates starch granules, thereby preventing their release.

Total starch results demonstrate the progression of starch granule digestion as digesta passes through the digestive tract of birds (Fig.2). Starch content (Fig.2ia) was greatest in the gizzard, with decreased levels within the upper small intestine and then less again in the digesta of the lower small intestine, as starch becomes hydrolysed by endogenous amylases. As expected, the glucose content (Fig.2ib) of gizzard digesta was minimal as starch digestion does not occur in this tract section. Conversely, a higher glucose content was found in the upper small intestine, as this is the major site of starch digestion. By the lower small intestine, glucose levels fall again as monomers are absorbed into enterocytes. The content of starch and its digestion product, in any tract section, were not significantly affected by treatment ($P > 0.05$). Total starch results are also supported by SEM imaging (Fig.2ii) where starch granules appear to become more hydrolysed and are fewer in number as digesta enters more distal tract sections. Interestingly, a small number of starch granules escaped digestion and entered the caeca (Fig.2iid) for all treatment groups. Therefore, even when granules are released from their surrounding matrices, some are still resistant to digestion.

Conclusions

In the current study there was no indication of greater cell wall destruction or starch digestion occurring in birds fed a xylanase supplemented diet. Starch fraction measurements and SEM imaging may not give a full picture of xylanase action due to the *ad libitum* feeding of birds and the complex environment of the digestive tract. Resistant starch granules that enter the caeca confirm the important issue of incomplete starch digestion in broilers. Further investigation is needed for a better understanding of the mechanisms of xylanase action on starch digestion in broilers fed a maize-based diet.

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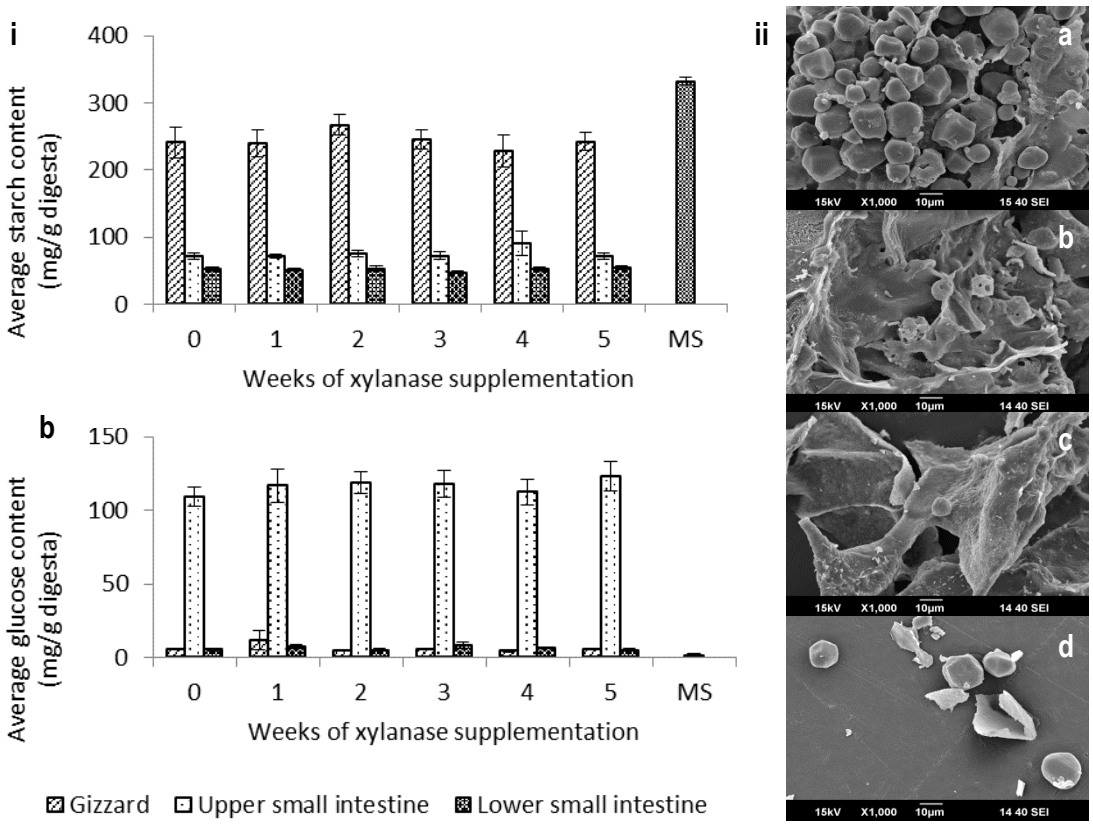

Figure 2. Progression of starch granule digestion through the tract of broilers. Graphs (i) show starch (a) and glucose (b) content of digesta taken from the gizzard, upper and lower small intestine of birds fed a xylanase-supplemented maize-soy diet (MS) for 0 (control), 1, 2, 3, 4 or 5 weeks before slaughter. SEM images (ii) show starch granule digestion as digesta moves from the gizzard (a) through to the upper (b) and lower (c) small intestine and into the caeca (d).

POSTER PRESENTATIONS

Additives

P-001 (ID 94)

THE EFFICACY OF CERTAIN FEED ADDITIVES FOR THE PREVENTION OF *CAMPYLOBACTER JEJUNI* INFECTION IN BROILER CHICKENS

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Summary

The efficacy of acidifiers (lactic and formic acids) and probiotics containing *Pediococcus acidilactici* (*P. acidilactici*) and *Saccharomyces boulardii* (*S. boulardii*) in reducing of *Campylobacter jejuni* (*C. jejuni*) infection in broiler chickens was investigated. Three birds out of 103 day old chick were euthanized for *C. jejuni* re-isolation. One hundred were assigned into 4 equal groups each; 25 birds. Groups 1, 2 and 3 were fed on ration containing acidifiers, *P. acidilactici* and *S. boulardii*, respectively; from day old till 5 weeks old. Group 4 was control. At 2 weeks old, each bird was orally challenged with *C. jejuni*. Groups were kept for 3 weeks recording signs and mortalities. Faecal swabs as well as liver and intestine were collected for *C. jejuni* shedding and re-isolation; respectively. Post-mortem lesions scoring of *C. jejuni* were calculated. Results cleared more severe signs of greenish diarrhoea in control than treated birds with no mortalities in all groups. Significant ($p<0.05$) reduction of *C. jejuni* shedding and re-isolation rates as well as lesions scoring was seen in groups treated with *S. boulardii* and *P. acidilactici* followed by acidifiers over control group. In conclusion, both acidifiers and probiotic preparations greatly reduced *C. jejuni* infection in broiler chickens.

Keywords: *C. jejuni*, Acidifiers, Probiotics, Chickens, Protection

Introduction

Campylobacter jejuni (*C. jejuni*) infection is considered as one of the most important bacterial disease causing acute human enteric infection in developing countries (SHANE, 2000). Natural alternatives are widely used nowadays to control enteric infections in poultry with promising results (MOUNTZOURIS et al., 2010). Using of different organic acids to reduce *C. jejuni* intestinal colonization was studied successfully **(**SANTOS et al., 2008**)**. Competitive exclusion compounds including probiotics are used effectively to protect chicks from intestinal pathogens colonization like *C. jejuni* **(**WILLIS and REID, 2008).

The aim of this study was to investigate the efficacy of acidifiers (lactic and formic acids) and probiotic preparations containing *Pediococcus acidilactici* (*P. acidilactici*) and *Saccharomyces boulardii* (*S. boulardii*) in reducing of *C. jejuni* infection in broiler chickens.

Materials and Methods

Three out of 103 day old, meat type chicks were euthanized to prove their freedom of *C. jejuni* infection. One hundred chicks were assigned into 4 groups each; 25 birds. Chicks of groups 1, 2 and 3 were fed on ration containing lactic and formic acids (1 kg/ton), *P. acidilactici* (100 g/ton) and *S. boulardii* (100 g/ton); respectively; from day old till 5 weeks old. Chicks of group 4 were kept as control. All groups were orally challenged with 0.5 ml containing $5X10^5$ *C. jejuni/* bird at 2 weeks old. Birds were kept for 3 weeks for signs and mortalities. Faecal swabs and liver and intestine were collected from all groups at different intervals for *C. jejuni* shedding and re-isolation; respectively. At 5 weeks old, a lesion scoring of C. jejuni after RABIE et al. (1998) was recorded. Microbiological examination of the organisms was done according to KONEMAN et al. (1995). Data were statistically analyzed and means were compared by one way ANOVA ($p<0.05$) using Post Hoc test according to SNEDECOR and COCHRAN (1980).

Results and Discussion

The results revealed no mortalities in all groups, but the severity of signs (depression and greenish diarrhae) was lower in treated than control birds. Figure (1) showed that the mean organ lesion score at 7, 14 and 21 days post infection was higher in acidifiers (2.04) than in *P. acidilactici* (1.40) and in *S. boulardii* (1.24) treated birds. Nevertheless, it was (3.04) in control group. Faecal swabs revealed presence of highly motile organisms. The rate of *C. jejuni* shedding decreased from 100 to 6.7% in acidifiers-treated group, from 100 to 0% in both *P. acidilactici* and *S. boulardii* treated birds, versus from 100 to 86.6% in control one (table 1). The rates of *C. jejuni* re-isolation showed descending patterns, viz. from 60 to 20% in acidifiers, from 80 to 20% in *P. acidilactici* treated groups, whereas it was 100% along 21 days post challenge in control group (figure 2).

Acidifiers and probiotics gave nearly similar degree of protection rate for *C. jejuni* infection in broilers (MOUNTZOURIS et al., 2010). PUCCI et al. (1988) mentioned that *P. acidilactici* can antagonize miceoaerophilic *C. jejuni* in their niches of intestine in which low dissolved oxygen tension exists. On the other hand, organic acids have a strong bactericidal effect on Campylobacter spp. (NASERI et al., 2012). EKLUND (1983) concluded that the un-dissociated acids diffused into the bacterial cell wall and released a proton that acidifies the cytoplasm. This result was supported by KROLL and PATCHETT (1991) and added that the cell death resulted from invisible denaturation of enzyme activity or DNA synthesis. Also, CHAVEERACH et al. (2004) showed that organic acids reduce the pH of crop to 3.8-4 and thus reduce the level of Campylobacter in the crop and the intestinal tract. However, treatments with viable probiotic bacterial cultures were effective in reducing *C. jejuni* in chickens due to bacteriocin (STERN et al., 2008). BLEHANT et al. (1989) explained the advantages of using yeast for combating enteropathogens as follows; yeast can grow at 37C, therefore be able to withstand the higher chickens body temperature, it is able to survive in low pH of the proventriculus and gizzard to reach the intestine and caeca where it can survive either aerobically and anaerobically and finally yeast is easily to be administered. Presence of mannose of *S. boulardii* on the intestinal cell wall may cause the yeast to act as a decoy for the attachment of pathogens (MILES, 1993), however RODRIGUES et al. (1996) attributed the protection to the ability of yeast to reduce the available amount of the produced toxins.

Conclusion

Both acidifiers and probiotic preparations could greatly assist in reduction and elimination of *C. jejuni* infection risk in broiler chickens.

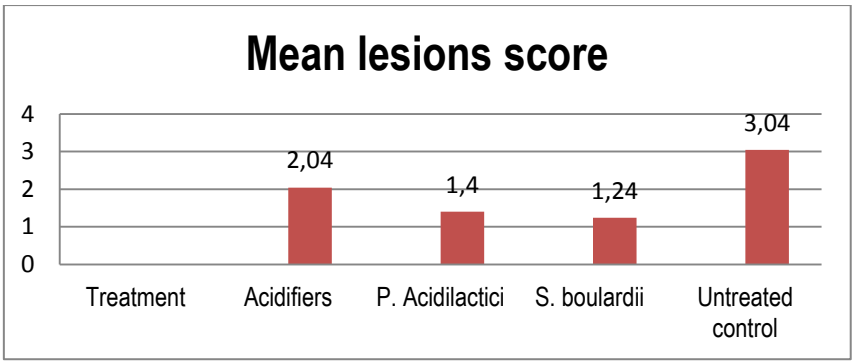

Figure (1): Post-mortem lesion scoring of *C. jejuni* in acidifiers and probiotic treated and untreated broiler chickens

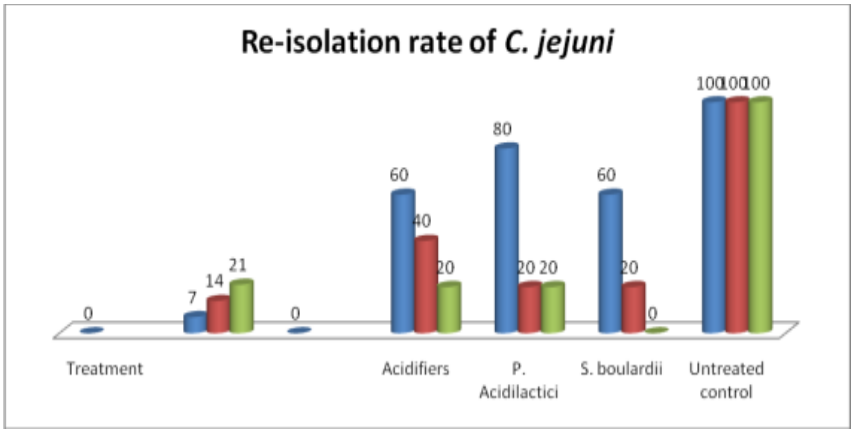

Figure (2): Re-isolation of *C. jejuni* in acidifiers and probiotic treated and untreated broiler chickens

Table (1) Shedding of *C. jejuni* in acidifiers and probiotic treated and untreated broiler chickens

Day post infection	Treatment											
	Acidifiers			*P. acidilactici*			*S. boulardii*			Untreated control		
	No. of birds	+ (%)	-	No. of birds	+ (%)	-	No. of birds	+ (%)	-	No. of birds	+ (%)	-
3	25	25 (100)	0	25	25 (100)	0	25	25 (100)	0	25	25 (100)	0
7	25	16* (64)	9	25	11* (44)	14	25	13* (52)	12	25	23 (92)	2
11	20	12* (60)	8	20	13* (13.3)	7	20	11* (55)	9	20	18 (90)	2
15	15	3* (20)	12	15	2* (13.3)	3	15	4* (26.6)	11	15	14 (93.3)	1
18	15	3* (20)	12	15	1* (6.7)	14	15	0* (0.0)	15	15	12 (80)	3
21	15	1* (6.7)	14	15	0* (0.0)	15	15	0* (0.0)	5	15	13 (86.6)	2

*Significant decrease over their untreated control group ($p<0.05$).
+= Positive
-= Negative

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P-005 (ID 28)

EFFECT OF SODIUM BENTONITE AND SEEDS OF *SILYBUM MARIANUM* ON LIVER ENZYMES, LYMPH ORGANS AND IMMUNE SYSTEM OF BROILER CHICKENS FED WITH AFLATOXIN

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Abstract

Aflatoxins (B1, B2, G1 and G2) are toxic groups of mycotoxins produced by Aspergillus flavus and Aspergillus parasiticus. Hepatotoxicity, weakening the immune system and mutagenic are the major effects of Aflatoxins in different animal species. In this study the effect of different levels of dietary sodium bentonite (SB), *Silybummarianum* seeds (SMS) and Aflatoxin B1 (AFB) on liver enzymes, lymph organs and immune system of broiler at the end of 42 days was studied. 224 Ross 308 broiler chicks in a completely randomized design based on factorial experiment (2 × 2 × 2) with 2 levels AFB (0and 500ppb), 2 levels SMS (0 and 0.5%) and 2 levels SB (0 and 0.5%) with 8 treatments, 4 replicates and 7 chickens per each. The experimental diets were fed from the first day. The results showed that compared with the control group, diets containing AFB significantly increased AST (Aspartate Aminotransferase) and ALT (Alanine Aminotransferase) however significantly decreased levels of immunoglobulin G ($p \leq 0.05$). SB with AFB causes a significant decrease in serum ALT levels and increase immunoglobulin G significantly ($p \leq 0.05$). Combination of SB, *SMS* and AFB decreased significantly serum AST compare to use each of them alone ($p \leq 0.05$). The results showed that the combination of SB and the SMS are more beneficial as separate application of them for deceased toxic effect of Aflatoxin B_1 in diets.

Keywords: Aflatoxin B1, Broiler, Immune system, Liver enzymes, Lymph

Introduction

Mycotoxins are metabolites from moulds. Aflatoxins, a group of closely related biologically active mycotoxins, are produced by strains of *Aspergillus flavus* and *Aspergillus parasiticus* (sujatha et al., 2003). Fungi or mould growth in feedstuff is associated with the utilization of nutrients from the host. Consequently, alterations in the nutritional content of the feedstuff are expected. The extent of mould growth determines the degree of depletion in the nutrient content of the feedstuff. The germ of the grain is the main site for *Aspergillus* sp. development, which leads to a greater potential for the synthesis of AFB (brekke et al., 1975). Layered alumino silicates, such as sodium bentonite, have been found effective in counteracting mycotoxins (eraslan et al., 2005). The effects of *silymarin* or various products made from the seed of the plant *Silybum marianum.* containing silymarin have also been tested on farm animals. The hepatoprotective activities were assessed on the basis of selected biochemical blood indicators in chicken broilers that had been exposed to the effects of aflatoxins (sujatha et al., 2003). The purpose of this study was to evaluate the changes of liver enzymes, lymph organ and immune system of broiler chickens when different levels of sodium bentonite and *Silybum marianum* seeds were used as a feed additive in their diet.

Material and Methods

Two hundred and twenty four of day old male Ross 308 broiler chickens obtained from a local hatchery were randomly distributed among eight treatments with four replicate groups per treatment and 7 chickens per replicate. the nutritional requirement of the chicks was based on Ross 308 company for starter (0-10 days) and grower (11-24 days) diets. Birds were distributed into the following eight treatments: 1) control 2)diet with (%) 500ppb aflatoxin B_1, 3) diet with 500ppb Aflatoxin B_1 and 0.5% *Silybummarinum* seeds, 4) diet with 500ppb aflatoxin B_1 and 0.5% Sodium Bentonite, 5)diet with 500ppb Aflatoxin B_1 and 0.5% *Silybummarinum* seeds and 0.5% Sodium Bentonite, 6)diet with 0.5% *Silybummarinum* seeds, 7) diet with 0.5% Sodium Bentonite, 8) diet with 0.5% *Silybummarinum* seeds and 0.5% Sodium Bentonite. At 42 day of age, two birds per pen were randomly selected and 2 mL of blood samples were obtained by wing puncture and centrifuged at 3000 rpm for 20 minto separate serum. Aspartate aminotransferase(AST), alanine aminotransferase

(ALT), Imnoglubulin G and M and relative organ weight (bursa and spleen) were analyzed using the general linear model procedure of SAS.

Results

The effect of experimental treatments on different parameters in Table 1 showed that compared with the control group, diets containing AFB significantly increased AST and ALT however significantly decreased levels of immunoglobulin G ($p \leq 0.05$). The increased serum AST and ALT activities observed by feeding naturally contaminated diets in the present study could be due to hepatic degeneration and subsequent leakage of enzymes into circulation (afzali and devegowda., 1999). SB with AFB causes a significant decrease in serum ALT levels and increase immunoglobulin G significantly ($p \leq 0.05$). Aflatoxin effects on humoral antibody levels compared to non-specific effects on the material seen below. However, interruption or reduction in antibody titer test has been reported against and reduce the amount of specific immunoglobulins (IgA and IgG) have been reported (campbell., 1983). These results are parallel with the findings reported (16), who observed a decrease in these parameters and an inhibition in protein synthesis during the aflatoxicosis in poultry. All treatment not significantly effects on relative weight of bursa and spleen. Combination of SB, ***SMS*** and AFB decreased significantly serum AST compare to use each of them alone ($p \leq 0.05$).

Conclusions

Dietary inclusion of sodium bentonite had not any adverse effects on serum biochemical characteristics and it can also improve the values of ALT in broiler chickens also results of the present study showed that consumption of level of SMS from 0.5 % in poultry diets can reduce the toxicity of AFB_1 in broiler chickens. But results showed that the combination of SB and the SMS are more beneficial as separate application of them for decreased toxic effect of Aflatoxin B_1 in diets.

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Table 1. effect of different treatment on liver enzymes (U/l), lymph organ (gr/kg live weight) and imnuglubolin G and M (mg/dl)

				AST	ALT	IGG	IGM	Bursa	Spleen
Main effects	Aflatoxin (ppm)	0		179.31^{b}	6.68^{b}	1.06^{b}	2.18	0.15	0.13
		500		208.90^{a}	8.87^{a}	1.75^{a}	1.56	0.14	0.13
	p-value			0.00	0.00	0.02	0.05	0.09	0.53
	Silybum (%)	0		196.18	8.25^{a}	1.56	2.18	0.15	0.13
		0.5		192.03	7.31^{b}	1.25	1.56	0.15	0.12
	p-value			0.25	0.03	0.28	0.05	0.77	0.42
	Bentonite (%)	0		206.58^{a}	8	1.06^{b}	2	0.15	0.12
		0.5		181.63^{b}	7.56	1.75^{b}	1.75	0.15	0.14
	p-value			0.00	0.31	0.02	0.42	0.87	0.07
	SEM			2.49	0.29	0.20	0.21	0.00	0.00
Dual interaction effects	Aflatoxin * silybum								
	0	0		178.50	6.62^{b}	1.37	2.75	0.15	0.13
	0	0.5		180.13	6.75^{b}	0.75	1.62	0.16	0.13
	0	500		213.86	9.87^{a}	1.75	1.62	0.14	0.13
	0.5	500		203.93	8.87^{b}	1.75	1.50	0.14	0.12
	p-value			0.11	0.01	0.28	0.12	0.77	0.23
	Aflatoxin * ben								
	0	0		175.58^{b}	6.37^{c}	1.12^{b}	2.25	0.16	0.12
	0	0.5		183.03^{b}	7^{bc}	1^{b}	2.12	0.15	0.14
	0	500		237.58^{a}	9.62^{a}	1^{b}	1.75	0.14	0.12
	0.5	500		180.21^{b}	8.12^{ab}	2.58^{a}	1.37	0.14	0.13
	p-value			0.00	0.01	0.00	0.69	0.66	0.74
	Silybum * ben								
	0	0		215.72^{a}	8.37	1.12	2.25	0.14	0.12
	0	0.5		176.63^{c}	8.12	2	2.12	0.15	0.14
	0	500		197.45^{b}	7.62	1	1.75	0.15	0.12
	0.5	500		186.62^{bc}	7	1.50	1.37	0.14	0.13
	p-value			0.00	0.66	0.51	0.69	0.11	0.89
	SEM			3.52	0.42	0.28	0.31	0.00	0.00
Treatments	AFB * silybum * Ben								
	0	0	0	177.12^{c}	6.25	1.75^{ab}	2.50	0.14	0/12
	0	0	0.5	179.87^{c}	7	1^{b}	3	0.17	0.14
	0	0.5	0	174.5^{c}	6.5	0.50^{b}	2	0.17	0.12
	0	0.5	0.5	186.22^{c}	7	1^{b}	1.25	0.14	0.14
	500	0	0	254.32^{a}	10.50	0.50^{b}	2	0.15	0.13
	500	0	0.5	173.40^{c}	9.25	3^{a}	1.25	0.14	0.14
	500	0.5	0	220.85^{b}	8.75	1.5^{ab}	1.50	0.14	0.11
	0.5	0.5	500	187.02^{c}	7	2^{ab}	1.50	0.15	0.12
	p-value			0.01	0.88	0.00	0.12	0.05	0.99
	SEM			4.98	0.59	0.40	0.43	0.00	0.01

Means within a column that do not have a common superscript are significantly different ($P<0.05$).

SEM= Standard error of means. AFB= Aflatoxin B1. Ben= Bentonite.

P-008 (ID 146)

COMPARATIVE EFFICIENCY OF TWO MARIGOLD-BASED YELLOW PIGMENTS ON EGGS TARGETING PASTA INDUSTRY

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Abstract

This study compared the pigmenting efficiency of 2 liquid marigold-based pigments (XAMACOL® 15-X15 and XAMACOL® Max-XM obtained through a new technology developed by Novus Int. Inc.) on eggs intended for the pasta industry. A total of 194 Hyline W-36 laying hens aged 55 weeks and fed a white basal diet were used. Trial included 5 weeks of xanthophyll depletion (days -35 to -1) and 4 weeks of experimental diets (days 0 to 28). Two products were tested: X15 (15 mg total xanthophylls-TX/g product) and XM at 15 and 12 mg TX/g product (XM15 and XM12). There were 10 treatments: X15 and XM15 at 4 supplementation levels (20, 40, 60, and 80 mg TX/kg feed), XM12 at 48 mg TX/kg feed, and a negative control (no yellow pigment added). All treatments included 2 mg/kg feed of red xanthophylls from paprika. Measurements: egg yolk color (Minolta CR400 and Yolk Color Fan-YCF) for all eggs laid on days 21-24, and β-carotene equivalents in whole liquid eggs laid on days 25-28. XM15 showed significantly higher pigmentation (redness, ratio redness/yellowness, and YCF) in the egg yolk and higher β-carotene equivalents content in whole liquid egg compared to X15. No differences were observed between 400 g product/100 kg feed of X15 vs XM12 in any of the variables measured, although the concentration of xanthophylls was 20% lower when using XM12 than X15 (48 vs 60 mg TX/kg feed). XM technology showed higher pigmenting efficiency compared to X15; therefore lower levels of XM can be used to reach equivalent results.

Keywords: carotenoids, layers, pigments, yellow xanthophylls, egg yolk

Introduction

Highly colored egg yolks are required for the food processing industry in applications such as pasta production. Carotenoids are compounds responsible for pigmentation in poultry, but only the fat soluble carotenoids (xanthophylls) have this property (Hencken, 1992). Poultry cannot synthesize these compounds and therefore must obtain them from the diets. To achieve optimal yolk coloration, laying hen diets should be supplemented with yellow and red xanthophylls (Belyavin and Marangos, 1987). The main yellow pigments used in poultry feed are marigold extracts and β-apo-8'-carotenoic acid ethyl ester (apo-ester), which are derived from natural and synthetic origin, respectively (Sirri et al., 2007). In recent years, there has been a notable trend toward the use of natural, rather than synthetic, supplements in animal nutrition (Galobart et al., 2004). XAMACOL® 15 (X15) is a traditional, liquid marigold-based pigment (Novus Int. Inc.), and XAMACOL® Max (XM) is a new liquid marigold-based product obtained through a new technology. This study compared the pigmenting efficiency of 2 liquid marigold-based pigments (X15 and XM) on eggs for pasta production.

Material and Methods

This study was conducted at Green Acres Research facility (Novus Int Inc., MO, USA). A total of 194 Hyline W-36 laying hens aged 55 weeks were kept individually in battery cages in an environmentally controlled room. The trial lasted 9 weeks with 5 weeks of xanthophyll depletion (day -35 to day -1) during which the animals received a common basal diet low in xanthophylls (based on wheat and soybean meal), and 4 weeks of feeding the experimental diets (day 0 to day 28). All rations (provided in mash form) were designed to meet or exceed all dietary recommendations for W-36 (based on Hy-line guide 2009-11).

Two products were tested: X15 (15 mg total xanthophylls-TX/g product) and XM at 2 different concentrations of active ingredients (15 and 12 mg TX/g product, XM15 and XM12, respectively). Treatments arose from the addition of the test product to the basal diet at the corresponding dosages. The 10 treatments included: X15 and XM15 at 4 supplementation levels (133, 267, 400, and 533 g product/100 kg feed, corresponding to 20, 40, 60, and 80 mg TX/kg), XM12 at a single dose (400 g product/100 kg feed, corresponding to 48 mg TX/kg), and a negative control (no yellow pigment

added). Each treatment included 2 mg/kg of red xanthophylls from paprika. Each treatment had 19-21 replicates of 1 hen except the negative control, which had 14 replicates.

General performance data were recorded (BW d0 and d29, FI d14 and d29, egg weight d0 and d21-28, egg production daily). Egg yolk color was assessed by a Minolta CR-400 colorimeter (CIELab scale {illuminant D65, 2º standard observer}) and YCF (using Egg Analyzer®, ORKA Food Technology, Israel) for all eggs laid during days 21-24. β-carotene equivalents in whole liquid eggs were measured in eggs laid during days 25-28.

All data were statistically analyzed using the appropriate procedures of SAS software package (version 9.2, SAS Institute Inc., Cary, NC, USA). Data were previously explored to discard any possible outlier. Differences were declared at $P<0.05$.

Results

No differences in performance were detected among treatments, which were considered normal for the conditions of this experiment (BWd0 = 1.53 ± 0.02 kg, BW d29 = 1.57 ± 0.02 kg, ADFI = 96.8 ± 0.56 g, egg weight = 65.1 ± 0.26 g/d, laying rate = 81 ± 0.7 %, FCR = 1.49 ± 0.01 g/g, mean ± SE, respectively). Because there were no differences in feed intake, differences in pigmentation are considered to be treatment related.

A treatment effect was observed in all egg yolk color variables and β-carotene equivalents when 10 treatments were compared. All treatments showed higher values compared to negative control, expect for luminosity (CIE L*) that was lower (data not shown).

Results from the factorial analyses (2 products: X15, XM15; 4 levels: 20, 40, 60, 80 ppm) showed higher pigmenting efficiency of XM15 compared to X15 (Figure 1). XM15 presented lower luminosity (CIE L*), and higher values in redness (CIE a*), redness/yellowness ratio (CIE a*/CIE b*), YCF and β-carotene equivalents compared to X15. No changes in yellowness (CIE b*) were observed among the two products at any dose.

An additional analysis was performed including only the three treatments with an inclusion level of 400 g product/100 kg feed (including X15, XM15, and XM12); these results are presented in Table 1. XM15 showed higher values in redness (CIE a*), redness/yellowness ratio (CIE a*/CIE b*), YCF and β-carotene equivalents compared to both X15 and XM12. XM12 demonstrated equivalent performance to X15 in the variables measured, despite having a 20% lower concentration of xanthophylls compared to X15 (48 vs 60 mg TX/kg feed).

Conclusions

XM technology showed higher pigmenting efficiency compared to X15; therefore, lower levels of active ingredients from XM can be used to reach equivalent results.

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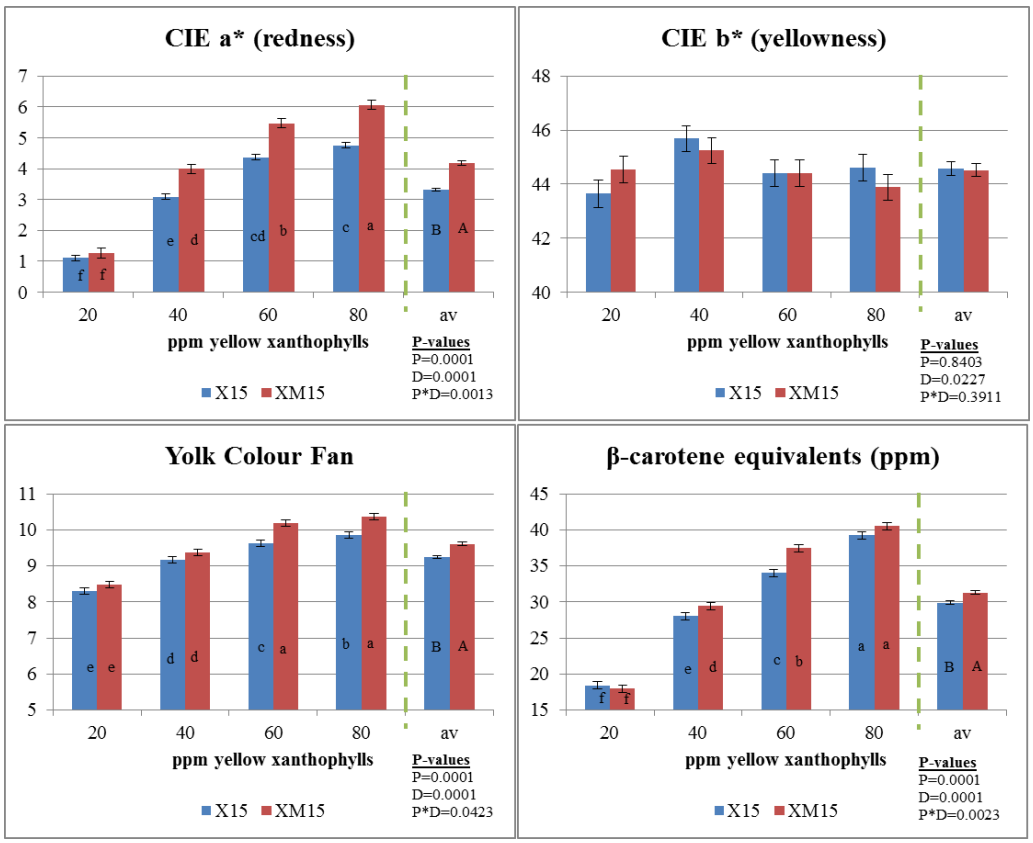

Figure 1. Effect of X15 and XM15 on egg yolk color (CIE-Lab system and yolk color fan scores) and on β-carotene equivalents in whole liquid egg.

Table 1. Effect of X15, XM15, and XM12 at 400 g product/100 kg feed on egg yolk color (CIE-Lab system and yolk color fan scores) and on β-carotene equivalents in whole liquid egg.

Yellow xanthophylls	**X15** (60 ppm)	**XM15** (60 ppm)	**XM12** (48 ppm)	SEM	***P*-value**
CIE L*	48.6	47.9	48.3	0.32	0.2793
CIE a*	4.37 b	5.46 a	4.64 b	0.145	0.0001
CIE b*	44.4	44.4	43.6	0.53	0.4907
CIE a*/CIE b*	0.099 b	0.124 a	0.108 b	0.0039	0.0001
YCF	9.62 b	10.19 a	9.63 b	0.087	0.0001
β-carotene (ppm)	34.0 b	37.5 a	34.6 b	0.49	0.0001

P-010 (ID 277)

EFFECTS OF FEEDING ORIGINAL XPC ON *SALMONELLA TYPHIMURIUM* OR *S. HEIDELBERG* PREVALENCE AND LOAD IN BROILER CHICKENS

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Abstract

Two *Salmonella* challenge studies were conducted in Cobb chicks and tested feeding XPC, a *Saccharomyces cerevisiae* fermentation product. Both were grown in floor pens; 11 pens with 25 chicks each (n=550; Study 1) or 8 pens with 50 chicks each (n=1200; Study 2). Two treatments were fed in Study 1: 1) Control or 2) 1.25 kg XPC/tonne from 0-21d and 0.62 kg XPC/tonne from 22-42d. In Study 2, the same two treatments were fed, in addition to a third: 1.25 kg XPC/tonne from 0-42d. All Birds in Study 1 were orally gavaged with a nalidixic acid resistant (NAR) strain of *S.* Typhimurium (10^9 CFU) at 7d. In Study 2, one-half of birds in each pen (n=25) were tagged and orally gavaged with NAR *S.* Heidelberg (10^9 CFU) at 0d. In Study 2, the 25 untagged birds in each pen were non-challenged and used to assess horizontal transmission (HT). In Study 1, fecal samples were collected at 14d and cecal samples collected at 35d from 3 birds per pen. In Study 2, cecal samples were collected from 10 birds per pen at 42d (5 tagged and 5 HT). Fecal and cecal samples were analyzed for *Salmonella*. FCR was statistically improved ($P<0.05$) in chickens fed XPC. *Salmonella* load was reduced ($P<0.05$) in feces of XPC fed birds in Study 1 (2.14 vs. 2.96 $\log_{10}$ CFU/g in XPC vs. Control, respectively). Cecal *Salmonella* prevalence (% positive) was reduced in birds fed 1.25 kg XPC/tonne (0-42d; $P<0.05$) in Study 2. Based on these results, XPC-fed chickens had improved FCR and lower prevalence and numbers of *Salmonella*.

Keywords: Salmonella Typhimurium, Salmonella Heidelberg, Food Safety, Chickens

Introduction

Salmonella is one of the leading causes of foodborne illness (Batz et al, 2012). Traditional methods for controlling *Salmonella* (pathogen) contamination have been focused within the processing plant, trying to prevent cross contamination through washing and evisceration (Berghaus et al, 2013). It has been suggested that if colonization of pathogens such as *Salmonella* could be controlled in the birds' intestinal tract prior to slaughter (preharvest), then contamination of processed birds will also be reduced. Research has shown that biosecurity and pest management measures may improve preharvest control of pathogens, but additional measures (i.e. dietary, vaccination) may be needed to have the optimal preharvest pathogen control (Dale et al, 2015). The objective of this research is to evaluate the effects of feeding Original XPC™, a *Saccharomyces cerevisiae* fermentation product, on fecal and cecal *Salmonella* Heidelberg and Typhimurium colonization and transmission following an oral challenge.

Materials and Methods

Two independent studies were recently conducted, in which broilers were orally gavaged with approximately 10^9 CFU of nalidixic acid resistant strains of *Salmonella* Typhimurium (Experiment 1) and *Salmonella* Heidelberg (Experiment 2). Experiments 1 and 2 were conducted by Colorado Quality Research in Wellington, CO and Southern Poultry Research Group near Nicholson, GA, respectively.

Experiment 1: Salmonella Typhimurium Trial

Cobb chicks (n = 550) were assigned to one of 2 feed treatments (Control vs. XPC), with 11 replicate floor pens per treatment and 25 birds per pen. Control birds received industry standard diets with salinomycin but no antibiotic growth promoter. The XPC treatment received the same rations with the addition of Original XPC at 1.25 kg/tonne in the starter feed and 0.62 kg/tonne in the grower and finisher feeds. All birds and feed were weighed by pen at 0, 14, and 35 d and feed conversion calculated.

At 7 d, the number of birds per pen was adjusted to 23 and then all birds were challenged with *S*. Typhimurium. At 14 d, fecal samples were collected from three birds per pen. At 35 d, three birds per pen were euthanized and the ceca removed. Feces and cecal contents were diluted with 0.1% peptone and either direct plated or enriched in tetrathionate

broth with brilliant green and potassium iodide and plated on MacConkey Agar containing nalidixic acid to enumerate *S*. Typhimurium.

Experiment 2: Salmonella Heidelberg Trial

Cobb chicks (n = 1200) were housed in 24 floor pens (50 birds/pen) and assigned to one of 3 feed treatments (8 replicates each). Control birds (T1) received industry standard diets with no antibiotics or coccidiostat. Treatments T2 and T3 received the control starter diet with the addition of XPC at 1.25 kg/tonne from 0-21 d. After 21 d, XPC was reduced to 0.62 kg/tonne for T2 birds, while T3 birds continued to receive 1.25 kg/tonne of XPC. All birds and feed were weighed by pen at 0, 21, 35 and 42 d, then feed conversion was calculated. All birds were vaccinated with Coccivac-B at 0 d.

At 0 d, 25-tagged birds per pen were challenged with *S*. Heidelberg. The remaining 25 birds per pen did not receive a tag or *S*. Heidelberg challenge, and were used to induce horizontal transmission (contact challenge between *S*. Heidelberg gavaged and non-gavaged birds). Ten birds per pen (5 tagged and 5 non-tagged) were sampled at 42 d and the ceca removed for *S*. Heidelberg colonization evaluation. Drag swabs (dragging sterile gauze swabs soaked in double strength skim milk) were taken from each pen (feces) at 42 d. Cecal and drag swab samples were diluted in buffered peptone water and spread on XLT-4 plates to determine *S*. Heidelberg positive samples.

Results

Body weight gains were not significantly different ($P > 0.05$) between treatments in both the broiler studies. Feed conversion was significantly improved ($P < 0.05$) by the addition of XPC through 14 and 35 d in Experiment 1 (Table 1). Feed conversion was also significantly improved ($P < 0.05$) with 1.25 kg XPC/tonne (T3) through 35 d and with both XPC inclusion levels (T2 and T3) through 42 d (Table 1).

Fecal *S.* Typhimurium numbers (log10 CFU) were significantly reduced ($P = 0.051$) by 14 d when XPC was fed (Table 2). By 35 d, the *S.* Typhimurium numbers (CFU; Table 2) in the cecal contents were low and not significantly different ($P > 0.05$) between treatments. In Experiment 2, no statistical differences ($P > 0.05$) in *S*. Heidelberg positive drag swabs were observed between treatments (Table 3). However, prevalence of *S.* Heidelberg positive ceca was significantly reduced ($P < 0.05$) when 1.25 kg/tonne (T3) XPC was fed continuously, and intermediate when 0.62 kg XPC/tonne (T2) was fed during grower/finisher period (Table 3).

Table 1. Feed conversion ratio (FCR) of broilers in both experiments.

	Experiment 2 - FCR		Experiment 1 - FCR	
Bird Age:	0-14 d	0-35 d	0-35 d	0-42 d
T1; Control	1.38[a]	1.62[a]	1.68[a]	1.79[a]
T2; XPC (1.25 and 0.62 kg/tonne)[1]	1.35[b]	1.61[b]	1.66[ab]	1.77[b]
T3; XPC (1.25 and 1.25 kg/tonne)[1]	-	-	1.64[b]	1.75[b]
P value	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$

[ab] Values within a column with different superscripts are statistically different ($P < 0.05$).
[1] Starter and grower/finisher dietary inclusion rates, respectively.

Table 2. Broiler *Salmonella* Typhimurium (ST) in feces and ceca at 14 and 35 day of age, respectively, in Experiment 1.

Treatment	ST fecal numbers at 14 d		ST cecal numbers (CFU/g)
	CFU/g	Log10 CFU/g	
Control	51,094	2.96[a]	94
XPC (1.25 and 0.62 kg/tonne)[1]	6,623	2.14[b]	20
P value	$P > 0.05$	$P = 0.051$	$P = 0.12$

[ab] Values within a column with different superscripts are statistically different ($P = 0.051$).
[1] Starter and grower/finisher dietary inclusion rates, respectively.

Table 3. Broiler *Salmonella* Heidelberg (SH) prevalence in fecal swabs and ceca at 42 day of age in Experiment 2.

Treatment	SH fecal[2] prevalence (% Positive)	SH cecal prevalence (% Positive)
T1; Control	100	38.3[a]
T2; XPC (1.25 and 0.62 kg/tonne)[1]	100	33.3[ab]
T3; XPC (1.25 and 1.25 kg/tonne)[1]	88	17.5[b]
P value	$P > 0.05$	$P < 0.05$

[ab] Values within a column with different superscripts are statistically different ($P < 0.05$).
[1] Starter and grower dietary inclusion rates, respectively.
[2] Pen drag swabs collected from bedding material.

Conclusions

A reduction in *S.* Typhimurium numbers was observed in broilers fed XPC. *S.* Heidelberg prevalence was reduced in the ceca of broilers fed XPC. Improvements in feed conversion were observed in broilers fed XPC in both experiments.

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P-011 (ID 220)

VITAMIN D ENRICHMENT OF EGGS FOR HUMAN HEALTH

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Abstract

Vitamin D is an essential component of vertebrate nutrition and epidemiological surveys confirm a chronic vitamin D insufficiency in the human population. Eggs are one of the few natural sources rich in vitamin D, containing both vitamin D_3 (D_3) and 25-hydroxyvitamin D_3 (25(OH)D_3). Can additional dietary vitamin D significantly increase the vitamin D content of table eggs? In order to establish the potential for enrichment of eggs with D_3 and 25(OH)D_3 a total of 162 hens were fed three levels of D_3, (2500 and 5000 IU/kg) in combination with three levels of 25(OH)D_3 (0, 34.5 and 69 µg/kg). Egg yolks were analysed for both their D_3 and 25(OH)D_3 content and egg production, egg weights and feed efficiencies recorded.

The concentration of D_3 and 25(OH)D_3 in egg yolk significantly increased with increasing dietary concentration. There was no significant difference in egg mass, egg weight or feed efficiency. Depending on the dietary concentrations used, it was possible to produce an egg with between 100-500 IU vitamin D, providing scope to help meet the recommended daily requirement of vitamin D for children or adults. The dietary addition of higher levels of D_3 and 25(OH)D_3 produced eggs with sufficient vitamin D to help meet the recommended daily requirements of adults and children without detrimental effect on production parameters.

Keywords: vitamin D, vitamin D_3, eggs, 25-hydroxyvitamin D_3, laying hens

Introduction

Vitamin D is an essential nutrient in vertebrate nutrition for the maintenance of good health and well being however epidemiological studies in most countries indicate a widespread vitamin D insufficiency in the population which potentially may have a detrimental effect on health. For example in Germany, a comprehensive survey was undertaken by Hintzpeter *et al*. (2008) which showed a widespread deficiency in vitamin D. This data is shown in Table 1.

Table 1. Prevalence of vitamin D-deficient and replete states by gender and season[a]

Extracted from*: Vitamin D status and health correlates among German adults.(Hintzpeter et al., 2008)*

	Severe deficiency (<12.5 nmol/l)	Moderate deficiency (12.5–25 nmol/l)	Mild deficiency (25–50 nmol/l)	Replete (>50 nmo/l)
Men				
Total (%)	2.2	13.4	41.2	43.2
May–October all ages (%)	1.1	8.7	35.4	54.8
Nov–April all ages (%)	3.3	18.1	46.8	31.8
Women				
Total (%)	1.9	15.1	40.8	42.2
May–October all ages (%)	0.9	11.5	42.4	45.2
Nov–April all ages (%)	2.7	18.8	39.3	39.2

[a] Categories of serum 25OHD levels as described by Lips (2004). The sum of each row is 100%.

Egg yolks are rich in vitamin D (Parrish and Richter, 1979) because of an efficient transfer by the domestic fowl of vitamin D from the feed into the yolk and unlike other animal-based foods the egg yolk contains both D_3 and 25(OH)D_3, in fact eggs contain higher levels of 25(OH)D_3 than any other animal-based food such as fish and meat (Mattila et al., 1995). The fact that eggs contain 25(OH)D_3 is significant because in human nutrition 25(OH)D_3 has five times the relative biological vitamin D activity as compared to D_3 (Koshy and Van Der Slik, 1979, Reeve et al., 1982, Mattila et al.,

1993). The objective of this study was to evaluate the efficiency of deposition of vitamin D into egg yolks following the supplementation of both D_3 and $25(OH)D_3$ in the feed of the laying hen.

Materials and Methods

A total of 162 Isa-Brown laying hens of 58 weeks of age were divided into nine treatment groups of 18 birds each, being composed of six replicates of three birds per replicate. Each bird was housed separately in cages measuring 25 x 50 x 50 cm^2, with three adjacent cages forming the replicate unit. Prior to the commencement of the trial all birds were fed for 4 weeks on the control diet with 2,500 IU D_3/kg (acclimatisation period). During the subsequent trial period of 9 weeks, the birds were fed a mash diet *ad libitum* with the only difference between treatment groups being the inclusion of various levels of vitamin D_3 and $25(OH)D_3$. The photoperiod regime was 16 hours of light and 8 hours of dark. Water was supplied *ad libitum.* An outline of the nine dietary treatments is shown in Table 2 and the inclusion rates of D_3 and $25(OH)D_3$ are stated in terms of both international units and µg/kg of feed.

Table 2. Outline of dietary treatments

Die t	Diet Description	Added D_3 †IU/kg	Added $25(OH)D_3$††µg/kg
1	Normal D_3	2,500	0
2	Normal D_3 + 34.5 µg $25(OH)D_3$	2,500	34.5 µg/kg
3	Normal D_3 + 69.0 µg $25(OH)D_3$	2,500	69.0 µg/kg
4	Medium-high D_3	5,000	0
5	Medium-high D_3+ 34.5 µg $25(OH)D_3$	5,000	34.5 µg/kg
6	Medium-high D_3+ 69.0 µg $25(OH)D_3$	5,000	69.0 µg/kg
7	High D_3	10,000	0
8	High D_3 + 34.5 µg $25(OH)D_3$	10,000	34.5 µg/kg
9	High D_3 + 69.0 µg $25(OH)D_3$	10,000	69.0 µg/kg

†DSM Nutrition I Products Pty Ltd Rovimix® D_3 500 ††DSM Nutritional Products Rovimix®HY·D®

Throughout the 9 week trial all eggs were collected and weighed for each replicate group. The feed supplied to each replicate group was weighed out weekly and on completion of the experiment all remaining feed was weighed and feed conversion efficiency determined. At the end of the 9 week trial a total of five eggs per replicate (30 eggs per treatment) were collected and their yolks separated, weighed, blended and freeze dried prior to analysis for D_3 and $25(OH)D_3$ content. The average weight of the egg yolks per replicate was used to calculate the D_3 and $25(OH)D_3$ content of egg yolk per replicate (6 replicates per treatment).

Results

After 9 weeks of feeding, the analytical results for the concentration of both D_3 and $25(OH)D_3$ in egg yolk for each replicate group are shown in Table 3. The D_3 content per kg of egg yolk was increased *(P*<0.01) with the addition of higher concentration of D_3 (5,000 IU D_3/kg and 10,000 IU D_3/kg) compared with the low concentration of D_3 (2,500 IU D_3/kg) in layer feed. However, the higher dietary levels of D_3 did not significantly change the $25(OH)D_3$ content of egg yolk. In contrast, the addition of $25(OH)D_3$ (34.5 µg $25(OH)D_3$/kg and 69.0 µg $25(OH)D_3$/ kg) in the diet of laying hens not only significantly increased the $25(OH)D_3$ content of egg yolk *(P<0.01)* but also significantly increased the D_3 content of egg yolk *(P<0.01)*. Consequently there was a significant positive interaction *(P*<0.05) between D_3 and $25(OH)D_3$ when $25(OH)D_3$ was included in the diet.

Not surprisingly the highest concentration of total vitamin D in egg yolk (in terms of IU per 100 g of egg yolk) was achieved at the highest level of D_3(10,000 IU D_3/kg feed) in the feed in combination with the highest level of $25(OH)D_3$ (69 µg $25(OH)D_3$/kg feed) in the feed.

Table 3. Effect of various levels of D_3 and $25(OH)D_3$ supplementation on D_3, $25(OH)D_3$ and total vitamin D content of egg yolk

D_3 (IU/kg)	$25(OH)D_3$ (µg/kg)	D_3 per 100g yolk (µg)	$25(OH)D_3$ per 100 g yolk (µg)	Total vit D† per 100 g yolk (IU)	Total vit D[1] per egg yolk (IU)
2500	0	6.48^{cd} (±1.462)[2]	1.61^{f} (±0.757)	580^{e} (±201.7)	96^{f} (±33.1)
2500	34.5	5.96^{cd} (±1.444)	3.30^{d} (±1.448)	898^{d} (±315.2)	152^{e} (±50.2)
2500	69.0	4.90^{d} (±0.532)	4.49^{c} (±1.242)	1094^{cd} (±246.2)	185de (±41.4)
5000	0	10.51^{c} (±2.270)	2.06^{ef} (±0.752)	832de (±186.1)	146^{e} (±39.6)
5000	34.5	7.43^{cd} (±1.673)	4.51^{c} (±1.011)	1199^{c} (±261.5)	213^{cd} (±50.1)
5000	69.0	8.07^{cd} (±3.022)	5.81^{b} (±0.656)	1484^{b} (±172.1)	256^{bc} (±27.5)
10000	0	26.17^{ab} (±8.280)	3.01^{de} (±0.392)	1649^{b} (±325.1)	270^{b} (±56.4)
10000	34.5	23.60^{b} (±5.208)	3.71^{cd} (±0.481)	1686^{b} (±173.0)	285^{b} (±25.6)
10000	69.0	30.85^{a} (±5.384)	8.05^{a} (±1.117)	2845^{a} (±251.3)	478^{a} (±39.40)
Pooled SEM		*1.648*	*0.381*	*99.3*	*16.98*
Model P		*<0.01.*	*<0.01*	*<0.01*	*<0.01*
Main Effects					
2500		5.78^{c}	3.13^{c}	858^{c}	144^{c}
5000		8.67^{b}	4.12^{b}	1172^{b}	205^{b}
10000		26.87^{a}	4.93^{a}	2060^{a}	344^{a}
P		*<0.01*	*<0.01*	*<0.01*	*<0.01*
	0	14.39^{a}	2.23^{c}	1021^{c}	171^{c}
	34.5	12.33^{a}	3.84^{b}	1261^{b}	217^{b}
	69.0	14.61^{a}	6.12^{a}	1808^{a}	307^{a}
	P	*N.S.*	*<0.01*	*<0.01*	*<0.01*
Interaction Terms					
D^3*$25(OH)D_3$		*N.S.*	*N.S.*	*P<0.01*	*P<0.01*

Means in columns with no common superscript differ significantly

†Total vitamin D (IU) is calculated by the addition of D_3 (µg/kg egg yolk) and $25(OH)D_3$ (µg/kg egg yolk) where 1.0 µg of D_3 equals 40 IU vitamin D and 1.0 µg of $25(OH)D_3$ equals 200 IU vitamin D.

Conclusion

Epidemiological surveys indicate a recognised chronic insufficiency of vitamin D within the human population. Vitamin D is essential for good skeletal health, healthy skin and a sound immune system and chronic insufficiencies may cause many diseases including osteoporosis. There are few naturally occurring foods which contain significant dietary levels of vitamin D, however the modern commercial laying hen has been shown to be unique in its ability to efficiently transfer vitamin D metabolites from its diet into egg yolk. Egg yolk represents one of the few foods which is rich in both D_3 and $25(OH)D_3$. This experiment has demonstrated that a single egg from a laying hen supplemented with increased levels of D_3 and $25(OH)D_3$ has the potential to contribute significantly to the recommended daily intake of vitamin D for both adults and children without detrimental effect on production parameters.

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P-012 (ID 198)

EFFECT OF NUCLEOTIDES ENRICHED YEAST EXTRACTS ON VILLUS LENGTH AND GOBLET CELL COUNTING IN BROILER INTESTINE

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Abstract

Dietary nucleotides have a key nutritional role in the development of tissues with a high renewal rate such as the intestine. In periods of fast growth, these nutrients can become conditionally essential so that exogenous supply may benefit animal development. Taking into account the dramatic changes that occur in the chicken intestine after hatching, our aim was to investigate two indicators of intestinal health (villus development and goblet cell counting) in animals fed four different diets supplemented with nucleotides. These diets were supplemented with yeast extracts containing different nucleotide concentrations (0-0.6 %). Cryostatic slices of the duodenum and ileum of 10-day-old male Ross chickens were prepared and treated with periodic acid-Schiff to stain mucus glycoproteins. Villus length and the number of goblet cells per epithelial length were measured in micrographs of known magnification. The results obtained indicate that beneficial effects of supplemented nucleotides are exerted in the duodenum by increasing the number of goblet cells from 12 to 22 % ($p<0.05$) and in the ileum by increasing villus length from 9 to 19 % ($p<0.05$). In conclusion, the inclusion of nucleotides-enriched yeast extracts in the starter diet for broilers may have beneficial effects by increasing absorptive surface area and improving the role of mucus in intestinal barrier function.

Keywords: poultry, nutrition, absorptive surface area, epithelial barrier function, mucus

Introduction

Nucleotides have significant effects on the development of tissues with a high replacement rates, such as the immune system and gastrointestinal tract (Gardiner et al., 1995). During the early stage of development, a period of fast growth, the availability of nucleotides can limit the maturation of these tissues and nucleotides can become conditionally essential so that exogenous supply may benefit animal development. In this sense, Jung and Batal (2012) have described in the jejunum of 14-day-old chickens fed a diet supplemented with nucleotides an increase in villus length, an effect which has been associated to an improvement of animal growth and conversion rate (Rutz et al., 2006; Esteve-García et al., 2007; Liotta et al. 2010; Jung and Batal, 2012). Taking into account the dramatic changes that occur in the chicken intestine after hatching (Geyra et al., 2001), our aim was to investigate in 10-day-old chickens, the effect of increasing dietary nucleotide supplementation on two indicators of intestinal health, villus length as an indicator of the absorptive available surface area and goblet cell counting as an indicator of epithelial barrier function.

Material and Methods

Male Ross 308 chickens (Granja Crusvi, Montblanc) were fed *ad libitum* from hatching until day 10 with balanced diets (Institut de Recerca i Tecnologia Agroalimentàries, IRTA; 3 replicates per treatment, 33 birds per replicate in floor pens) supplemented with yeast extracts containing increasing nucleotide concentrations (0, 0.2, 0.4 and 0.6 % in feed, Phileo-Lesaffre Animal Care, France, Control and treatments 0.2, 0.4 and 0.6, respectively). Villus length and goblet cell counting were determined in 6 animals per treatment (2 birds per replicate, randomly selected). After the animals were euthanized by cervical dislocation, the duodenum and the ileum were washed with ice-cold saline (NaCl 9 g/L, 4°C). Intestinal portions (5 mm in length) were taken and fixed in 3.0 % paraformaldehyde in 0.1 mol/L PBS (pH 7.4) during 2 h at room temperature. After fixation, the samples were cryoprotected in ice cold sucrose gradient in 0.1 mol/L PBS, mounted in optimal cutting temperature (Aname, Barcelona) and stored at -20 °C. Four groups of seven serial transverse cryostatic sections (10 µm, CM 3050 S, Leica, Wetzlar, Germany) were obtained from each intestinal portion at intervals of 300 µm to guarantee the observation of different villi. The samples were treated with periodic acid-Schiff to stain glycolproteins from mucus (Shao et al., 2013) and mounted with ProLongol (Life Technologies, Carlsbad, CA, E.U.A.).

The images were captured with an Olympus BX41 microscope (Tokyo, Japan) at two magnification levels: 20 x for villus length and 100 x for goblet cell counting. Villus length was measured using the *lamina propria* as the base (Uni et al., 1995) in images processed by IMAT software (Centres Científics i Tecnològics of the Universitat de Barcelona). Goblet cell counting was performed in printed images of known magnification and refereed to epithelial length. The animal was used as the experimental unit. The statistical analysis was performed by ANOVA followed by a post-hoc test comparing the mean values of the treatments with the negative treatment (Control). $P < 0.05$ was considered to denote significance.

Results

The results of villus length revealed (Figure 1A), as we have previously observed for Leghorn chickens (Mitjans et al., 1997), higher values in the duodenum than in the ileum ($P < 0.05$). Regarding the effect of the different treatments, the statistical analysis of the duodenal data indicated a progressive decrease for treatments 0.2 and 0.4 and no effect for the highest nucleotide concentration (treatment 0.6). In the ileum, the comparison of the supplemented diets with the Control revealed a progressive increase with nucleotide concentration.

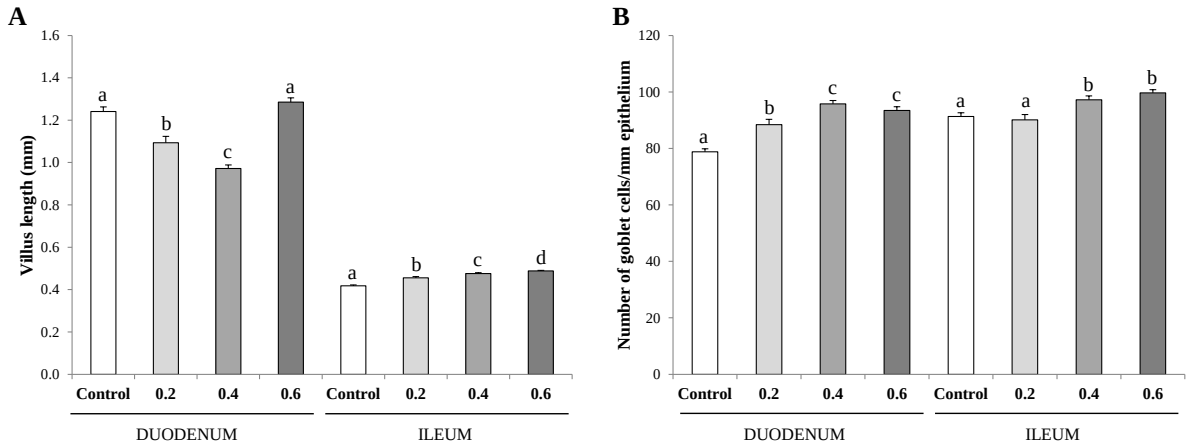

Figure 1. Villus length (A) and number of goblet cells (B) in the duodenum and ileum of 10-day-old chickens feed the different treatments. Results were expressed as mean ± SEM (n = 6 animals). Different letters denote significant differences ($P < 0.05$) within de duodenum and the ileum.

The values of goblet cell counting (Figure 1B) for the Control treatment were higher in the ileum than in the duodenum ($P < 0.05$). As for the comparison between treatments, the statistical analysis revealed in the duodenum, an increase for treatments 0.2 and 0.4 and no differences between treatments 0.4 and 0.6 and in the ileum, no effect for treatment 0.2 and a similar increase for treatments 0.4 and 0.6.

Conclusions

The data obtained indicate that the best results were found for the highest nucleotide concentration (0.6 %) which induced no changes in villus length and a 22 % increase in the number of goblet cells in the duodenum, and in the ileum, an increase of 19 % in villus length and 9 % in goblet cell counting. In conclusion, the inclusion of nucleotides-enriched yeast extracts in the starter diet for broilers may have beneficial effects by increasing absorptive surface area and improving the role of mucus in intestinal barrier function.

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P-013 (ID 193)

EFFECT OF THE INCLUSION OF NA-BUTYRATE IN DIETS BASED ON CORN OR WHEAT, WITH DIFFERENT AMEN CONTENT, ON GROWTH PERFORMANCE OF BROILER REARED UNDER POOR HYGIENE CONDITIONS

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Abstract

In total, 800 one-d-old, straight run Ross 308 broilers were used to study the inclusion of 0.1% of a chemically protected Na-butyrate (Butirex C4) in diets based on corn or wheat with high (HE) or low (LE) AMEn content (60 kcal/kg difference) on growth performance of broilers. Chicks were placed in their respective pens immediately after the removal of the previous batch of birds, without any cleaning or disinfection. The experimental design was completely randomized with 8 treatments arranged as a 2 x 2 x 2 factorial with 2 cereals sources (corn vs. wheat), 2 AMEn concentration (HE vs. LE), and 2 levels of Na-butyrate (0 vs. 0.1%). Each treatment was replicated 4 times (a pen with 25 chicks). The trial lasted 40 d. From d 0 to 20, ADG ($P < 0.05$) and FCR ($P < 0.01$) were better in broilers fed corn than in broilers fed wheat. Also, FCR ($P < 0.01$) was improved in broilers fed the HE diets. Na-butyrate supplementation increased ADG ($P < 0.001$) and ADFI ($P < 0.001$) but did not affect FCR. Cumulatively (0 to 40 d of age), chicks fed corn tended to have better FCR than chicks fed wheat ($P < 0.10$) and chicks fed the HE diets had better FCR ($P < 0.05$) than chicks fed the LE diets. Na-butyrate increased ADG ($P < 0.01$) and ADFI ($P < 0.05$) of the birds. In summary, the supplementation with protected Na-butyrate improved ADG and ADFI from hatch to slaughter in broilers reared under poor hygiene conditions, independent of the main cereal and the energy content of the diet.

Keywords: broiler, cereal, growth performance, Na-butyrate

Introduction

The ban of use of antibiotics as “in feed” growth promoters in poultry diets has increased the interest on the utilization of other active feed additives (MATEOS et al., 2002). The inclusion in the diet of short chain fatty acids, such as butyric acid, might acidify the luminal contents and help in the control of the growth of the pathogen microbiota. Butyric acid, however, has a very unpleasant odor that reduces diet palatability (ANTONGIOVANNI et al., 2007). Consequently, this acid is frequently marketed as a salt, which is odourless and easier to handle. Several researches have compared diets based on corn or wheat in broilers (MASEY-O´NEILL et al., 2014). Broilers eat to satisfy their energy requirements and therefore, an increase in the AMEn content of the diet should result in a concomitant increase in feed intake and in an improvement in feed conversion (FCR) (SALEH et al., 2014). However, the effects of changes in the AMEn of the diet on average daily gain (ADG) are not well studied in broilers. The aim of this research was to study the effects of Na-butyrate supplementation on growth performance of broilers fed different type of diets when reared under dirty environmental conditions.

Material and Methods

In total, 800 one-d-old, straight run Ross 308 broilers were received from a commercial hatchery and randomly distributed in groups of 25 into 32 pens. Chicks were placed in the experimental barn immediately after the removal of the previous batch, without any cleaning or disinfection. The pens (1.1 m x 1.1 m) were bedded with wood shaving and provided with a hopper feeder and a bell drinker. The environmental conditions during the experiment were controlled automatically according to the age of the birds. The feeding program consisted in 3 diets supplied from 1 to 20 d (starter), 20 to 35 d (grower) and 35 to 40 d (finisher) of age. All diets met or exceeded the nutritional requirements for broilers (FUNDACIÓN ESPAÑOLA DESARROLLO NUTRICIÓN ANIMAL, 2008) and were presented as 3 mm Ø pellet. Within each of the 3 feeding periods, all diets were formulated to have the same AMEn:indispensable amino acid ratio. The diets were based on corn or wheat and contained 3,000 and 2,940 kcal/kg for the started diets, 3,140 and 3,077 kcal/kg for the grower diets and 3,170 and 3,107 kcal/kg for the finisher diets, for the high (HE) and low (LE) energy diets, respectively. Each of these 4 experimental diets by periods was supplemented or not with 0.1% Na-butyrate. Body weight of the chicks and feed consumption were determined by pen on d 1, 20, 35 and 40, and mortality was re-

corded and weighed as produced. From these data, ADG, average daily feed intake (ADFI) and FCR were determined. The experimental design was completely randomized with 8 dietary treatments arranged as a 2 x 2 x 2 factorial with 2 cereal sources (corn vs. wheat), 2 nutritional levels of the diet (differing in 60 kcal AMEn/kg) and 2 levels of inclusion of Na-butyrate (0 vs. 0.1%). Each treatment was replicated 4 times, and the experimental unit was a pen with 25 chicks. At d 20, after the growth performance control, the number of birds per pen was reduced to 15 to fit the European legislation on rearing density of broilers. Excess of birds were removed at random within each pen.

Results

Mortality was 2.1% and was not related to treatment. From 0 to 40 d of age, FCR tended to be better in broilers fed corn than in broilers fed wheat (1.60 vs. 1.63; P = 0.094) and in broilers fed the HE diets than in broilers fed the LE diets (1.60 vs. 1.63; P < 0.05) (Table 1). Na-butyrate inclusion increased ADG (75.8 vs. 73.6 g; P < 0.01) and ADFI (122.1 vs. 119.2 g; P < 0.05). From 0 to 20 d of age, broilers fed the corn based diets had greater ADG (41.3 vs. 40.5 g; P < 0.05) and better FCR (1.34 vs. 1.39, P < 0.01) than broilers fed the wheat based diets. Broilers fed the LE diets had greater ADFI (56.8 vs. 55.0 g, P < 0.05) than chicks fed HE diets. Broilers fed Na-butyrate supplemented diets showed greater ADG (42.1 vs. 39.7 g; P < 0.001) and ADFI (57.8 vs. 54.0 g; P < 0.001) than broilers fed the control un-supplemented diet, but FCR was not affected.

Conclusions

In summary, FCR was improved with corn or high energy diets. The addition of 0.1% of Na-butyrate in broilers feed is especially recommended at early ages (from 0 to 20 d of age) after this age, the effect is diluted.

Table 1. Effects of the main cereal, AME_n concentration, and inclusion of Na-butyrate (Butyrate) in the diet on growth performance from 0 to 40 days.

	0 - 20 d			20 - 35 d			35 - 40 d			0 - 40 d		
	ADG[1] (g)	ADFI[2] (g)	FCR[3]	ADG (g)	ADFI (g)	FCR	ADG (g)	ADFI (g)	FCR	ADG (g)	ADFI (g)	FCR
Cereal												
Corn	41.3	55.5	1.34	108.4	174.3	1.61	110.6	217.4	1.97	75.1	120.3	1.60
Wheat	40.5	56.4	1.39	107.5	175.5	1.63	110.5	216.0	1.95	74.3	121.2	1.63
AME_n, kcal/kg												
2,940	40.8	56.8	1.39	108.5	177.0	1.63	110.3	217.2	1.97	74.9	122.0	1.63
3,000	41.0	55.0	1.34	107.3	172.8	1.61	110.8	216.1	1.95	74.6	119.3	1.60
Butyrate, %												
-	39.7	54.0	1.36	106.4	173.6	1.63	111.1	216.6	1.96	73.6	119.2	1.62
0.1	42.1	57.8	1.37	109.4	176.3	1.61	110.1	216.7	1.97	75.8	122.1	1.61
SEM[4]	0.265	0.482	0.0109	1.017	1.725	0.0124	1.846	2.205	0.0244	0.515	0.940	0.00963
						P-value						
Cereal	0.034	0.248	0.004	0.649	0.658	0.278	0.992	0.659	0.617	0.367	0.606	0.094
AME_n	0.704	0.021	0.005	0.427	0.115	0.223	0.716	0.723	0.393	0.703	0.064	0.045
Butyrate	<0.001	<0.001	0.472	0.064	0.305	0.344	0.437	0.973	0.356	0.009	0.046	0.650

[1] Average daily gain
[2] Average daily feed intake
[3] Feed conversion ratio
[4] Standard error of the mean (n=16). The experimental unit was a pen with 25 or 15 birds per pen from 0 to 20 d and 20 to 40 d of age, respectively
[5] The interactions among main effects were not significant in all cases (P > 0.05)

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P-014 (ID 195)

EFFECT OF FEEDING DIFFERENT COMBINATIONS OF ORGANIC ACIDS, MONO-GLYCERIDES AND PROBIOTICS ON CAMPYLOBACTER COLONIZATION IN BROILERS

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Abstract

An experiment was conducted within the EU-FP7 project CAMPYBRO in order to evaluate the effect of a combination of a blend of mono-glycerides and organic acids (MGOA) with a probiotic alone (*B. subtilis* C-3102, P) or with a multispecies probiotic (PS) and a plant extract based on garlic (G) added to the feed on *Campylobacter* counts in broilers. There were three treatments applied from 1 to 42 d of age, T1: Positive controls (*Campylobacter*, no additives), T2: T1+MGOA at 2.5%+P at 100 g/t and T3: T1+MGOA at 2.5%+PS at 1,000 g/t+G at 1,000 g/t. A total of 126 one-day-old Ross 308 broilers (half male and half female) were divided into the experimental treatments. At 14 d of age, all broilers were orally gavaged with 100 µl of a solution containing 1 x 10^5 CFU/ml of ST-45 *C. jejuni* strain. On days 21, 35 and 42, ceca from 12 birds per treatment were collected and *Campylobacter* counts determined (ISO 10272). Data were analysed with a Kruskall-Wallis test (SPSS v.19.0). At 21 d, the two combinations used consistently decreased the *Campylobacter* counts compared with T1 (by 3-log; $P < 0.05$). At 35 and 42 d, only T2 (MGOA+P) decreased the colonization of *Campylobacter* compared with T1 (3.05 *vs* 7.15 $\log_{10}$ CFU/g; P = 0.001 and 4.41 *vs* 8.39 $\log_{10}$ CFU/g; P = 0.003; for T2 *vs* T1 at 35 and 42 d, respectively). The results suggest that the supplementation of a blend of mono-glycerides with organic acids together with a probiotic, especially *B. subtilis*, can effectively decrease *Campylobacte*r in broilers.

Keywords: mono-glycerides, organic acids, probiotics, *Campylobacter jejuni*, broiler

Introduction

Campylobacter is the major cause of bacterial gastroenteritis in humans worldwide. The number of confirmed cases of campylobacteriosis in the European Union has followed a significant increasing trend in the last years, along with a clear seasonal trend. In 2013, it was the most commonly reported zoonosis with 214,779 confirmed human cases (EFSA, 2015). Few studies have evidenced a positive role of probiotics in preventing or reducing the shedding of *C. jejuni* in *in vivo* trials (MORISHITA et al., 1997; SANTINI et al., 2010), although *in vitro* studies reported a strong antimicrobial activity of several probiotic strains toward this pathogen (FOOKS and GIBSON, 2002; CHAVEERACH et al., 2004). A wide range of plant extracts and compounds have demonstrated strong bactericidal activity against *Campylobacter spp*. *in vitro* (FRIEDMAN et al., 2002; ROBYN et al., 2013). However, when evaluating *in vivo* results, data are not so conclusive. Also, *in vitro* studies have demonstrated that organic acids, medium chain fatty acids, or their monoglycerides have a strong bactericidal effect on *Campylobacter spp.* (CHAVEERACH et al., 2002; HERMANS et al., 2010). However, inconsistent results have been reported in *in vivo* trials (SOLIS DE LOS SANTOS et al., 2010; HERMANS et al. 2012). Therefore, it is clear that there exist differences in the results *in vitro* and *in vivo*. The objective of this work was to evaluate the effect of a combination of a blend of mono-glycerides and organic acids (MGOA) with a probiotic alone (*B. subtilis* C-3102, P) or with a multispecies probiotic (PS) and a and a plant extract based on garlic (G) added to the feed on *Campylobacter* counts in broilers.

Material and Methods

A total of 126 Ross 308 broilers (50% male and 50% female) from 1 to 42 days of age were used and allocated to the experimental treatments. There were three treatments applied from from 1 to 42 d of age, T1: Positive controls (*Campylobacter*, no additives), T2: T1 + MGOA at 2.5% + P at 100 g/t and T3: T1 + MGOA at 2.5% + PS at 1,000 g/t + G at 1,000 g/t. The trial was carried out in an Animal Biosafety Level 2 Unit for broilers. Broilers were housed in cages in groups of three. The individual bird was the experimental unit. Mash (non-pelleted) feeds were fed *ad libitum* with no added coccidiostat, growth promoter or veterinary antibiotics. Diets were cereal based diets similar to those used commercially. Prior to the infection of the animals, caecal samples of 9 birds randomly selected were collected to verify that chicks were all *Campylobacter*-free. At 14 d of age, all broilers were orally gavaged with 100 µl of a solution containing

1 x 10^5 CFU/ml of ST-45 *C. jejuni* strain. On days 21, 35 and 42, ceca were collected from 12 birds per treatment and *Campylobacter* counts determined (ISO 10272). Detection limit was 2 logs, and animals below limit of detection were assigned 2 logs for statistical analysis. Data were analysed with a Kruskall-Wallis test (SPSS v.19.0). Statistical significance is declared at $P \leq 0.05$, with $0.05 < P \leq 0.10$ considered as a near-significant trend.

Results

At 21 d, the two combinations used consistently decreased the *Campylobacter* counts compared with T1 (by 3.5-log P = 0.010 and by 3.0-log P = 0.032, for T2 *vs* T1 and T3 *vs* T1, respectively). At 35 and 42 d, only T2 (MGOA+P) decreased the colonization of *Campylobacter* compared with T1 (3.05 *vs* 7.15 $\log_{10}$ CFU/g; P = 0.001 and 4.41 *vs* 8.39 $\log_{10}$ CFU/g; P = 0.003; for T2 *vs* T1 at 35 and 42 d, respectively). This decrease was mainly due to the broilers below detection level, 75 and 8% and 58 and 17% in T2 and T3 at 35 and 42 d, respectively. Birds on T3 showed a non-significant reduction of 1.81-log compared to T1.

Conclusions

Under our experimental conditions, it is concluded that: i) Both the supplementation of a combination of a blend of MGOA with a probiotic alone or with a multispecies probiotic and a garlic extract, significantly decrease *Campylobacter* counts in the ceca of infected birds at 21d, ii) Only the blend of MGOA together with the probiotic based on *B. subtilis* significantly decreased *Campylobacter* counts at 35 and 42d (a decrease by 4-log was reached at the end of the fattening period), and iii) the blend of MGOA with the multispecies probiotic and the garlic extract reached a non-significant decrease of 1.81-log in relation to controls.

The results suggest that the supplementation of a blend of MGOA together with a probiotic, especially *B. subtilis*, can effectively decrease *Campylobacter* in broilers.

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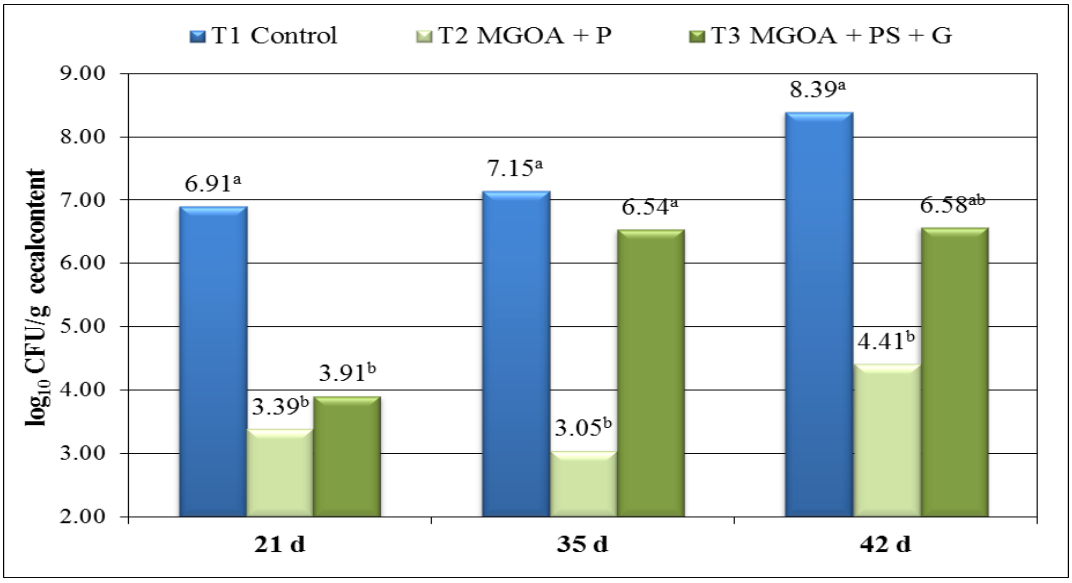

Figure 1. Effect of dietary treatment (T1: Control, T2: T1 + MGOA at 2.5% + P at 100 g/t and T3: T1 + MGOA at 2.5% + PS at 1,000 g/t + G at 1,000 g/t) on *Campylobacter* counts ($\log_{10}$ CFU/g) in the caeca of broilers at 21, 35 and 42 days of age

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P-016 (ID 51)

THE EFFECTS OF DIETARY PLANT ALKALOIDS SUPPLEMENTATION WITH VARIOUS LEVELS OF DIGESTIBLE LYSINE–METHIONINE AND CALCIUM–NON PHYTIC PHOSPHORUS RATIO ON PRODUCTIVE PERFORMANCES, CARCASS CHARACTERISTICS AND MEAT QUALITY OF BROILER

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Abstract

This experiment was conducted to evaluate the effects of plant alkaloids (Sangrovit® Extra), digestible lysine-methionine (Lys-Met) and calcium-non phytic phosphorus (Ca-NPP) ratio on productive performances, carcass characteristics and meat quality of broiler. A 2x2x2 factorial arrangement of treatments comprising 2 dietary Lys-Met ratio (1.0:052% and 0.90:0.47%) and 2 levels of Ca-NPP ratio (1.09:0.49% and 0.98:0.44%) with and without Sangrovit® Extra (non and 60 mg/kg feed) was used. Cobb mixed sex broiler chicks (n = 360, 1-d old) were randomly divided into 8 dietary treatments (5 replicates of 9 birds per treatment). Birds were given 2 phase feeding regimen consisting of starter and grower periods. The results had shown that supplementation of plant alkaloids (Sangrovit® Extra) at the levels of 60 mg/kg feed tend to improve survival rate and significantly improve wing percentage (P<0.05). The dietary digestible Lys:Met ratio at the levels of 1.0:0.52% had better growth performance in term of weight gain (WG), average daily gain (ADG), feed efficiency and improve breast percentage when compare with the level of 0.90:0.47%. It also found that breast meat from 0.90:0.47% of digestible Lys:Met had significantly higher a* (redness) value than the control group (P<0.01). The interaction effect (P<0.01) between Sangrovit® Extra and digestible Lys:Met ratio were observed on WG, ADG and feed conversion rate (FCR). It was concluded, based on the data, that supplementation of Sangrovit® Extra at the levels of 60 mg/kg feed could improve survival rate and carcass yield in term of wing percentage. Digestible Lys:Met ratio at the levels of 1.0:0.52% had better growth performance while the level of 0.90:0.47% had higher significantly a* value of breast meat.

Keywords: digestible lysine-methionine ratio, calcium-non phytic phosphorus ratio, plant alkaloids, broiler

Introduction

In recent years, the potential of the emphasis on alternative feed additive such as phytobiotics in reducing the extraction of N and P by farm animal to protect the environment, have received interest in the use of low protein, amino acid-supplemented diets for various kind of poultry (Keshavarz and Austic, 2004). The objective of this study was conducted to evaluate the effect of plant alkaloid (Sangrovit® Extra) in form of quaternary benzophenanthridine alkaloids (QBA) and protopine alkaloids (PA) from Phytobiotics (Thailand) Co; Ltd. supplementation with various levels of digestible lysine-methionine and calcium-non phytic phosphorus ratio on growth performance, carcass characteristics and meat quality of broilers.

Material and Methods

Three hundred and sixty Cobb mixed sex broiler chicks of 1 day of age in five replications per treatment (9 birds/replication) were used. A 2x2x2 factorial arrangement of eight experimental diets comprising 2 levels of digestible lysine-methionine ratio (1.0:052%; control and 0.90:0.47%) with 2 levels of Ca-NPP ratio (1.09:0.49%; control and 0.98:0.44%) and supplementation with plant alkaloid (Sangrovit® Extra) in the levels of 0 and 60 mg/kg feed of isoenergetic diets. The reduced-digestible lysine-methionine and calcium-non phytic phosphorus ratio had approximately 10 percentage units lower than their control counterparts. Dietary treatments met the minimum requirements for another digestible essential amino acids recommended by NRC (1994). Birds were reared in open-side house and were given 2 phase feeding regimen consisting of starter and grower period. Feed and water were provided *ad libitum* throughout the experimental period (42 days of age). In each period, body weight, feed intake were recorded. FCR and ADG were calculated of each period and overall period. At the end of the experiment, 2 birds per replicate were killed by cervical dislocation. Carcass, abdominal fat and meat yields were collected and calculated relative to the final body weight. Breast samples were evaluated for drip loss, cooking loss, Warner-Bratzler Shear force (WSF) and meat color in term

of L*(lightness), a* (redness) and b* (yellowness). Data were analyzed by ANOVA and compared the difference between dietary treatment means by Duncan's New Multiple Range Test (Steel and Torrie, 1980) for a factorial completely randomized design. A significance level of P<0.05 was used.

Results

Supplementation of plant alkaloids (Sangrovit® Extra) and various level of Ca-NPP ratio had no significant effect on growth performance of broilers. (Table 1). However, it was found that broiler fed with control group of digestible Lys:Met ratio had significantly more WG, ADG and feed efficiency than that the reduced-digestible Lys:Met ratio group. It was also found the interaction effect between Sangrovit® Extra and digestible Lys:Met ratio on WG, ADG and FCR (P<0.05). There has no differences (P>0.05) between treatments and interaction on eviscerated weight (EW) and abdominal fat. There were numerical trends for increasing wing yield as supplemented Sangrovit® Extra and increasing breast yield as due to the level of 1.0:0.52% digestible Lys-Met ratio in diet (P<0.05). The supplementation Sangrovit® Extra, level of digestible Lys:Met and Ca-NPP ratio in diets had no influence on drip loss and WSF of breast meat. However, Sangrovit® Extra supplementation had significantly higher (P<0.05) cooking loss than the control group (P<0.05). No differences in L*, a* and b* color exited between breast meat from broilers fed diets with various Sangrovit® Extra and level of Ca-NPP ratio. The a* value of meat from 0.9:0.47% digestible Lys:Met treatment was significantly higher (P<0.01) than the 1.0:0.52% digestible Lys:Met group (p<0.01). Results of this study were agreement with the report of Lilly et al. (2011) who reported that the dietary amino acid density in broiler feed had no effect on cooking loss and shear force of meat.

Conclusions

The present study had shown that supplementation of plant alkaloids (Sangrovit® Extra) at the levels of 60 mg/kg feed could improve survival rate and carcass yield in term of wing percentage. It was also found that dietary digestible Lys:Met ratio at the levels of 1.0:0.52% had better growth performance while the level of 0.90:0.47% has significantly higher meat color in term of a* (redness) value.

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Table 1. Effects of dietary plant alkaloids (Sangrovit® Extra) supplementation with various Level of digestible Lys-Met and Ca-NPP ratio on growth performance and carcass composition of broilers

Factors	WG[2] (g)	ADG[2] (g/d)	FCR[1]	EW (%)	Breast[1] (%)[1]	Wing[1] (%)	Drumstick (%)	Thigh (%)
Sangrovit®Extra (mg/kg feed)								
0	2184.90	52.02	1.83	81.91	23.70	9.57[b]	12.42	15.19
60	2176.70	51.83	1.82	78.18	24.91	10.45[a]	12.18	15.96
Digestible Lys:Met (%)								
1.0:0.52	2221.34[a]	52.89[a]	1.79[b]	79.50	25.13[a]	10.03	12.24	15.74
0.90:0.47	2140.26[b]	50.96[b]	1.86[a]	80.59	23.48[b]	9.99	12.36	15.41
Ca-NPP (%)								
1.09:0.49	2176.72	51.83	1.83	80.05	24.57	9.82	12.24	15.58
0.98:0.44	2184.88	52.02	1.82	80.04	24.04	10.20	12.68	15.57
Pooled SEM	70.88	1.69	0.07	4.45	2.01	0.96	1.62	1.42

[1] [a,b] Means within a column with different superscripts differ significantly (P<0.05)

[2] [a,b] Means within a column with different superscripts differ significantly (P<0.01)

Table 2. Effects of dietary plant alkaloids (Sangrovit® Extra) supplementation with various Level of digestible Lys-Met and Ca-NPP ratio on meat quality of broilers

Factors	Drip loss (%)	Cooking loss[1] (%)	WSF (kg)	Meat color		
				L*	a*[2]	b*
Sangrovit®Extra (mg/kg feed)						
0	9.43	29.30[b]	2.65	53.71	4.99	15.65
60	10.64	31.92[a]	2.70	54.79	4.66	15.48
Digestible Lys:Met (%)						
1.0:0.52	10.23	30.04	2.67	54.22	4.44[b]	15.52
0.90:0.47	9.84	31.19	2.68	54.27	5.21[a]	15.61
Ca-NPP (%)						
1.09:0.49	9.95	29.75	2.62	54.35	4.94	15.22
0.98:0.44	10.12	31.48	2.73	54.15	4.71	15.91
Pooled SEM	2.89	2.76	0.47	2.05	0.87	1.17

[1] [a,b] Means within a column with different superscripts differ significantly (P<0.05)

[2] [a,b] Means within a column with different superscripts differ significantly (P<0.01)

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P-017 (ID 329)

EFFICACY OF COATED CALCIUM BUTYRATE ON PERFORMANCE, EGG QUALITY AND INCUBATION PARAMETERS IN BROILER BREEDERS

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Abstract

Organic acids salts are alternatives for antibiotic growth promoters, banned since 2006. Besides antibacterial and anti-inflammatory properties, it is know that butyric acid is an energy source for epithelial cells, improving gut morphology and absorption of nutrients in the intestinal tract. For an effective dietary application specific coating is required to by-pass crop and/or stomach and to insure gradually release in the intestinal tract. Objective of this trial was to determine for the first time the efficacy of coated calciumbutyrate in broiler breeders, on performance and incubation parameters.

Two dietary treatments with 5 replicates of 31 broiler breeders and 3 roosters were used. Dietary treatments consisted of control diet and control diet supplemented with coated calciumbutyrate (*Globamax Enhanced,* Sanluc International nv). Productive performance of broiler breeders, egg quality, and incubation parameters were investigated.

Supplementing feed with coated calciumbutyrate resulted in significant increased laying rate and egg mass, resulting in 12.8% more eggs per hen. Also feed conversion was significantly lower in coated calciumbutyrate Although hens produced more eggs, egg shell and egg quality parameters remained equal. In coated calciumbutyrate a numerical higher haugh unit was seen. Fertility, hatchability and chick quality were each time numerically better after 1,2 and 3 months of treatment. Also late embryo mortality was lower. Therefore, a positive effect of coated calciumbutyrate was seen on performance, internal egg quality and some incubation parameters.

Keywords: broiler breeders, calcium butyrate, production, incubation

Introduction

Organic acids are considered potential alternatives to the banned antibiotic growth promoters. It is well recognized that butyric acid is an energy source for the epithelial cells of the villi, improving the gut morphology and absorption of nutrients in the small intestines. Moreover, the efficacy of butyrate likely will be improved if it is protected from immediate absorption in the upper tract by a coating. Several trials have been executed with calciumbutyrate (CB) to judge its effect on the zootechnical performances of broilers and diet's digestibility. Coated calciumbutyrate (CCB) showed a consistent positive effect on broilers' performances during different trials. Moreover, an amino acid sparing effect was demonstrated while it enhanced the metabolic energy value of wheat based diets.

This experiment was designed to determine the effect of supplementing the coated commercial CB (*Globamax Enhanced*, Sanluc International nv) at a dosage of 1000gram/tonne on 1) the productive performance of broiler breeders, 2) their egg quality, 3) fertility, embryonic mortality and hatchability and on 4) chick quality of their progeny.

Materials and Methods

Birds and Housing. Ross 308-broiler breeder hens (Belgabroed nv, Merksplas, Belgium) were housed in the poultry experimental facility at the Institute for Agricultural and Fisheries Research (ILVO) under standard conditions of lighting, heating and ventilation. The breeders were randomly placed in 10 pens, 31 hens per pen and 3 cockerels.

Dietary Treatments. The trial was designed as a randomized complete block design with 2 dietary treatments and 5 replicates each. The first treatment was a control treatment (Tr1) and treatment 2 consisted of the control treatment supplemented with Globamax Enhanced (1000gram/tonne). The feed was a wheat-corn based diet. During the trial period, feed was restricted based on body weight in order to obtain a body weight gain recommended by the Ross Management Guide (Aviagen EPI BV, Roermond, the Netherlands).

Performance Measurements. 10 hens per pen were weighed individually on a 2-weekly basis and the average body weight was calculated per pen. Eggs were collected and counted daily. Laying rate (%), egg mass and feed conversion ratio (FCR) was calculated All pens were monitored daily for hen mortality and morbidity.

Egg Quality Measurement and Incubation Parameters After 1, 2 and 3 months, 120 eggs (60 eggs/treatment) were collected and analyzed for egg quality. Haugh unit was calculated using the following formula (MONIRA et al., 2003): $HU = 100 \log (H - (1.7 \times W^{0.37}) + 7.6))$. Egg shell deformation was assessed under a 500 mg load, in micrometers. Yolk color was determined using a 15-point scale color fan of DSM. The thickness of the eggshell, after the removal of the egg content and egg membranes, was measured with a micrometer at 3 different areas. An average value was calculated based on these 3 measurements. At each timepoint, 1000 eggs per treatment were collected and incubated to assess fertility, hatchability, embryonic mortality and chick quality. From 75 chicks of each treatment chick quality was scored as described by TONA et al. (2003).

Statistical analysis incubation parameters and broiler breeders performance were analysed using a factorial ANOVA to investigate the effect of dietary treatment and age and their interaction using Statistica 10 (StatSoft Inc., Tulsa, OK, USA). The egg quality measurements, with egg as a single replicate, were analyzed using a factorial ANOVA. Significant differences between treatments were tested with LSD test. Differences due to treatments or age were considered significant if the P-value was < 0.05.

Results

The main results of the broiler breeders experiment are presented in table 1. No dietary effect was found on body weight and egg weight. Laying rate (*P*=0.018) and egg mass (*P*=0.002), however, were significantly higher if hens were fed the Globamax Enhanced supplemented diet. This effect was noticed weekly. As feed intake was equal for all diets, FCR was only influenced by laying rate, resulting in a significantly lower feed conversion if broiler breeders were given the Globamax Enhanced supplemented diet. No age or treatment effect was observed on the incidence of cracked, soft-shelled and floor eggs.

It is obvious from the results that egg quality did not change with increasing age of the hens, eggs became only heavier and more yellow. Probably, duration of the trial was too short to observe an age effect. There was also no significant effect of diet on egg quality. For the internal egg quality, a numerical increase was seen if breeders were fed the Globamax Enhanced supplemented diet compared to their control counterparts.

Table 1: Effect of age and treatment (T) on egg weight (g), laying percentage (%), egg mass (g) and feed conversion ratio (FCR) of broiler breeders.

Age	T	Egg weight (g)	Laying percentage, %	Egg Mass, g	FCR
Age effect	55	70.4	55.4^{ab}	39.2^{ab}	4.67
	56	69.8	54.8^{ab}	38.2^{ab}	4.70
	57	70.6	56.3^{a}	39.7^{a}	4.46
	58	70.3	48.6^{ab}	34.2^{ab}	5.24
	59	72.7	48.7^{ab}	35.4^{ab}	5.40
	60	71.1	44.2^{b}	31.4^{b}	6.55
	64	69.7	46.9^{ab}	32.6^{ab}	5.71
T effect	A	70.8	47.4^{b}	33.5^{b}	5.78^{b}
	B	70.5	54.0^{a}	38.1^{a}	4.71^{a}
P-value	Age	NS	0.008	0.01	NS
	T	NS	0.018	0.002	0.04
	A x T	NS	NS	NS	NS

$^{a-b}$ means with the same letter are not significantly different from each other at P ≤ .05.

Egg weight at start of the incubation was comparable between dietary treatments. Chick weight was consequently also comparable between both groups of broiler breeders. For each incubation, at the age of 57, 62 and 66 weeks of age, numerical higher percentages of fertility and hatchability and lower embryonic mortality were obtained for the Globamax Enhanced supplemented fed breeders. The quality of chicks was very high although age of broiler breeders was high. As broiler breeders were already more than 50 weeks of age at the start of the trial, low fertility, hatchability percentage and high embryonic mortality percentages were reached. If broiler breeders were 45 weeks of age, cockerels were spiked, by replacing the worst cockerel out of the 3, by a younger new cockerel for the last time. The worse fertility and hatchability results in this trial, might thus be a consequence of the bad performance of the cockerels as the trial started about 10 weeks after spiking. It is known that both fertility and hatchability improve after cockerels are spiked.

Conclusions

It can be concluded that a positive effect after supplementation of coated calcium butyrate was seen on performance of the broiler breeders, their internal egg quality and some incubation parameters.

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P-018 (ID 239)

HISTOLOGICAL STUDIES ON THE EFFECT OF PREBIOTICS ON BURSA OF FABRICIUS AND CAECAL TONSILS OF BROILERS

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Summary

Evaluation of effects of prebiotics on histological structure of bursa of Fabricuis and cecal tonsils of broiler chickens was studied. A total of 4000 Arbor Acres broilers from local company (Ommat) were raised from 1-35 days old, and divided into two equal groups; control and treated one. The treated birds received water mixed with (INMUNAIR® 17.5) based prebiotic at inclusion rate 0.5-1 ml/ litter while the control ones received water free from a prebiotic. It was found that the used prebiotic enhanced the growth rate levels and immunity in treated birds as well as increased the cellular density of bursal lymph nodules with increasing in epithelial surface area. In addition, significant appearance of ileum and cecal tonsils with increase of musculosa thickness, villus and intestinal crypt depth and length were observed. It was noticed an increase in mucin dynamics in bursa of Fabricius and caecal tonsils of treated group. Conclusion, By adding prebiotics to poultry diets, Zootechnical performance body weight, feed conversion ratio and GIT health status were significantly improved.

Keywords: Prebiotic, Immunity, Broilers, Performance, Histology.

Introduction

A prebiotic substance has been defined as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of limited number of bacteria in the colon CHOUDHARI (2008). Several studies have shown that addition of prebiotics to the ration of broilers, layers and pigs improved performance through improving gut microflora (XU et al., 2003). Using of compounds that may have prebiotic effects is a possible way to improve intestinal health and animal performance in the absence of antibiotic growth promoters (KIM et al., 2011). From the available literature, the study of the effects of prebiotics on the Bursa of Fabricius and caecal tonsils of chickens is limited. So, the present study was conducted to evaluate the effect of prebiotics supplementation on bursa of Fabricius and gut performance as well as growth performance of broilers.

Material and Methods

The commercial corn soya bean starter and grower finisher diets were offered to the birds from one day old to 21 and from 22 to 35 days of age, respectively. The treated birds were received drinking water mixed with inactivated cells of *Propionibacterium acnes* and lipopolysaccharide from *E. coli* (INMUNAIR® 17.5) based prebiotic. Administration in drinking water was at a rate of 0.5-1 ml / Litter, while the control bird received water free from prebiotic.

Administration of the product according to the recommendation of the manufacturer.

Table (1): Administration guidelines of INMUNAIR® 17.5 (*)

Animal	Age	Prophylactic use Breeding	Booster of antibiotic treatment	Dose
Broiler	During the first 5 days, or days 3, 4 and 5	1 x 500 ml / 2.000 l. water		1 ml / 10 k bw
	3rd week of life	1 x 500 ml / 2.500 l. water (during 2 days)		0.5 ml /10 k bw
	Variable		0.5 ml / 10 kbw (during antibiotic treat.)	

Immediately after sacrificing birds, specimens of bursa of Fabricius, ileum and caeci at the level of cecal tonsils were collected and fixed in 10% neutral buffered formalin solution. The trimmed sections were processed and stained by histological and histochemical stains according to BANCROFT and STEVENS (1996).

Results

Growth performance: The result showed that dietary treatment of broiler chickens by (INMUNAIR® 17.5) at inclusion rate of 1 ml / litter showed significant higher body weight in the treated birds than control ones (Tables 2).

Table (2): Feed intake and growth performance

	Average body weight gain (gm/bird/day)	Average feed intake (gm/bird/day)	Feed conversion ratio
days	1-21 22-35 1-35	1-21 22-35 1 -35	1-21 22-35 1-35
Control	25 80 50.5	42.5 156.6 89.7	1.7 1.95 1.77
Prebiotic	25 98 55.5	42.6 166.12 92.1	1.7 1.69 1.65

Feed conversion ratio was calculated by the average body weight gain divided by corresponding average feed intake.

Discussion

The effect of prebiotics on body weight gain showed some controversial results. The available information for different prebiotic used in poultry industry showed conflicting results. ORTIZ et al., (2009) and ALZUETA et al., (2010) found that by using inulin (Chicory extract) as dietary inclusion with dose up to 20g/kg did not significantly affect the body weight gain, feed intake or feed conversion ratio of broiler chickens. In contrast to these findings, REBOLE et al., (2010) showed that the inclusion of inulin in control diet improved the body weight gain of birds. In the mean time, using others preboitic in several studies like Fermacto prebiotics (PIRAY et al., 2007 and KHAKSAR et al., 2008) has effect on growth performance. The current study showed that feeding INMUNAIR® 17.5 had no significant effect on body weight gain in starting period with improvement by bird getting older and significant effect gained in week 4th and 5th. In earlier study by ZELENIKA et al., (2007), it was found that using INMUNAIR® 17.5 in broiler induced significant improvement of zootechnical performance.

Conclusion

By adding prebiotics to poultry diets, Zootechnical performance body weight, feed conversion ratio and GIT health status were significantly improved. Moreover, Prebiotics improve immune system to reduce colonization by pathogens.

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P-020 (ID 100)

EFFECT OF BEE POLLEN ON GROWTH PERFORMANCE AND IMMUNE STATUS OF YOUNG BROILER CHICKS

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Abstract

This study was carried out to evaluate the effect of supplementing a corn-soybean meal-based diet with bee pollen (BP) on growth performance and immune response. A total of 160 Ross 308 male broiler chicks were assigned to 4 dietary treatments (0, 10, 15 and 20 g kg $^{-1}$ of BP feed inclusion), consisting of 4 pens of 10 birds each, and were fed experimental diets from 1 to 21 d of age. Then, all birds received a common finisher diet from 22 to 35 d of age. The antibody response to sheep red blood cells (SRBC) was measured at 35 d. In general, average daily feed intake and relative weight of gizzard were not affected by the dietary groups compared to the control birds (P>0.05). Dietary supplementation increased (P<0.01) body weight, average daily gain (ADG), and improved feed conversion ratio (FCR). Relative pancreas, liver, bursa, intestine and spleen weights significantly increased (P<0.01) at 20 g kg^{-1} of BP. Inclusion of 20 g kg^{-1} of BP in the diet significantly increased (P<0.01) antibody response against SRBC at 35 d of age. In conclusion, these findings demonstrate that dietary supplementation with BP improved broiler growth performance and immunity. Thus, BP may be used as alternative feed additives in the poultry industry to improve immune response of young broiler chicks.

Keywords: bee pollen, broiler chick, immune response, performance

Introduction

The bee pollen (BP) is created in the male sexual organ of the flower – anthers with the purpose of fertilizing the stigma. It is the main source of proteins for bees (FAZAYELI-RAD et al., 2015). Honeybee collects pollen and mixes it with its own digestive enzymes. Although there is no specific chemical composition, BP is a rich source of protein (25%), essential amino acids, linolenic, palmitic and linoleic acids, flavonoids, carotenoids and phytosterols (XU et al., 2009). Bee pollen contains several antioxidant active compounds such as catalase, peroxidase and superoxide dismutase. Moreover, BP has antibacterial properties and increases the body's resistance to infection. Bee pollen makes the plumage of birds fuller and more vivid in color. OLIVEIRA et al. (2013) found that inclusion of BP increased number of humoral immune cells, phagocytes and red blood cells, and accelerated antibody production in broilers chickens. It seems that BP contains most of the essential nutritional elements necessary for growth and development in monogastric animals and humans (FAZAYELI-RAD et al., 2015). Therefore, the present study was conducted to evaluate the effects of BP on growth performance, relative weight of visceral organs, and immune response in broiler chicken.

Material and Methods

One hundred and sixty 1-d-old male broiler chicks of the Ross strain were obtained from a commercial hatchery and were used in this experiment, which lasted for 35 d. The chicks were individually weighed upon arrival and divided into 16 groups of 10 birds balanced for body weight. Birds had ad libitum access to feed and water during the experimental period and light was provided for 24 h throughout the experiment. Four corn-soybean meal-based diets were fed from 1 to 21 d of age. The diets included basal diet supplemented with 0, 10, 15 and 20 BP g kg^{-1} of feed. The BP was mainly pollen collected by the honey bee from *Amygdalus scoparia* in the spring season. A common finisher diet (12.68 MJ metabolizable energy/kg and 190 g crude protein/kg) was fed from 22 to 35 d of age to all birds.

The BW and feed consumption for each cage were determined and were used to calculate data on average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR). At 21 d of age, three randomly chosen birds per replicate (12 birds per treatment) were killed by cutting the carotid artery and jugular vein, and processed manually and collections were made following a 4-h fast. The weights of the pancreas, liver, gizzard, intestine, bursa and spleen were recorded. The organs were cleaned with physiological saline solution, dried with filter paper and weighed. The weight of each organ was expressed relative to the total body weight (g/100 g BW). At 28 days of age, 3 birds from each pen were inoculated with a volume of 0.2 mL from a 8% suspension of sheep red blood cell (SRBC). Seven days after each injection, blood was collected in nonheparinized tubes by puncturing the brachial vein. Serum was obtained

by centrifuging at 2,500 × g for 15 min at 25°C, and stored at −30°C until assayed. Individual serum samples were analyzed for antibody responses against SRBC by ELISA technique using commercial kits, and the plates were read at 405 nm on an ELISA reader. The collected data were subjected to analysis of variance (ANOVA) using general linear model (GLM) of SAS (SAS Inst. Inc. Carry, NC) software and the corresponding means were compared by Tukey-Kramer's test. For the different statistical tests, significance was declared at $P< 0.05$.

Results

The effect of dietary BP on growth performance is summarized in Table 1. The mean BW at 21 and 35 days of age, ADG and FCR were significantly improved by addition of BP in the diet ($P<0.01$), and it was maximized at 15 g kg^{-1} supplemental BP. No change in relative gizzard weight was observed with dietary BP, while inclusion of 20 gkg^{-1} of BP in the diet, significantly increased the relative weight of pancreas ($P<0.05$), liver ($P<0.01$), intestine ($P<0.01$), bursa ($P<0.05$), spleen ($P<0.01$), and antibody response against SRBC ($P<0.01$) at 35 d of age compared with birds fed the control diet (Table 2).

Conclusions

The present study indicates that the addition of BP to diets improves the growing performance of broiler chickens compared with the un-supplemented, control treatment. The positive effect of BP on growth performance of broiler chickens could be related to the high contents of nutrients, among which essential amino acids, polyunsaturated fatty acids (PUFA), and some trace minerals (FAZAYELI-RAD et al., 2015) and these nutrients can be easily absorbed as BP components. It has been shown that nutrients content of BP pass from the stomach into the blood stream within 2 h after ingestion (XU et al., 2009).

During the experiment, we noticed that two important digestive organs, the liver and pancreas, were larger in the pollen group compared to the control group. The BP may promote effective contact between the digestive enzymes and their corresponding substrates, leading to significant modifications of the structure and function of intestine and organs (OLIVEIRA et al., 2013). To adapt to those changes, the activities of the intestinal secretory mechanisms may be enhanced. Thus, this may lead to increases in the size of the gastro-intestinal tract (GIT), pancreas, and liver. OLIVEIRA et al. (2013) indicated that this increased size of intestine and GIT could be an adaptive response to an increased need for digestive enzymes. It seems that BP raised digestive enzymes secretion in the gut for better digestion. OLIVEIRA et al. (2013) also implied that the reduction in relative organ weight had a direct economic benefit, as the dressing yield of broilers should increase proportionally. BP is rich in nutrients, such as proteins, amino acids, vitamins, and trace elements, among others, that promote faster cell proliferation and differentiation in the immune system of birds. According to WANG et al. (2007) dietary BP may stimulate the T lymphocytes formation, as well as the division, proliferation and activity of thymus cells. WANG et al. (2007) found that the thymus, spleen and bursa were heavier in broilers fed the diets with BP. To our knowledge, there is no published data on effect of the BP that was used in this study on antibody response to SRBC. The SRBC, a non-pathogenic antigen, is classified as a thymus-dependent antigen that obviously needs the help of T lymphocytes to produce antibodies. Bee pollen that is rich in flavonoids extends the activity of vitamin C, acts as antioxidant and may therefore enhance the immune function (WANG et al., 2007). Bee pollen is confirmed an interesting resource, able to improve productive performance and immune status of broiler chickens. Standing our results, BP could be used as a supplement in the young broiler chicks during the starter phase with positive effects on growth rate and feed conversion ratio, without increasing the final cost of feed. In addition, it could be a strategy to improve resistance to disease in broiler chickens and organic production.

Table 1. Effect of bee pollen (BP) supplementation on average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) of broiler chicks during 0 to 21d of age

	BW at 21 d (g)	ADG (g)	ADFI (g)	FCR (g/g)
Basal diet (BD)	632[b]	27.89[b]	46.67	1.67[a]
BD+ 10 BP g kg^{-1}	713[a]	31.69[a]	48.80	1.54[b]
BD+ 15 BP g kg^{-1}	727[a]	32.38[a]	46.94	1.45[c]
BD+ 20 BP g kg^{-1}	725[a]	32.3[a]	48.07	1.49[bc]
Pooled SEM	19.30	0.83	0.89	0.03
P value	0.003	0.002	0.486	0.001

[a-c]Mean values within a column with no common superscript differ significantly from each other (P<0.05).

Table 2 Effect of bee pollen (BP) supplementation on relative weight of organs (g/ 100 g BW) at 21 day of age, and SRBC responses at 35 day of age

	Pancreas	Liver	Gizzard	Intestine	Bursa	Spleen	SRBC (log^2)
Basal diet (BD)	0.23[b]	1.97[b]	1.59	2.43[b]	0.18[b]	0.15[b]	6.25[b]
BD+ 10 BP g kg^{-1}	0.23[b]	1.99[b]	1.61	2.45[b]	0.20[a]	0.15[b]	6.50[b]
BD+ 15 BP g kg^{-1}	0.24[ab]	1.98[b]	1.61	2.45[b]	0.20[a]	0.16[b]	7.37[ab]
BD+ 20 BP g kg^{-1}	0.25[a]	2.05[a]	1.63	2.62[a]	0.21[a]	0.20[a]	8.13[a]
Pooled SEM	0.01	0.01	0.01	0.03	0.01	0.01	0.41
P value	0.019	0.001	0.10	0.002	0.034	0.001	0.010

[a-c]Mean values within a column with no common superscript differ significantly from each other (P<0.05).

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FAZAYELI-RAD, A.R., N. AFZALI, H. FARHANGFAR, M.R. ASGHARI, 2015: Effect of bee pollen on growth performance, intestinal morphometry and immune status of broiler chicks. Europ. Poult. Sci. **79**, DOI: 10.1399/eps.2015.86.

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P-021 (ID 101)

EFFECT OF BEE POLLEN ON INTESTINAL MORPHOMETRY AND SELECTED BLOOD PARAMETERS IN BROILER CHICKS

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Abstract

This study was conducted to determine the effect of bee pollen (BP) supplementation on intestinal morphometry and selected blood metabolites. A total of 160 Ross 308 male broiler chicks were assigned to 4 dietary treatments (0, 10, 15 and 20 g kg $^{-1}$ of BP feed inclusion), consisting of 4 pens of 10 birds each, and were fed experimental diets from 1 to 21 d of age. In general, phosphorus concentration in serum and morphometric indices of duodenum were not affected by the dietary groups compared to the control birds (P>0.05). Dietary supplementation decreased total serum cholesterol and triacylglycerides levels (P<0.01) at 20 g kg^{-1} of BP. Addition of BP to the diets increased (P<0.01) villus length, crypt depth and villus length to crypt depth ratio in the jejunum compared to the control diet. In conclusion, these findings demonstrate that dietary supplementation with BP improved morphometric indices. Therefore, the present study suggests that a level of 15 g/kg BP supplementation in the diet is optimal for absorptive capacity in broilers.

Keywords: bee pollen, broiler chick, morphometric indices, blood parameters

Introduction

Pollen grains, a fine powder-like material, are the male seeds of a flower blossom which has been gathered by the bees and to which special elements (such as; Fe, Cu, Zn, Cr and Mn) from the bees has been added (FAZAYELI-RAD et al., 2015). Like propolis, other well-known honey bee products, the exact chemical composition depends on the plants the worker bees gather the pollen from, and can vary variable (OLIVEIRA et al., 2013). Addition of BP to the broiler chicken diet reduced cholesterol and triacylglycerides. Also, High-density lipoproteins (HDL) increased, whereas low-density lipoproteins (LDL) decreased (ATTIA et al., 2011). In addition, BP was able to improve gut health and normal gastrointestinal functions in poultry, protect kidney, and decrease triacylglycerides, cholesterol, creatinine, and blood urea N in rats (ATTIA et al., 2014). The present study was conducted to determine the effects of BP as dietary supplement on growth intestinal morphometry and blood biochemical parameters of broiler chickens.

Material and Methods

One hundred and sixty 1-d-old male broiler chicks of the Ross strain were obtained from a commercial hatchery and were used in this experiment, which lasted for 21 d. The chicks were individually weighed upon arrival and divided into 16 groups of 10 birds balanced for body weight. Birds had ad libitum access to feed and water during the experimental period and light was provided for 24 h throughout the experiment. Four corn-soybean meal-based diets were fed from 1 to 21 d of age. The diets included basal diet supplemented with 0, 10, 15 and 20 BP g kg^{-1} of feed. The BP was mainly pollen collected by the honey *bee* from *Amygdalus scoparia* in the spring season.

At 21 days of age, 5 mL of blood was collected from the killed birds (the same birds were used for slaughtering) in non-heparinised tubes and kept on ice slashes until they were subjected to serum collection by centrifuging the whole blood sample at 2,500×g for 15 min. Before serum was collected, blood samples were held at room temperature for 4 h and were then refrigerated overnight. The concentrations of Ca, P, triacylglycerides and total cholesterol in serum samples were subsequently analyzed using colorimetric methods on an automatic biochemical analyzer, following manufacturer instructions for the corresponding reagent kit.

Fragments of approximately 5 cm were obtained from each of the following segments of the small intestine: the duodenum and the jejunum to evaluate the intestinal morphology. The fragments from each segment were then placed on polystyrene sheets, opened longitudinally, washed in saline solution, fixed in 10% formaldehyde solution, dehydrated and embedded in paraffin. The measurements of villus length and crypt depth were taken using a light microscope and a system that analyses computerised images, and the data were used to calculate the villus length to crypt depth ratio. The height of 30 villi and the depth of 30 crypts were measured from each segment and replicate, and the means were utilized for statistical analysis. The collected data were subjected to analysis of variance (ANOVA) using general linear

model (GLM) of SAS (SAS Inst. Inc. Carry, NC) software and the corresponding means were compared by Tukey-Kramer's test. For the different statistical tests, significance was declared at $P < 0.05$.

Results

The effect of dietary treatment on cholesterol, triacylglycerides, Ca, and P concentrations in serum are presented in Table 1. At 21 d of age, none of the dietary treatments significantly altered the serum Ca and P concentrations. In this study, birds fed the dietary BP had lower cholesterol and triacylglycerides concentrations ($P<0.01$) compared with values for the control group. The effects of dietary BP on duodenal and jejunal criteria are presented in Table 2. Regarding the duodenal morphology, inclusion of BP in the diets did not have a significant effect on duodenum measured criteria. There were significant differences between treatments for jejunum morphometry. In this regard, feeding both 10 and 15 g kg^{-1} of BP increased villus length and villus: crypt ratio as compared to those fed control and 20 g kg^{-1} BP fed birds ($P< 0.05$). Jejunal crypt depth was higher in birds fed 15 g kg^{-1} BP compared to control group ($P< 0.05$).

Conclusions

Reduction of total cholesterol and triacylglycerides concentrations is the most beneficial effect on the lipid profile. It has been reported that bee pollen in a diet normalizes cholesterol and triacylglycerides levels in blood (FAZAYELI-RAD et al., 2015). After regular consumption of bee pollen, reports have been made of reduced cholesterol and triacylglycerides (ATTIA et al., 2014). The decrease in plasma lipids and cholesterol could be due to phospholipids and PUFA particularly linolenic acid (ATTIA et al., 2014). The small intestine is the main site for digestion and absorption of nutrients. The intestinal villi in the pollen group were longer and thicker than those of the control group. This enhanced intestinal villi development greatly increases the functional surface area of the mucosa for nutrient absorption in the small intestine. As a consequence, the absorption efficiency of the small intestine is accordingly increased. In addition, higher villus-to-crypt ratios suggest higher digestive and absorptive capacities (FAZAYELI-RAD et al., 2015). Thus, the increased ratios obtained here can be attributed to the beneficial effect of the BP in controlling proliferation of pathogenic bacteria and avoiding possible damage to the intestinal mucosa, which could result in reduced dimensions of the villi (ATTIA et al., 2011). Furthermore, the glands of the small intestine play an important role in the digestion of nutrients. The increased function of these glands also significantly contributes to the absorption of the nutrients in small intestines. Thus, addition of BP to the basic daily diet stimulates the digestive and absorptive functions of broilers and, in turn, promotes growth and development (ATTIA et al., 2014). Standing our results, morphometric indices of broilers were greater as the level of BP increased. Therefore, the present study suggests that a level of 15 g kg^{-1} BP supplementation in the diet is optimal for maximizing absorptive capacity in broilers. In addition, it could be a strategy to decrease cholesterol in broilers meat.

Table 1. Effect of bee pollen (BP) supplementation on blood parameters (mg/dL) at 21 day of age

	Cholesterol	Triacylglycerides	Calcium	Phosphorus
Basal diet (BD)	126.10^{a}	64.63^{a}	9.19	9.13
BD+ 10 BP g kg^{-1}	110.25^{b}	47.25^{b}	9.23	9.31
BD+ 15 BP g kg^{-1}	105.62^{b}	45.75^{b}	8.54	8.64
BD+ 20 BP g kg^{-1}	98.13^{b}	59.63^{ab}	8.38	8.39
Pooled SEM	3.66	4.03	0.33	0.26
P value	0.001	0.005	0.184	0.163

$^{a-c}$Mean values within a column with no common superscript differ significantly from each other ($P<0.05$).

Table 2. Effect of bee pollen (BP) supplementation on intestinal morphometry at 21 day of age

	Duodenum			Jejunum		
	Villus length (µm)	Crypt depth (µm)	Ratio	Villus length (µm)	Crypt depth (µm)	Ratio
Basal diet (BD)	1225	178	6.89	1148[b]	158[b]	7.26[b]
BD+ 10 BP g kg^{-1}	1219	175	6.96	1187[a]	160[ab]	7.41[a]
BD+ 15 BP g kg^{-1}	1248	181	6.88	1202[a]	162[a]	7.42[a]
BD+ 20 BP g kg^{-1}	1235	175	7.06	1165[ab]	159[b]	7.33[ab]
Pooled SEM	2.4	0.6	0.02	1.3	1.1	0.05
P value	0.087	0.092	0.102	0.021	0.014	0.035

[a-c]Mean values within a column with no common superscript differ significantly from each other (P<0.05).

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P-023 (ID 332)

EXAMINATION OF BROILER CHICKEN VILLI EVOLUTION AFTER HERBAL EXTRACT FEEDING

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Introduction

Intestinal digestion of birds depends on the ingredients of the consumed feed (BARNES et al. 1972; ENGSTÖM 1992). The stability of the intestinal flora and the integrity of the intestinal epithelium are important for optimal digestion and absorption (COLEO et al. 1984, HORVÁTH-PAPP 2008). The absorption of the nutrition and the utilization of the feed depend on the condition of the villi and the epithelial cells covering them (YANG et al. 2009, COLEO et al 1984).

The aim of our study was to evaluate the differences in the individual morphological parameters of the villi in broiler chicken apropos of feeding with herbal extract supplemented feed. Changes in the villi significantly influence the digestive and the absorptive processes in the intestine, which affect the feed conversion and thus have an economic importance in broiler production.

Material and methods

For the study four groups of 115 broiler chicken were considered, out of the four three groups were infected with 2ml 10^8CFU/ml *Clostridium Perfringes* bacteria three consecutive times (18, 21 and 24 days of age) using a crop probe. The negative control group was not infected and was fed with feed having the nutritive value specified in the technology instructions, ad libitum. The positive control group was fed the same feed, but was infected with *Clostridium Perfringes*. The feed of the other two groups were supplemented with Zn-bacitracin (1kg/T) and with complex plant extracts (Herbanoplex) (1kg/T) respectively.

The chickens were slaughtered according to animal rights laws and a small piece from the small intestine was fixed in 8% formaldehyde, and was segmented following paraffin embedding. After hematoxilin-eozin dying the length and the width of the villi as well as the depth of the crypts were investigated.

Results and discussion

Values of the measured villi parameters are summarized in table 1.

Table 1. The development of the parameters of the villi in case of the different experimental settings

	Length of villi	Width of villi measured in the middle	Depth of the crypts
Negative control	2,21	0,196	0,330
Positive control	2,09	0,184	0,273
Treated with plant extracts	2,30	0,234	0,323
Treated with Zn bacitracin	2,20	0,189	0,285

Examining the results, we found that the villi parameters were more favorable in case of the group where the feed was supplemented with herbal extracts, then in case of the other groups. The villi were 5 % longer and 20 % wider as compared to the other groups. Moreover the crypts between the villi were deeper as well.

These results show that when the chicken consumed feed supplemented with herbal extract the absorptive surface of the intestine showed an improved development, which in turn results in a positive effect on feed conversion ratio and body weight improvement. We think that the additive containing complex plant extracts had a synergic positive effect on the villi and the epithelial cells when mixed into the feed. At the same time these results suggest the possibility to decrease antibiotics use with the application of herbal extracts.

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P-027 (ID 200)

EFFECT OF DIETARY INCLUSION OF MEDIUM CHAIN FATTY ACIDS AND BUTYRATE ON PERFORMANCE OF LAYING HENS AND FATTY ACIDS PROFILE; SENSORY AND TEXTURAL PROPERTIES OF EGGS

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Abstract

The aim of the present study was to investigate the effect of dietary supplementation of the mixture comprising medium chain fatty acids, butyrate, essential oils and *Saccharomyces cerevisiae* on laying hens' performance, fatty acids profile, sensory and textural properties of eggs. For the period of 8 weeks, 36 Hisex Brown laying hens were randomly assigned to 3 dietary treatments: 1) control diet, 2) diet supplemented with the mixture of medium chain fatty acids, butyrate, essential oils and *Saccharomyces cerevisiae (Lumance®)* at the level of 0.5 g/kg of feed, 3) diet supplemented with the same mixture as in treatment 2, but at a higher amount (1.5 g/kg of feed). All laying hens were kept under the same conditions. Sensory and textural attributes of the eggs (1 days and after storage for 28 days) were also evaluated. The results of the present study showed that neither 0.5 g/kg nor 1.5g/kg supplementation of feed additive affected egg production and feed consumption. No significant difference in saturated, unsaturated and polyunsaturated fatty acids between the treatments was observed. The diet supplemented with 0.5 g/kg of tested feed additive decreased toughness of egg yolk (1 days). After 28 days of storage, the hardness and solidity of egg white were higher in the 2nd and 3rd treatment groups ($P<0.05$). The inclusion of 1.5 g/kg feed additive had no significant effect on sensory properties of eggs, except the hardness – the egg white was decreased and egg yolk was increased compared with other treatments ($P<0.05$).

Keywords: laying hens, MCFA, butyrate, fatty acids, sensory and textural properties

Introduction

One of the most important quality criteria of table eggs are sensory characteristics. Sensory attributes and chemical composition of eggs can easily be influenced by feeding of hens (HUOPALAHTI et al., 2007), e.g. by the choice of feeding stuffs and feed additives.

Previous studies reported that organic acids such as fumaric, propionic and butyric acids and their salts have shown variable effects on egg production and egg quality parameters. These discrepancies would be related to the source, the amount of organic acids used, location, environmental condition and the composition of the diets (GRASHORN et al., 2013).

However, little information has been published on the synergetic effects of the *Lumance®*on fatty acids profile, sensory and textural properties of eggs as feed additive in laying hens diets. Therefore, the aim of this study was to investigate the effects of dietary supplementation of the *Lumance®* on laying hens performance, fatty acids profile, sensory and textural properties of eggs.

Material and Methods

Feeding trial was conducted on 36 laying hens of *Hisex Brown* strain at the age of 30 weeks divided into 3 groups (12 laying hens in each group) and fed a compound feed diets (Control group), supplemented with the mixture of medium chain fatty acids, butyrate, essential oils and *Saccharomyces cerevisiae* (Experimental I group, *Lumance®,* 0.5 g/kg of feed) and supplemented with the same mixture as in Experimental I group, but at a higher amount (Experimental II group, 1.5 g/kg of feed). During the feeding trial, the laying hens were held in the individual cages with stationary drinking-bowl and feed box under the same feeding and holding conditions. The birds were fed 8 weeks with wheat-soybean meal diet 125 g per day. Diets composition was adjusted to the respective requirements of the National Research Council (NRC, 1994).

The boiling process ensured that the inherent egg flavor was preserved. The group consisted of 6 probands in this research. The probands were selected and trained in accordance with LST ISO 8586-1. The estimation was performed according to the requirements of LST ISO 8589 in the institute cabins of the Laboratory of Sensory Analysis. For preparation of the sample for texture analysis, the modified WOODWARD and COTTERILL (1987) method. The samples texture characteristics were determined with the universal texture analyzer Universal Testing Machine Instron 3343 (Instron Engineering Group, High Wycombe, UK) equipped with a 1kN load cell and Bluehill software.

Extraction of lipids for fatty acid analysis was performed with chloroform/methanol (2:1 v/v) as described by FOLCH et al. (1957). Fatty acid methyl esters were prepared using the procedure of CHRISTOPHERSON and GLASS (1969). The FAMEs were analysed using a gas liquid chromatograph (GC–2010 SHIMADZU) fitted with flame ionization detector.

Statistical analysis. The results of the experiment were analysed using the 1-way ANOVA test, and significant differences between groups were determined by Duncan's multiple range test. Statistica 8.0. for Windows™ software was used. Differences were considered significant at P<0.05.

Results

Productivity parameters of laying hens of 30–38 weeks of age in the Experimental I group the *Lumance*® increased egg laying intensity by 1.46–4.91% compared with the Control group. The results of increasing egg production percentage due to adding organic acids mixture is in agreement with those of SOLTAN (2008) who found that organic acid supplementation increased egg production by 5.77% compared to control group.

No significant difference in saturated, unsaturated and polyunsaturated fatty acids in egg yolk between the treatments and different days of storage was observed.

The results of albumen and yolk texture analysis are presented in Table 1. Due to the effect of *Lumance*®, the egg albumen after 28 days of storage was more harder in experimental groups by 2.99–4.49 N and resilient 15.53–26.23 N compared to the control group (P<0.05). Statistically significant differences were found in Experimental I group (0.5 kg/t *Lumance*®) after 28 days of egg storage, egg yolk springiness was by 0.95 mm less compared to the Control group.

Summing up the results of albumen and yolk texture analysis, it may be stated that the use of different *Lumance*® levels supplement in laying hen diet had a minor effect on the texture characteristics of yolk and albumen.

Table 1. Effect of dietary inclusion of medium chain fatty acids and butyrate on egg texture characteristics storage 1 and 28 days

Characteristic	Storage time in days	Groups		
		Control	Experimental I +0.5 kg/t *Lumance*®	Experimental II +1.5 kg/t *Lumance*®
Albumen				
Hardness, N	1	10.7±1.29^{a}	11.4 ±2.78^{a}	11.37±2.48^{a}
	28	8.84±1.96^{a}	13.33±0.52^{c}	11.83±1.55^{bc}
Cohesiveness	1	0.47±0.02^{a}	0.46±0.10^{a}	0.44±0.09^{a}
	28	0.52±0.04^{a}	0.57±0.02^{a}	0.56±0.04^{a}
Springiness, mm	1	8.64±0.44^{a}	8.03±0.57^{a}	7.64±0.60^{a}
	28	8.11±0.39^{bc}	8.35±0.17^{c}	8.01±0.29^{abc}
Recilience, N	1	43.39±6.83^{a}	44.99±9.13^{a}	40.01±9.74^{a}
	28	37.39±9.57^{ab}	63.62±1.76^{d}	52.92±5.76^{cd}
Yolk				
Hardness, N	1	14.91±4.76^{ab}	22.78±6.42^{b}	24.88±10.30^{b}
	28	19.40±3.26^{a}	22.17±2.48^{ab}	17.27±5.71^{a}
Cohesiveness	1	0.36±0.03^{bc}	0.36±0.05^{bc}	0.42±0.01^{c}
	28	0.54±0.03^{ab}	0.59±0.01^{b}	0.58±0.02^{ab}
Springiness, mm	1	6.47±0.11^{d}	5.52±0.20^{c}	6.15±0.11^{d}
	28	7.01±0.46^{a}	6.82±0.16^{a}	6.84±0.31^{a}
Recilience, N	1	34.33±7.76^{ab}	46.49±9.52^{b}	44.17±7.93^{b}
	28	74.80±9.77^{a}	89.81±9.77^{a}	68.73±9.58^{a}

a, b, c, d – values within each column with the same superscript are not different at P<0.05

Determination of egg sensory attributes shows that the insertion of the different levels of *Lumance*® in laying hens compound feeds statistically influenced the hardness of the egg protein after 28 days of storage (from 0.75 to 1.17 N) compared to the Control group. Egg yolk hardness in the Experimental I group after 28 days of storage increased by 1 N compared to the Control group.

Conclusions

Supplementation of different levels *Lumance*® and different time of storage had no significant impact on the laying hens performance, fatty acids profile and sensory properties except textural properties of eggs.

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P-028 (ID 214)

CANTHAXANTHIN FROM CAROPHYLL® RED CAN IMPROVE THE VITELLINE MEMBRANE STRENGTH IN STORED EGGS FROM BREEDER HENS

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Abstract

Hatchability is certainly a key performance and profitability parameter for chick producers. Storing hatching eggs is a current practice and this can damage hatchability results. Canthaxanthin, as an antioxidant, is particularly effective for supporting hatchability. The vitelline membrane strength (VMS) is very important to the early embryo survival that canthaxanthin can promote it. The experiment involved a flock of 3,800 breeders (males and females) separated into two adjacent blocks. Feeding experiment started at 18 weeks of age with a control diet as 3000 IU/kg Vitamin D_3 and no Carophyll® supplementation and experimental diet as 1,600 IU/kg vitamin D_3, 37.5 µg/kg 25-hydroxy-vitamin D_3 (HyD®) & 6 mg/kg canthaxanthin (Carophyll® Red 10%). Vitamin D_3 or 25-hydroxy-vitamin D_3 were not expected to have any influence on the VMS. Eggs were collected at 40/45 weeks and stored 7 days. The VMS was evaluated using a compression machine. Stiffness and breaking strength were significantly increased with the supplementation of canthaxanthin. Thanks to the unique capabilities of high deposition rate in the yolk and singlet oxygen quenching capabilities, canthaxanthin from Carophyll® can improve the VMS in complement to Vitamin E and we hypothesized that it contributes to explain its influence on early embryo viability and hatchability in breeder performance.

Keywords: canthaxanthin, vitelline membrane, stored eggs, broiler breeders, strength

Introduction

Hatchability is certainly a key performance and profitability parameter for chick producers. At commercial level, there is often a bigger gap between fertility and hatchability than the genetics standard settings. It is well known that hatchability declines with age, partly explained by a lower egg quality. Storing hatching eggs for extended periods is a current practice in the broiler breeder industry due to uncoordinated demand for hatchlings in the market. The egg storage can also damage the hatchability and these increases with old flocks. Canthaxanthin is particularly effective for supporting hatchability. DSM was the first company to get the approval for canthaxanthin use in breeder diet (EU regulation No 684/2014). Canthaxanthin can improve early embryo survival as shown in a breeder study where the embryonic mortality was significantly reduced from 12.66% to 10.81% (Souza et al, 2008).

Vitelline membrane separates the yolk content from the albumen. The vitelline membrane strength (VMS) is reduced in aged eggs (Kirunda and McKee, 2000). The protein elasticity is crucial for VMS. Oxidation reduces the protein elasticity and the VMS. The vitelline membrane protects the embryo (pH 6) from the high pH of the albumen (pH 9.4). If it is too weak then the embryo will die. The embryo progressively gets surrounded by the yolk sac and is totally protected in the yolk sac between day 3 and 4 of incubation (Sadler, 1995).

Material and Methods

The objective of this experiment was to study the influence of Canthaxanthin from Carophyll® Red on the VMS in eggs from breeder hens. The experiment involved a flock of 3,800 breeders (males and females). They were placed in a laying house equipped with a floor system (1/3 slat, 2/3 litter) and separated into two adjacent blocks. Control diet contained 3,000 IU Vitamin D_3. It was based on corn, wheat, soya and sunflower meals; Vit E 60 IU/kg, Vit A 14,000 IU/kg, Se 0.3 ppm (including 0.05 organic Se). Supplementation with MaxiChick® started 3 weeks before the laying period (at 18 weeks), males and females both supplemented with 1,600 IU/kg vitamin D_3, 37.5 µg/kg 25-hydroxy-vitamin D_3 (HyD®) & 6 ppm canthaxanthin. Vitamin D_3 or 25-hydroxy-vitamin D_3 were not expected to have any influence on the VMS. Average hatchability rates (% fertile eggs) were 72.2 % in the control and 77.6 % in the MaxiChick® group from 21 to 51 weeks of age (the flock was killed at 51 weeks because of sanitary concerns). Eggs were collected at 40/45 weeks and stored 7 days. The VMS was evaluated using a compression machine MTS Synergy 200 a cylindrical probe with a diameter of 2 mm. Compression speed was 42 mm/mn. Each egg was cracked and the egg content was spread

on a plastic plate. The probe moved towards the yolk to penetrate through the yolk vitelline membrane in the equatorial region and the VMS was measured. Stiffness (or rigidity) is the property of a solid body to resist deformation. Breaking strength (or fracture strength) is the maximum force applied on the yolk before the breaking of the membrane occurs. Statistics were run with R 3.0.2 software. Data were analyzed for normality test (Shapiro) and were significantly different when $p<0.05$.

Results/discussion

The VMS was significantly increased by the MaxiChick® supplementation.

Table 1. Vitelline membrane strength according to the treatment

	Control	MaxiChick®	Statistics (T test)
Number eggs	209	172	
VM Stiffness (mN/mm)	7.0 +/- 1.0	7.2 +/- 0.9	P<0.05
VM breaking strength (mN/mm)	42.4 +/- 8.2	44.4 +/- 7.7	P<0.05

Oxidation occurs in any bodies living in an atmosphere with oxygen O^2. Radical oxygen species (ROS) are derived from the use of O^2. They are unstable and react with lipids, protein and cause damages to the tissues. Singlet oxygen $^1O_2$ is a very reactive ROS and canthaxanthin quenches the energy of $^1O_2$ and release it as heat. No chemical reaction is involved and the carotenoid is not consumed. Canthaxanthin has a high singlet oxygen quenching capacity among commercially available carotenoids (Cantrell et al, 2002) because of its long symmetric chain of double bonds carbon (Figure 1). Canthaxanthin is also used to regenerate oxidized Vitamin E to be able to capture a new ROS (Surai, 2012). During the first 3-4 days of egg incubation, the main generation of ROS occurs inside the yolk coming from the oxidation of unsaturated fatty acids and the cell division of the embryo (Surai, 2012). Increasing dosages of Vitamin E from 50 to 150 IU/kg could strengthen the vitelline membrane (Scheideler et al, 2010). Selenium had no significant effect on VMS (Scheideler et al, 2010; Monsalve, 2004). Selenium is not an antioxidant per se. Selenium forms part of the active site of the Glutathione peroxidase which main function is the protection of the albumen and not the yolk or the vitelline membrane (Surai, 2002). Vitamin C is a well-known antioxidant but it is not deposited in the egg and not produced by the embryo before 3 days of incubation. Beta-carotene is not efficiently transferred to the yolk of chicken eggs. All-trans-canthaxanthin from Carophyll® is well absorbed and deposited in the yolk. According to Grashorn and Steinberg, 2002, the deposition rate was 40% in the yolk. The diets for breeders do contain some carotenoids from corn but in a low amount. Therefore vitamin E and canthaxanthin are the only antioxidants present in high enough quantities to be of any protective use in the yolk during the first 3-4 days of incubation.

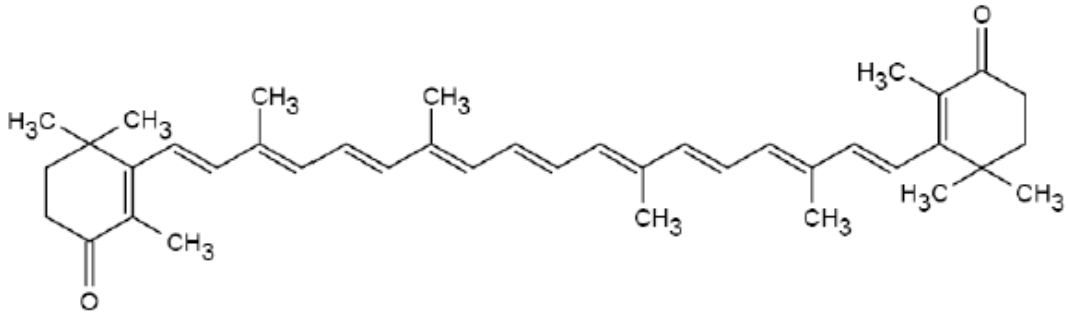

Figure 1. Canthaxanthin

Conclusions

Thanks to the unique capabilities of high deposition rate and singlet oxygen quenching capabilities, canthaxanthin from Carophyll® can improve the vitelline membrane strength in complement to Vitamin E and we hypothesized that it contributes to explain its influence on early embryo viability and hatchability in breeder performance.

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P-029 (ID 244)

SUPPLEMENTATION OF BUTYRIC ACID PRODUCING PROBIOTIC VERSUS DIETARY BUTYRATE

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Abstract

In this study the efficacy of Miya-Gold®, a probiotic containing viable spores of *Clostridium butyricum*, on foot pad lesions versus dietary butyrate supplementation was tested.

Foot pad lesions were compared between a group supplemented with coated calcium butyrate and a group which received the probiotic Miya-Gold® in the feed at a concentration of $2.5*10^8$ CFU/kg.

Broilers supplemented with butyrate showed a significant higher water to feed ratio, which resulted in a higher number of foot pad lesions compared to the group supplemented with Miya-Gold®. If any lesions were present, Miya-Gold® birds showed mainly signs of small superficial lesions while 35% of the butyrate group had deep large lesions.

Keywords: *Clostridium butyricum*, probiotic, foot pad lesions

Introduction

Contact dermatitis of the foot pads is an important commercial condition in the rearing of broilers. The condition impairs the welfare of the animals while economic costs are incurred as a result of carcass downgrading.

Foot pad dermatitis is mainly linked to wet litter. Enhanced gut health, by inhibiting pathogens to infiltrate the lower part of the gut, can reduce the incidence and severity of pododermatitis by affecting the litter moisture content.

Butyric acid is known to stimulate gut health (Guilloteau et al, 2010). However, targeted release at the hind part of the gut by dietary supplementation of butyrate is uncertain. The probiotic Miya-Gold®, which contains viable spores of the anaerobic bacteria *Clostridium butyricum*, delivers the butyric acid produced by the Clostridium at the lower part of the GIT (Okamoto et al., 2000).

Material and Methods

One thousand seven hundred, commercial, one-day-old broilers (male, Ross 308) were divided at random in two groups with 5 replicates per group. The broilers were housed in a standard environment at LVFZ Kitzingen (Germany) under commercial rearing conditions. A three-phase feed programme was used and the birds were kept to 35 days of age.

One group received feed supplemented with coated calcium butyrate (0.75 kg/mton of feed in starter; 0.5 kg/mton of feed in grower and finisher). The other group received feed supplemented with Miya-Gold® in starter, grower and finisher at a concentration of $2.5*10^8$ CFU/kg.

The animals were individually weighed at day 0 and at 35 days of age. Feed intake and water intake were registered daily on pen level. At the end of the trial foot pad lesions were scored on a scale of 0 to 2 (0=no lesions, 1=mild, superficial lesions, 2= severe lesions, ulcers or swollen foot pads).

Results

There were no significant differences in weight gain, feed conversion and survival rate between both treatments. On average, daily gain for both treatments was 59 g/day with a feed conversion of 1.55. Mortality was 2.8% for the butyrate and the Miya-Gold® group.

A significant lower water to feed ratio was seen for the birds supplemented with Miya-Gold® versus the butyrate group (table 1). As a result, Miya-Gold® birds had significant less foot pad lesions (56% showed no lesions) than the birds supplemented with butyrate (14% showed no lesions). If any lesions were present, Miya-Gold® birds showed mainly signs of small superficial lesions while 35% of the butyrate group had deep large lesions (table 2).

Table 1. Water/feed ratio*

	butyrate	**Miya-Gold®**
Total feed intake (kg)	3.238[a]	3.157[b]
Total water intake (l)	6.702[a]	6.142[b]
Water/feed ratio	2.070[a]	1.946[b]

Different letters mean statistically different at $p < 0.05$.
*recalculated per bird

Table 2. Foot pad lesions

	butyrate	**Miya-Gold®**
0: no foot pad lesions (% of birds)	14[a]	56[b]
1: small superficial lesions (% of birds)	51[a]	35[b]
2: deep and large lesions (% of birds)	35[a]	9[b]

Different letters mean statistically different at $p < 0.05$.

Conclusions

Although the dietary butyrate supplement was coated, probably the butyric acid was not released in the second part of the gut resulting in minimal influence. The obligate anaerobic *Clostridium butyricum* in Miya-Gold® produces butyric acid only in the anaerobic regions of the gut and thus at the targeted hind part.

A significant lower water to feed ratio was observed by supplementing Miya-Gold®, resulting in less and less severe foot pad lesions compared to the group receiving the coated butyrate.

In commercial management systems, Miya-Gold® exerts extra advantages versus dietary butyrate supplementation by improving the health of the birds.

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P-031 (ID 196)

EFFECT OF MEDIUM CHAIN FATTY ACIDS ON BROILER PERFORMANCE IN ENERGY REDUCED DIETS

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Abstract

It is known that medium chain fatty acids (MCFA) have a positive effect on broiler performance when added on top of a control diet. In this experiment a total of 480 Ross 308 one-day-old male broilers were used to determine whether a carefully balanced mixture of medium chain fatty acids [Aromabiotic® Poultry containing 60% MCFA] can replace energy in the diet without impairing performance. A completely randomized design was applied with three experimental treatments. A standard corn/wheat/soy based diet was used as a positive control (PC) and Aromabiotic® Poultry was supplemented to a -150 kcal reduced control diet (NC) at a dosage of 0.16%, 0.12% and 0.08% to the starter, grower and finisher diet respectively. The experimental design was applied to 24 pens (20 broilers/pen) with 160 broilers per treatment until 38 d. The supplementation of the negative control diet (NC) with Aromabiotic® Poultry improves body weight significantly (2196 g vs 2108 g; $P < 0.05$) and brings back growth performance to the original level of the positive control (2196 g vs 2187 g). WAFCR is numerically improved with Aromabiotic® Poultry (1.65 vs 1.73; trend) and again no significant differences were observed with the positive control (1.65 vs 1.63). Although the latter difference is not significant the numerical difference is still there. It is concluded that Aromabiotic® Poultry is an interesting functional feed ingredient for replacing energy but further research is needed to calculate the exact energy equivalent.

Keywords: MCFA, Aromabiotic, reducing energy, antibacterial, intestinal morphology

Introduction

For years the focus of broiler breeding companies has been the improvement of growth rate and feed conversion. This 'less for more' strategy has put tremendous stress on the health of the animals. To be able to reach the increased potential of genetic selection birds not only need a well-balanced diet but also benefit to a large extent from functional feed ingredients. With oil and fat prices around 800 euro / MT, energy is the major cost factor in diets for high performing animals. Broiler chickens have traditionally been fed relatively high-energy diets, because, in addition to promoting efficient feed utilization, it is also assumed that this type of diet maximizes growth rate (Leeson and Summers, 1991). If medium chain fatty acids are added to a broiler feed, it is known that performances of broilers will be better. With an inclusion rate of 0.8 g/kg Aromabiotic® Poultry (containing 60 % medium chain fatty acids (MCFA)), an increase in weight of 90 g and a decrease of 5 points WAFCR have been shown (Deschepper *et al*., 2013). Since it is well known that FCR depends strongly on the energy density of the diet (Holsheimer and Veerkamp, 1992), it was the aim of this experiment to determine to what extent Aromabiotic® Poultry can replace energy in broiler diets.

Material and Methods

In the described trial, a total of 480 male broiler Ross 308 chicks were used (Origin was Belgabroed Hatchery, Merksplas, Belgium). At the beginning of the trial, all transport boxes are weighed and the average weight of the chickens is determined. After weighing the day-old chickens individually, 24 groups are made. Only the day-old chicks with a weight of the average weight + or – 3 g could participate in this trial. The birds were housed in an experimental farm in commercial conditions in Meigem, Belgium. There were 3 treatments: a positive control with 2800 kcal/kg, 2900 kcal/kg and 2950 kcal/kg in the starter, grower and finisher feed, a negative control (positive control - 150 kcal / kg) and a negative control + Aromabiotic® Poultry. Aromabiotic® Poultry is delivered by Nuscience and contains 60 % MCFA (C6, C8, C10, C12) on a support of silicium dioxide. The dosage of Aromabiotic® Poultry was respectively 1.6; 1.2 and 0.8 g/kg for the starter, the grower and the finisher. There were 8 replicates of 20 birds / replicate per treatment (480 birds in total). A randomized block design was used. The broilers were vaccinated the 1st day of age against Newcastle Disease (NDW, spray) and Infectious Bronchitis (Poulvac IB Primer, spray). At 16 days of age the vaccination against Newcastle Disease was repeated with La Sota (Clone 30, drinking water). No other treatments were administered to the animals during the entire study period. The diet was wheat/corn/soy based and was formulated as shown in table 1. All treatments had a 3-phase feeding scheme (starter-grower-finisher).

Table 1: Wheat/corn/soy based diets, positive control and negative control (-150 kcal / kg)

Nutrient	Starter	Starter -150 kcal/kg	Grower	Grower -150 kcal/kg	Finisher	Finisher -150 kcal/kg
ME broiler (kcal/kg)	2800	2650	2900	2750	2950	2800
Crude protein (g/kg)	215	215	203	203	190	190
Crude fat (g/kg)	59.7	31.9	58.4	31.4	58.7	31.7
Ca (g/kg)	9.0	9.0	7.2	7.2	6.4	6.4
Dig P (g/kg)	4.1	4.1	3.2	3.2	2.8	2.8
Dig Lys (g/kg)	10.4	10.4	9.6	9.6	8.0	8.0
Dig M+C (g/kg)	7.6	7.6	7.3	7.3	6.1	6.1
Dig Thr (g/kg)	6.8	6.8	6.2	6.2	5.5	5.5

Daily the animals and housing facilities were inspected for the general health status, constant feed and water supply as well as temperature and ventilation, dead birds, and unexpected events. Daily mortality and cullings were recorded for each pen. Corrections for mortality calculating zootechnical performances were done using the number of 'broiler days' (number of broilers x days alive). For each trial, average pen weight was recorded at day 38. Feed intake was recorded. Feed conversion, daily growth rate and daily feed intake per bird were calculated. All parameters were subjected to a 2-factorial (treatment, block) analysis of variance (ANOVA, JMP).

Results and discussion

At arrival, one-day old Ross 308 birds showed a good quality with an average body weight of 43.8 g. The results of the trial are summarized in Table 2.

Table 2: Zootechnical performances

	Positive control	Negative control (-150 kcal / kg)	Negative control + Aromabiotic® Poultry
Average daily gain (g / bird / day)	54.9^{a}	52.9^{b}	55.2^{a}
Feed intake (g / bird / day)	91.4	91.5	93.2
Body weight (g / bird)	2187^{a}	2108^{b}	2196^{a}
WAFCR (2500 g)	1.63^{a}	1.73^{b}	1.65^{ab}

^{a,b} numbers on one row with different superscripts are significantly different ($P < 0.05$)

As expected, reducing energy has a negative effect on the end weight (2108 g vs 2187 g, $P < 0.05$) and on the WAFCR (1.73 vs 1.63, $P < 0.05$). These results are similar to the observations of Holsheimer and Veerkamp (1992) who found a lower end weight and a higher FCR when low energy diets are used. If Aromabiotic® Poultry is supplemented to this low energy diet, technical performances are almost back at the same level of the positive control group. The weight of the Aromabiotic® Poultry group is significantly different from that of the Negative control group (2196 g vs 2108 g, $P < 0.05$) and not significantly different from the Positive control group (2196 g vs 2187 g). The WAFCR of the Aromabiotic® Poultry group is not significantly different from that of the Negative (1.65 vs 1.73) and Positive control group (1.65 vs 1.63). Numerically the WAFCR of the Aromabiotic® Poultry group is clearly improved, but the Aromabiotic® Poultry group was not able to reach the original level of the Positive control group.

It is clear that Aromabiotic® Poultry has a positive effect on the technical performances in an energy-reduced diet, possibly due to the antimicrobial activity of MCFA (Kabara *et al*, 1972; Skrivanova *et al*; 2006; Batovska *et al*, 2009). This may lead to a lower intestinal infection pressure and an improved intestinal morphology, resulting in better digestive and absorptive capacities (Deschepper *et al*, 2003). On the one side, the energy that the birds usually use for this antibacterial activity will be taken over by Aromabiotic® Poultry, and this spared energy can be used for extra growth and a better WAFCR. On the other side, the energy from the feed will be absorbed more efficiently due to the improved intestinal morphology. In this case, if we look at the WAFCR, the energy that can be replaced will be around 120 kcal / kg.

Conclusions

In conclusion, Aromabiotic® Poultry proved to be very effective in replacing energy in broiler diets.. Further research, however, is needed to determine the exact energy value Aromabiotic® Poultry is able to replace (without hampering zootechnical performance), in a given situation.

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P-032 (ID 325)

GROWTH PROMOTING EFFECT OF *ASPERGILLUS LUCHUENSIS* IN BROILER

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Abstract

Four experiments were conducted to assure the growth promoting effect of a fungus *Aspergillus luchuensis* in broiler chickens (Ross 308). After optimum feeding levels of the fungus culture (Koji) was determined in experiment 1, sex difference of the growth promoting effect was tested in experiment 2. In experiment 3, the effects of Koji with different xylanase activities on the growth of the chicken were examined, and the concerted effect of *A. luchuensis* and *Clostridium butyricum* was evaluated in experiment 4. The Koji of *A. luchuensis* and *C. butyricum* culture were mixed with the basal experimental diet. The basal diet was formulated mainly by corn and soybean meal. The birds were raised using steel cages in a temperature controlled room with 20h light and given experimental diets ad libitum during 16 days experimental period. Significant growth promoting effect was observed when the level of the Koji was 0.04% while there was little effect when the levels were 0.002, 0.02 and 0.2%. The growth promoting effect was higher but not significantly in male birds than female birds. The Koji of *A. luchuensis* contained significant xylanase activity and the growth rate of the birds given Koji with higher xylanase activity tended to be higher than those given Koji with lower activity. Finally, we showed that simultaneous dose of *A. luchuensis* and *C. butyricum* accelerated the growth more than the birds given *A. luchuensis* alone.

Keywords: broiler, growth performance, *Aspergillus luchuensis*

Introduction

The present Koji (fungus culture) is a solid state fermentation product of harmless *Aspergillus* using cereals such as rice, barley and soybean as substrates. In Japan, Koji has been widely used for processing foods such as Miso, Shoyu and Sake. Koji contains many kinds of enzymes such as amylase, protease and lipase and these enzymes play essential roles in processing foods. Black Koji (culture of black *Aspergillus*) is mainly used in Okinawa and southern Kyusyu for the production of shochu a kind of liquor. Black *Aspergillus* produces citric acid and thus lowers pH of the fermented products. We have already reported that dietary supplementation of a black Koji (*Aspergillus luchuensis*) improves performance and meat quality in broiler (Saleh et al, 2011, 2012). We have also shown that digestibility is improved and intestinal profile of short chain fatty acids such as acetate, propionate and butyrate is changed by the Koji (Hioki et al in press). The present study was conducted, 1)to determine the optimal feeding level of the Koji, 2)to examine the sex difference of the effect, 3)to show the relation between xylanase activity and growth promoting effect and also 4)to show the concerted effect of *A. luchuensis* and butyric acid bacteria (*C. butyricum*) on the performance in broiler.

Material and Methods

1. Peperation of black Koji

Rice was soaked and drained and then steamed for 1 hour. After cooling down to 36 °C the rice was inoculated with *A. luchuensis* (Kawachi genichiro shouten, Japan) and fermented at 34-38°C for 5 days.

2. Animal experiment

In all the experiments, Ross 308 broiler chickens and commercial basal diet (Japan Scientific Feeds Association) were used. Water and feed were given ad libitum. Pairs of male and female birds (n=6) were raised in steel cages in the experiments except experiment 2. In experiment 2, male and female birds (n=6) were raised individually. The experimental periods were from 7 to 23 days of age for experiments 1 and 2, from 15 to 31 days of age in experiment 3 and from 12 to 28 days of age in experiment 4. Body weight and feed intake were measured every 4 days and feed conversion ratio was calculated. Experimental diets were prepared as shown below.

- Experiment 1 Basal diets supplemented with 0, 0.002%, 0.02%, 0.04% and 0.2% Koji (*A. luchuensis*) were prepared.
- Experiment 2 Basal diet supplemented with 0.04% Koji (*A. luchuensis*) was used.
- Experiment 3 Basal diets supplemented with 0.04% Koji (*A. luchuensis*) with high and low Xylanase (LX) activities were prepared
- Experiment 4 Basal diets supplemented with 0.04% Koji (*A. luchuensis*), 0.2% *C. butyricum* culture and both 0.04% Koji (*A. luchuensis*) and 0.2% *C. butyricum* culture were prepared

Results

All the results were shown together in Figure 1. Figure 1-A showed that the growth performance was the best when 0.04% Koji diet was given. The difference between control and the 0.04% group was statistically significant (Experiment 1). Figure 1-B shows that the effects of Koji was stronger in male than female (Experiment 2). Figure 1-C (Experiment 3) shows that Koji with high xylanase activity improved the performance more than that with the low xylanase activity. Figure 1-D (experiment 4) shows the concerted effect of *A. luchuensis* and *C. butyricum* on the growth performance.

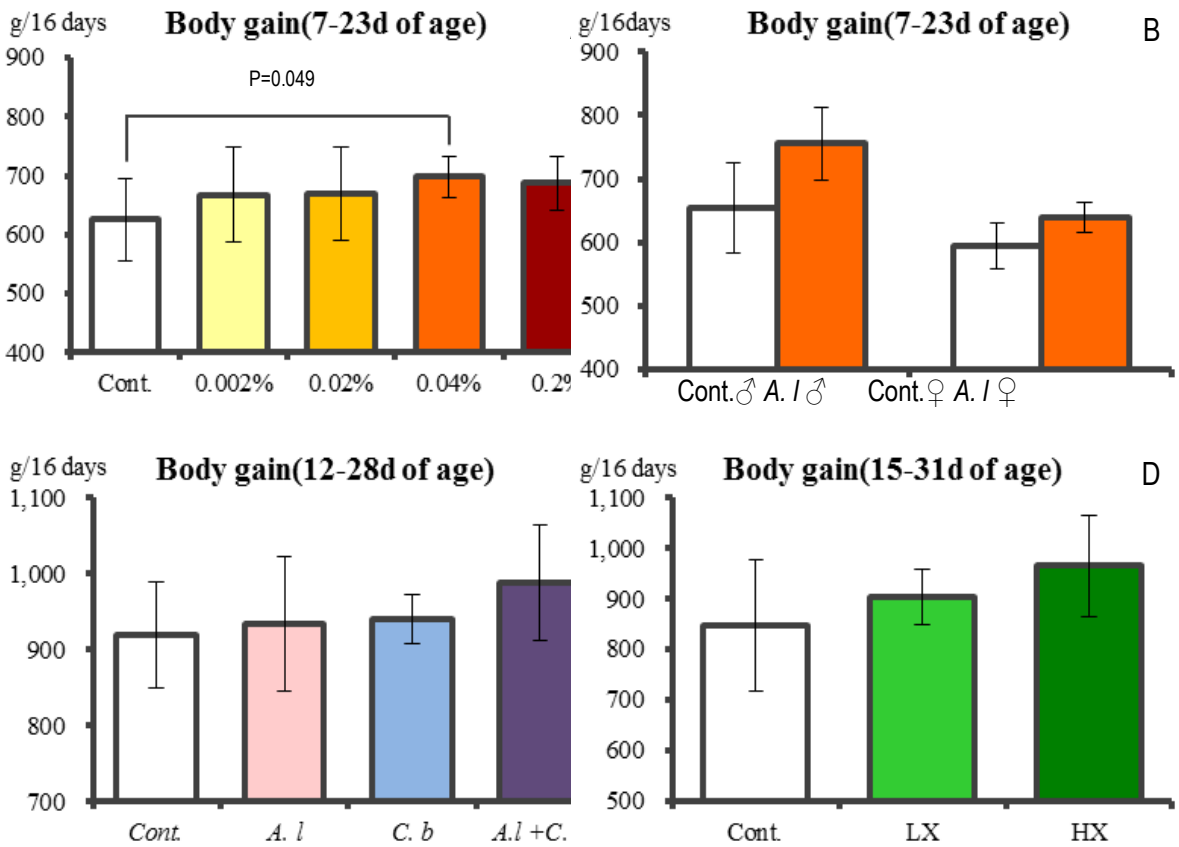

Figure 1. The differential effects of Koji on body weight gain due to feeding level(A), sex of chickens(B), xylanase activity(C) and combined supplementation with *C. butyricum*(D)

Conclusions

Growth promoting effect of Koji is highest at 0.04% feeding level especially in male chicken. The effect is higher when the xylanase activity is higher, and simultaneous feeding of *C. butyricum* stimulates the growth more.

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P-034 (ID 263)

EFFECT OF DIFFERENT LEVELS OF ARTICHOKE (*CYNARASCOLYMUS* L.) EXTRACT SUPPLEMENTATION IN DRINKING WATER ON THE IMMUNOLOGICAL RESPONSE AND YOLK RETENTION OF BROILER CHICKENS

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Abstract

This study was conducted to determine the effects of different levels of artichoke (*Cynarascolymus* L.) extract supplemented in drinking water on the immune response and yolk retention of broiler chickens. A total of 272 one-day-old broiler chicks (Ross 308) were randomly assigned to 16 battery cages and four treatment groups with 17 chickens per cage and 4 replicates per treatment. Treatments included the regular drinking water as control or the drinking water supplemented with 75 ppm. (T_1), 150 ppm. (T_2) and 300 ppm. (T_3) of artichoke. The drinking water was offered ad libitum throughout the study. Body weighted (BW) and yolk mass per body weight (YB) ratio were measured during experimental period. Blood samples were taken for measuring immune response. After 5 week, T_2 and T_3 showed higher hemagglutination inhibition titer against Newcastle disease virus (NDV) compared with control group ($P<0.05$). Other measured parameters, including BW, Y:B ratio and IgG against SRBC were not affected by the treatments ($P>0.05$). In conclusion, supplemented with 150 ppm. and 300 ppm. of artichoke in drinking water improved antibody-mediated immunity. Overall, high-level of artichoke appears to increase the immune response of broiler receiving the extract as supplement.

Keywords: Artichoke extract, broilers, body weight, yolk retention, immune response

Introduction

Rapid development of poultry production has increased stress and increased the risk of disease outbreaks in poultry industry. Under such conditions, antibiotics are used for therapy and growth-promotion in poultry drinking water, which have contributed to the increasing number of antibiotic resistant infection. Artichoke (*Cynarascolymus* L.**)** is a medicinal plant which rich in cynarin, flavonoids, and phenolic acids. Previously document nutritive effects of artichoke have been studied. Accordingly, the aim of this study was to evaluate different levels of artichoke in drinking water on the immunel response and yolk retention of broilers.

Material and methods

A total of 272 one-day-old broiler chicks (Ross 308) were randomly assigned to 16 battery cages and four treatment groups with 17 chickens per cage and 4 replicates per treatment. Treatments included the regular drinking water as control or the drinking water supplemented with 75 ppm. (T_1), 150 ppm. (T_2) and 300 ppm. (T_3) of artichoke. The drinking water was offered ad libitum in two phases: starter (1-7 days) and grower (15-24 days). Chickens were vaccinated following the standard broiler vaccination schedule during the whole experimental period. The chicken were weighted and collected for determine yolk mass:body weight ratio (Y:B ratio) at 1 and 7 days. In order to assay antibody responses to sheep red blood cell (SRBC), two chickens in each replicate were injected intramuscularly with 1mL, suspension of 7%SRBC in phosphate buffer saline on days 28. The hemagglutination inhibition (HI) test against newcastle's disease virus (NDV) was also set up to determine the antibody titer.

Results

As Table 1 indicates, Y:B ratio of broilers feeding diets containing artichoke at 0, 75, 150, and 300 ppm had no significant different. However, decreased Y:B ratio were observed in the chickens fed diet T_2. The results obtained in this study suggest that the presence of the yolk sac in a chick beyond day 7 should be considered as retained. The suitable rate retained is necessary for initiation of growth and the utilization of diet.

Table 1. The effects of different dietary additives on yolk mass:body weight ratio (Y:B ratio) in broiler chickens at 1 and 7 day.

Day	Dietary treatments			
	Control	Artichoke extract		
		75 ppm	150 ppm	300 ppm
1	9.34±2.18	8.29±2.81	8.54±3.12	7.11±1.38
7	0.18±0.09	0.14±0.11	0.11±0.07	0.14±0.09

The effects of experimental treatments on HI-titer against NDV is summarized in Table 2. Administration of artichoke had no effects on HI-titer against NDV as similar to other studies that found that artichoke had no effect on immune response (Fallen *et al*, 2013 and Tajodini *et al*, 2014).

Table 2. The effects of different dietary additives on Hemagglutination inhibition titer (HI-titer) against newcastle's disease virus (NDV).

Week	Dietary treatments			
	Control	Artichoke extract		
		75 ppm	150 ppm	300 ppm
0	5.50±1.22	6.75±0.65	6.13±1.10	6.63±0.85
1	5.25±0.87	5.50±0.41	5.00±0.41	5.25±0.29
2	3.63±0.75	4.25±0.29	3.25±0.87	3.38±0.63
3	4.13±1.25	5.00±1.47	4.50±1.00	4.75±0.87
4	4.88±1.25	4.75±0.87	3.38±1.31	4.00±1.22
5	5.00±0.41	5.25±0.96	6.33±0.63	6.78±0.71
6	4.75±0.29	4.63±0.75	5.38±0.48	4.75±1.32

Antibody titer against sheep red blood cell (SRBC) is summarized in Table 3. There were no significant different ($p<0.05$) in antibody titer among groups.

Table 3. The effects of different dietary additives on antibody titer against sheep red blood cell (SRBC).

Group	IgG level (u/mL)	
	7 days	14 days
Control	32.41±24.76	23.40±17.08
T_1 (75 ppm)	19.53±4.90	12.76±10.02
T_2 (150 ppm)	41.74±27.82	8.69±6.48
T_3 (300 ppm)	35.67±15.52	8.79±2.91

Conclusions

Regarding the present study, the effects of artichoke on the immune function are rare. However, high-level of artichoke appears to increase the immune response of broiler receiving the extract as supplement.

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P-035 (ID 330)

EFFECT OF A PHYTOGENIC FEED ADDITIVE ON PROTEIN AND AMINO ACID DIGESTIBILITY IN BROILERS

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Abstract

The objective of this study was to investigate the effects of a phytogenic feed additive (PFA; Biostrong 510, Delacon) consisting of essential oils, herbs and spices on ileal protein and amino acid digestibility in 21-day-old broilers. 72 day-old Cobb 500 broilers were randomly allocated to two dietary treatments. Each treatment was replicated 9 times with 4 birds per replicate. Birds were fed a corn/wheat/soy-based mash diet with 0 mg/kg PFA (control) or 150 mg/kg PFA (treatment). Feed and water were available for *ad libitum* intake. Titanium oxide was used as inert dietary marker (dose 5 g/kg) to enable measurement of the apparent ileal digestibility values on day 21. Ileal protein digestibility was significantly ($P<0.05$) improved by 5.1% in birds fed the PFA. Digestibility values for amino acids were increased by on average by 1 to 4 percentage units by the PFA, but effects were only statistically significantly demonstrated for four amino acids at $P<0.05$ and for three amino acids at $P<0.10$. Body weight gain and feed intake were not significantly different between the two treatments, whereas feed conversion ratio tended ($P<0.10$) to be better by dietary PFA supplementation. This study showed the tested PFA improved protein digestibility in broiler, being supported by amino acid digestibility values.

Keywords: phytogenic, broiler, digestibility, amino acids

Introduction

Phytogenic feed additives are plant derived and numerous growth and health promoting effects have been shown in poultry. These benefits mainly comprise effects on secretion of digestive enzymes and bile (JAMROZ et al., 2003; PLATEL and SRINIVASAN, 2004), and on intestinal morphology (AMAD et al., 2013). Positive effects on nutrient digestibility values were shown not to be age-depended in broilers (AMAD et al., 2011). Moreover, Utterback at al. (2013) demonstrated positive effects on amino acid digestibility in laying hens. However, data on the effects of phytogenic feed additives on ileal amino acid digestibility in broilers is limited. Therefore an experiment was done to investigate the effects of a phytogenic feed additive on amino acid digestibility in broiler. (UTTERBACK et al., 2013)

Material and Methods

A total of 72 day-old male Cobb 500 chickens were randomly allocated to two dietary treatments. Each treatment was replicated 9 times with each 4 broilers. Broilers were housed in stainless steel cages with a base area of 0.19 m².

Birds of the control group (CON) were fed a diet based on soybean meal, corn and wheat from day 1 till day 21. Treated birds (PFA) were fed the basal diet supplemented with a phytogenic feed additive (Biostrong 510, Delacon Biotechnik GmbH) at 150 mg/kg. Diets were formulated to meet the nutrient recommendations given by GFE (1999). Feed and water were available for ad libitum intake.

Production performance (body weight, feed intake and feed conversion ratio) was measured per cage from day 1 to 21 of age. For measuring apparent ileal amino acid digestibility birds were killed after stunning on day 21. Digesta were collected from the entire ileum (segment from Meckel's diverticulum to about 3 cm cranial to the ileocaecal junction). Ileal digesta were flushed with distilled water of the intestinal segment and were pooled per cage. Diets and ileal digesta were analyzed for crude protein, AA and Ti contents. Titanium Oxide (TiO_2) was supplemented to the diets as inert marker at 5 g/kg.

Performance and digestibility data were statistically analyzed using SAS. After checking the homogeneity of the variances, analysis of variance was performed (proc glm) and means were compared (Tukey test). The significance level was set at $p \leq 0.05$.

Results

The effects of the addition of the PFA on protein and amino acid digestibility are summarized in Table 1. PFA supplementation significantly ($P \leq 0.05$) improved crude protein digestibility by 5.1% in 21 day broiler. Digestibility of total amino acids (TAA) was also significantly improved in birds fed the PFA. Digestibility of Lys, Ala, Gly and Glu were significantly improved ($P \leq 0.05$) and digestibility of Pro, His, Leu and Asp tended ($P \leq 0.10$) to be improved by the PFA.

Table 1. Effects of a phytogenic feed additive (PFA) on protein and amino acid digestibility in 21-day old broilers, when compared to an unsupplemeted control (CON) diet.

	CON	PFA	P-value
Crude Protein	80.8 ± 2.3	84.9 ± 1.7	<0.001
Asp	78.6 ± 2.5	81.0 ± 3.0	0.075
Thr	74.6 ± 3.3	77.4 ± 3.7	0.116
Ser	80.6 ± 2.5	82.8 ± 2.9	0.109
Glut	82.6 ± 2.2	84.9 ± 2.2	0.040
Gly	76.1 ± 2.6	79.3 ± 3.2	0.034
Ala	79.0 ± 2.5	81.8 ± 2.9	0.046
Val	76.7 ± 2.6	79.0 ± 3.5	0.143
Iso	80.1 ± 2.4	81.6 ± 3.6	0.332
Leu	82.6 ± 2.0	84.6 ± 2.6	0.088
Tyr	77.2 ± 4.0	75.4 ± 5.7	0.437
Phe	85.3 ± 1.9	86.3 ± 2.4	0.305
His	85.3 ± 1.6	87.1 ± 2.1	0.058
Lys	83.8 ± 1.9	85.9 ± 2.1	0.042
Arg	88.3 ± 1.5	89.1 ± 1.9	0.338
Pro	84.2 ± 2.0	85.5 ± 2.4	0.075
Met	94.1 ± 1.7	93.5 ± 1.5	0.116
Cys	71.6 ± 2.9	72.9 ± 3.7	0.109
TAA	81.0 ±2.2	82.9 ± 2.8	0.040

Performance data for the whole trial period are shown in Table 2. Feed conversion ratio (FCR) tended to be improved ($P \leq 0.10$) by 4.7% in birds fed the PFA. No significant differences were observed between the two groups in body weight gain (BWG) and feed intake (FI).

Table 2. Effects of a phytogenic feed additive (PFA) on performance in 21-day old broiler, when compared to an unsupplemeted control (CON) diet.

	Con	PFA	P-value
BWG, g/d	750 ± 53	779 ± 53	0.277
FI, g/d	926 ± 87	916 ± 77	0.804
FCR, g/g	1.234 ± 0.074	1.176 ± 0.062	0.092

Discussion and Conclusions

In the current study the tested PFA (Biostrong 510, Delacon, Austria) significantly improved ileal protein digestibility. This finding is in line with the results shown by Amad et al. (2011). In their publication a significant improvement of ileal protein digestibility was shown in broilers at 21 days, 35 days and 42 days of age when fed a phytogenic feed additive. Utterback at al. (2013) have reported a positive effect of a phytogenic feed additive on amino acid digestibility in laying hens. Similar to that publication, results of the current study revealed that feeding the phytogenic additive part of the amino acid digestibilitiy values were significantly improved or tended to be. Improved protein and amino acid digestibility is most likely linked to an increased activity of pancreatic proteases through PFA feeding. Platel and Srinivasan (2004) have shown that the addition of phytogenic substances increases the activity of intestinal proteases in rats, whereas previous research has shown that the tested PFA increases activity of pancreatic enzymes in the lumen from 10 to 25 % (data not shown).

From the current study it is concluded, that protein and amino acid digestion was improved which resulted in an improved feed efficiency in broilers.

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P-036 (ID 331)

EFFECTS OF COMBINING PHYTOGENIC SUBSTANCES WITH ESTERS OF SHORT AND MEDIUM CHAIN FATTY ACIDS ON PRODUCTION PERFORMANCE IN BROILERS

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Abstract

An experiment was conducted to evaluate the effects of the combination of a phytogenic feed additive (PFA) and esters of short and medium chain fatty acids (SMFA) depending on duration of application on production performance in a subclinical necrotic enteritis infection in broiler chickens. A total 1'600 male Cobb x Cobb broilers were randomly allotted to four dietary treatments, each with 8 repetitions. Treatments were: infected non-treated (INT) and 3 groups infected treated (IT) with 750 mg/kg PFA+SMCFA till day 21 (T1), day 28 (T2) and day 35 (T3), all followed by PFA alone (150 mg/kg) till harvest at day 49. Birds were fed a corn/soy based diet for ad libitum intake. All birds were vaccinated against coccidiosis at day 1 (Coccivac-B) and challenged with a culture of C. perfringens on days 19, 20, and 21. On day 21 five birds per pen were necropsied for NE lesion scoring. Results on day 49 show that birds in all IT groups had significantly ($p<0.05$) better FCR compared to birds of the control. FCR between IT groups didn`t differ significantly. However, with prolonged application FCR was numerically improved. Body weight on day 49 tended ($p<0.10$) to increase when birds fed combination of PFA and SMCFA followed by PFA alone. On day 21 NE lesions were significantly reduced ($P<0.01$) in birds fed PFA and SMCFA. It was concluded that the application of this combination of PFA and SMCFA followed by PFA alone till harvest significantly improved production performance and reduced NE related gut lesions.

Keywords: necrotic enteritis, health, performance, broiler

Introduction

Currently the ban on the use of feed antibiotics is becoming common use in more and more countries. By the end of 2016 the USA will also ban in-feed antibiotics. Necrotic enteritis, caused by *Clostridium perfringens* is a disease in poultry that can have a high economic and animal welfare cost, and becomes more prevalent in birds fed AGP free diets (McDevitt et al., 2006). Thus, strategies to control and support intestinal health in broiler becomes more important. Some nutritional interventions, like modifications of feed composition and feed characteristics, that alleviate the consequences of intestinal disease challenges, decrease incidence of clostridial infections or simply improve nutrient availability in broilers, have been summarized (Van der Klis, 2014). Amad *et al.* (2013) have reported positive effects of a PFA containing thymol and anethol on gut morphology. Positive effects of thyme oil on intestinal integrity in broiler are also reported (PLACHA et al., 2014). In addition, SMFA exert direct antibacterial effects in the intestine. The objective of the present study was to investigate the effects of a combination of a PFA and SMFA on performance and gut lesions in broiler under a sub-clinical *C. perfringens* challenge.

Material and Methods

A total of 1'600 male Cobb x Cobb broilers were randomly allocated to 4 dietary treatments. Each treatment was replicated 8 times with 50 broilers each. Treatments were: infected non-treated (INT) and 3 groups infected treated with 750 mg/kg PFA+SMFA till day 21 (T1), day 28 (T2) and day 35 (T3), all followed by PFA alone (150 mg/kg) till harvest at day 49. Birds were fed a starter (day 0-21), grower (day 21-35) and finisher (35-49) corn/soy-based diet with or without additives according to treatments. Feed and water were provided for *ad libitum* intake.

All birds were spray-vaccinated at the trial facility with the label recommended dosage of Coccivac-B on day of hatch. On day 19, 20 and 21 all were challenged with *C. perfringens.* Each day fresh inoculum was prepared and administrated by mixing in the feed.

Production performance was measured per replicate per phase (data not shown) and over the total 49-day period. On Day 21, five birds from each pen were selected, sacrificed, group weighed, and examined for the degree of presence of Necrotic Enteritis lesions. The scoring was based on a 0 to 3 score, with 0 being normal and 3 being the most severe.Performance and lesion scores were statistically analyzed using SAS. After checking the homogeneity of the vari-

ances, analysis of variance was performed (Tukey test). Performance means were compared for all treatments whereas lesion scores were compared between non-treated and treated (T1, T2, T3). The significance level was set at $P \leq 0.05$.

Results

The effects of duration of supply of the PFA and SMFA are summarized in Table 1. Results show that with increased duration of supply BW and FCR were improved. Independent from duration of application, the combination of PFA and SMFA significantly ($P<0.001$) improved FCR. Body weight gain tended ($P<0.10$) to be improved with application of PFA and SMFA till day 35 (T3) compared to INT. In T1 and T2 was only numerically improved compared to INT.

Table 1. Effects of duration of supply of PFA in combination with SMFA on broiler performance on day 49

	Body weight (g)	Feed conversion ratio (feed/gain)
INT	2'639^{A}	1.941^{a}
T1 (till day 21*)	2'682^{AB}	1.852^{b}
T2 (till day 28*)	2'729^{AB}	1.816^{b}
T3 (till day 35*)	2'788^{B}	1.803^{b}
p-value	*0.087*	*<0.001*

* application of combination of PFA and SMCFA followed by PFA at 150 mg/kg till slaughter

Figure 1 shows the effects of duration of supply of the PFA and SMFA on necrotic enteritis (NE) gut lesions. Compared to the INT birds, treated birds had significant ($P=0.008$) lower NE related gut lesions on day 21.

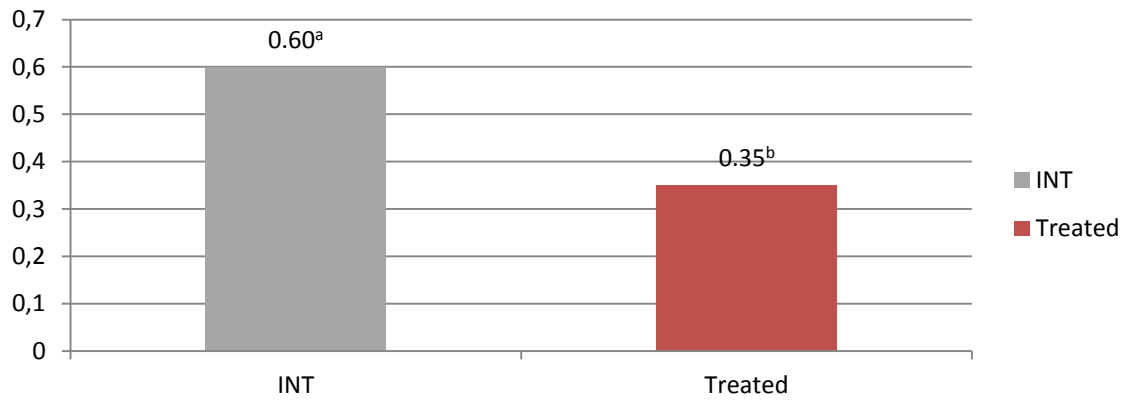

Figure 1: Effects of application of PFA and SMFA on necrotic enteritis related gut lesions in 21 day old broiler ($P=0.008$). Skrivanova et al. (2006) reported strong antibacterial effects of medium chain fatty acids against Gram-positive bacteria. Especially C. perfringens is very sensitive to these medium chain fatty acids. Such molecules prevent necrotic enteritis lesions due to growth inhibition or killing of C. perfringens in the gut. This supports the findings of the present study, which shows a significant reduction in NE related lesions. Performance results might be related to the positive effects of the PFA on performance as shown by Amad et al. (2011). From the current study it is concluded, that the combination of the tested PFA and esters of short and medium chain fatty acids reduces necrotic enteritis lesions and improves performance under a C. perfringens challenge.

Conclusions

In the current study the combination of PFA and SCFA (Biostrong Forte, Delacon, Austria) significantly improved FCR and significantly reduced necrotic enteritis related gut lesions.

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P-037 (ID 162)

A *BACILLUS SUBTILIS* BASED PROBIOTIC ASSIST IN OVERCOMING THE PERFORMANCE LOSS ASSOCIATED WITH DIETS REDUCED IN ENERGY

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Abstract

A study was carried out to evaluate the effect of a commercially available *Bacillus subtilis* based probiotic GalliPro® (GP) to corn/soybean based broiler diets with reduced energy levels. A total of 2160 Cobb 500 broilers were allocated to 6 treatments and reared over 42 days with *ad libitum* feeding. The positive control (100% ME) met NRC standards and GP was added with 8 x 10^5 CFU/g of feed (500 ppm). Treatments were as follows: 1) 100% ME; 2) 98% ME; 3) 96% ME; 4) 100% ME + GP; 5) 98% ME + GP; 6) 96% ME + GP. By day 42, each pair of energy level showed significant improvement in bodyweight with GP addition. The 100% ME control showed similar weight gain as 98% ME + GP and 96% ME + GP. The FCR at day 42 was likewise improved by GP for 98% ME + GP being statistical similar to 100% ME control (1.814). Overall mortality was significantly lower in both 100% ME + GP (3.89%) and 98% ME + GP (2.50%) groups, compared to the same energy level without GP addition (both 6.11%). In conclusion, these results indicate that addition of a *Bacillus* based probiotic has the ability to reduce mortality and overcome performance loss associated with diets reduced up to 4% in energy.

Keywords: broilers, *Bacillus subtilis* probiotic, dietary energy

Introduction

It is well known that the margins of the poultry industry and, therefore, its future prospects are largely dependent on feed costs, these being consequently one of the main concerns of poultry producers. Moreover, their goal today is to provide consumers with safe food, while promoting poultry health and welfare, in addition to carefully respect the environment. Given this challenging conjuncture, poultry producers really need tools to achieve all the referred objectives in the most competitive possible way.

The intestinal microflora has a great impact on avian performance, health and welfare. Furthermore, it is widely recognized that its profile can be modulated through dietary changes and by a variety of feed additives (Pedroso et al., 2013; Pan and Yu, 2014). In this sense, probiotics represent a valuable option to simultaneously improve production efficiency, avian health and welfare and poultry meat safety. GalliPro® (GP) is commercial heat stable *Bacillus* probiotic, which contains spores of *Bacillus subtilis* (>1.6 x 10^9/gram) as the active ingredient. The use of GP as a growth promoter is not a new concept. While there have been several studies focusing on the effects of GP on broiler performance, intestinal microflora profile, pathogen bacteria counts in the gut and immune response (Opalinski et al., 2007; Knarreborg et al., 2008; Knap et al., 2011; Lourenco et al., 2012), few studies have approached the contribution of this probiotic to dietary energy efficiency in chickens. The objective of this study was to determine if broiler chickens fed reduced energy (ME) diets supplemented with GP at 8x10^5 CFU's/gram of feed, perform similarly to broilers fed higher energy diets.

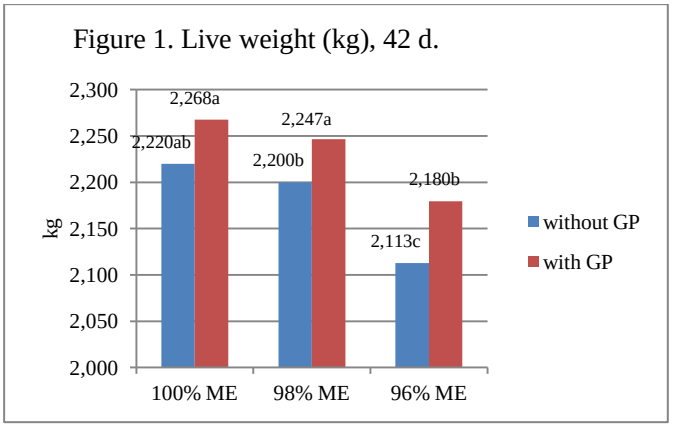
Material and Methods

This experiment consisted of 72 pens with 30 straight run Cobb 500 broilers in each pen (2160 birds in total). The 76 pens were further grouped into 12 location blocks with 6 pens per block with each pen within a block randomly representing one of 6 treatment groups identified as 100% ME, 98% ME and 96% ME (without GP) or 100% ME+GP, 98% ME+GP and 96% ME+GP (with 500 ppm GP). On Day 7, each pen was top dressed with exactly 1,8 kg of previously used broiler litter from a flock having no disease issues or exposure to direct fed microbials. Continuous lighting was provided from Days 0 to 42. Feeds for this study were made up of corn, soybean and meat/bone meal. Diets were formulated to provide 3 different ME levels for each of the starter, grower and finisher dietary phases (9 basal diets in total). Pen body weights and feed weights (for feed conversions) were recorded at Days 0, 21 and 42. All deaths and removed birds (mortality) were documented. Data was analysed by ANOVA. Each response variable was evaluated by

serial 2-tail t-tests comparing the means of each group to all others assuming unequal variances. The p-value of any comparison found to be less than or equal to a "p" value 0.05 ($p \leq 0.05$) was considered as statistically different.

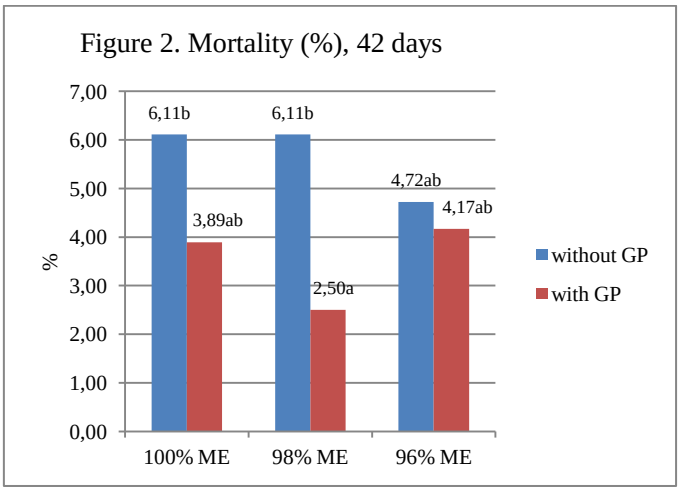
Results

At day 21, birds were heaviest ($p<0.05$) for 100% ME group and lightest ($p<0.05$) for 96% ME group, with all other groups being intermediate. Addition of GP in the diet did not improve live weight at 21 d. Live weight/bird of 100% ME + GP and 98% ME + GP groups was heavier ($p \leq 0.05$) than all other groups with the exception of the 100% ME group, which was not different ($p>0.05$). Final weights of each pair of 98%, and 96% ME treatment groups showed the GP-supplemented group having the heaviest ($p \leq 0.05$). This observation also shows that the 42d live weights of 96% ME + GP group was equal to that of the 98% ME group suggesting an energy uplift. Final live weights are shown in figure 1.



Means followed by different letters are significantly different ($p<0.05$)

GP addition to the diet resulted in significantly improved FCR ($p<0.05$), with the exception of the lowest energy diet (96% ME). Mortality of the 3 groups receiving GP were either numerically lower (100% ME + GP and 96% ME + GP) or significantly ($p \leq 0.05$) lower (98% ME + GP) than their same ME level counterparts. In figure 2, mortality results are shown.



Means followed by different letters are significantly different ($p<0.05$)

Conclusions

The goal of this study was to determine if broiler chickens fed low energy diets supplemented with GP, perform similarly to broilers fed higher energy diets or do similarly to non-supplemented broilers on the same energy level. Under these experimental conditions, the results of this study demonstrated that diets with 2% lower energy and supplemented with GP have final feed conversions and live weights equal to non-supplemented diets with 2% higher energy. Furthermore, GP addition to the diet significantly reduced mortality in the group with 98% ME and numerically in 100% and 96% ME groups. In view of these results, it can be concluded that GP supplementation in broiler diets results in dietary energy utilization uplift and in mortality reductions.

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P-040 (ID 250)

EVALUATION OF CHOLINE CHLORIDE REPLACEMENT BY A POLYHERBAL MIXTURE IN INTENSIVE BROILER PRODUCTION

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Abstract

Material and methods: In a 1200m^2 Colorado type house 26400 broilers ROSS PM3 were raised from which 820 males were divided into 20 cages inside the house; 2 batches of 10 repeats of 41 males were performed. Diet, based on corn, wheat and soybean meal was supplemented with choline chloride (CC group) or Natu B4® (B4 group) using a substitution ratio of 100g of Natu B4® for 430g of Choline chloride 70%. Feed consumption, body weight and FCR are measured at days 12, 24, 28 and 35. Blood analyses and liver weight are performed at day 35 on 43 animals per batch.

Results: Except for FCR which was significantly lower during the first 12 days for B4 group, final weights and FCR were similar at each age. Despite a low initial weight at day 1 (37.4 and 37.6 g), final weights at 35 days were heavy, 3255 and 3244g for CC and B4 group respectively. Final FCR were 1.54 for both groups. Most of blood parameters were similar in both groups (glucose, cholesterol, triglycerides, albuminemia, SGOT, etc..). Blood urea levels were significantly lower in B4 group. Liver weight were significantly lower in B4 group, 51.3 vs 55.4g.

Conclusion: Natu B4® is able to replace choline chloride supplementation allowing high level of performance. Natu B4® is a technical and economical substitute of choline chloride.

Introduction

Choline deficiency results in liver damage such as steatosis, hepatocarcinomas, inflammation, oxidative damage, abnormal phospholipid secretion, defects in lipoprotein secretion, Rinella et al, 2008. In animal production choline deficiency results also in reduction of zootechnical performance thus choline choride is often supplemented in animal feed. The purpose of this trial is to compare the zootechnical performance of standard choline chloride supplementation and its substitution by Natu B4®, polyherbal product rich in phosphatidylcholine.

Material and methods

Building and Animals

In a 1200m2 Colorado type house 26400 broilers ROSS PM3 were raised from which 820 males were divided into 20 cages inside the house. 2 batches divided into 10 repeats of 41 animals are performed. Feed consumption, body weight and FCR are measured at days 12, 24, 28 and 35. Blood analyses and liver weight are performed at day 35 on 43 animals per batch.

Feeding treatments

Two groups, CC & B4 are compared. CC group is supplemented in Choline chloride while Natu B4® is used in the B4 group, according the quantities reported in table 1

Table 1: Choline Chloride and Natu B4® content (g/T) in the different feed (age in days)

Group	g/T	1-12 d	12-21 d	21-28 d	28-35 d	1-12 d	12-21 d	21-28 d	28-35d
CC	Choline Cl	857	643	643	429	-	-	-	-
B4	Natu B4®	-	-	-	-	200	150	150	100

Results

Zootechnical performance

High performance levels were reached (table 2) with final weights at 35 days of 2355 and 2344 grams respectively for CC and B4 groups (1 day in advance versus genetic line standards). FCR, 1.54 for both groups at 35 days, is also better (-3.9%) compared to expected genetic line standard. A slight but significant improvement of FCR in the B4 group during the first 12 days was observed (1.02 vs 1.03).

Table 2: Zootechnical performance

	Day 12		Day 35	
	CC	B4	CC	B4
Weight g	421 ±10	425 ±-5	2355±218	2344±227
Feed Conversion	1.03±0.01[a]	1.02±0.01[b]	1.54±0.02	1.54±0.03

[a], [b] statistically different (P<0.05)

Blood parameters and liver weight

Glucose, total cholesterol, triglycerides, albumin, liver enzymes, were similar in the 2 groups. Urea and creatinine levels were significantly lower in B4 group. Liver weight is also significantly lower in the B4 group, 51.3g vs 55.4g, table 3.

Table 3: blood analysis (partial results) and liver weight at day 35

	CC	B4
Glucose g/L	2.23	2.23
Urea g/L [1] < 0.05	27.3 %[a]	50.0 % [b]
Urea g/L [1] ≥ 0.07	20.5 %	15.9 %
Creatinine mg/L[2] < 0.6	15.9 %[a]	34.1 %[b]
Creatinine mg/L [2] ≥ 0.6	84.1 %	65.9 %
Liver weight at 35 days (g) [3]	55.4±7.3[a]	51.3±7.1[b]

[1] Urea [a], [b] Khi 2 p = 0.036; [2] Creatinine [a], [b] Khi 2 p = 0.049; [3] Liver weight [a], [b] Kruskal-Wallis Batch p = 0.042

Discussion

Same zootechnical performance and similar blood parameters show an equivalency on the mode of action between Choline chloride and Natu B4®.

Natu B4® is characterised by its high content in phosphatidylcholine and Choline chloride is a precursor of phospatidylcholine. Lamaziere and Wolf, 2010, have given clear evidence that one important mode of action of phosphatidylcholine in the liver function is mediated through the peroxisome proliferator-activated receptor, PPARα nuclear receptors. Ip et al, 2004 have been able to correct a liver fibrosis and steatosis caused by a dietary deficiency in choline with a fibrate supplementation. Fibrates, a class of molecules used for the treatment of metabolic disorders (hypercholesterolemia) are also strong PPARα agonist, Staels et al, 1998. Among the different terpenoids present in Natu B4®, rutin has been also studied for its positive effect on liver cells. Wu and al, 2011, also found out the positive effect of rutin in the stimulation of PPARα and antioxidative enzymes

Nephroprotective activity of rutin through a reduction of blood creatinine and urea has been demonstrated by Sadeghnia et al, 2013. Demirbiliek et al, 2006, has also found a reduction of blood urea and creatinine with polyenylphosphatidylcholine, showing, on kidney protection, a similarity of action of this choline ester and rutin contained in Natu B4®.

Conclusion

Possibly explained by a similar PPARα activation, Choline chloride and Natu B4® provide the same performance in broiler growth. Natu B4® is even able to do a little better through an improvement of feed conversion in the early age, a higher reduction of liver weight at slaughter, and a better kidney protection by reducing urea and creatinine level.

In industrial conditions, Choline chloride can be substituted by a polyherbal mixture rich in Phosphatidylcholine.

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P-041 (ID 222)

EFFECT OF ARTICHOKE (*CYNARA SCOLYMUS* L.) EXTRACT SUPPLEMENTATION IN DRINKING WATER ON GROWTH PERFORMANCE OF BROILER CHICKENS

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Abstract

This study was conducted to determine the effect of artichoke (*Cynara scolymus* L.) extract (AE) supplementation in drinking water on growth performance of broiler chickens. A total of 272 day-old chicks were randomly distributed into four groups (control: without AE; AE-75: 75 ppm AE in drinking water; AE-150: 150 ppm AE in drinking water; AE-300: 300 ppm AE in drinking water). Each group was containing four replicates of 17 birds. All chicks were offered mash feed and drinking water *ab libitum*. Artichoke extract was provided on day 1-7 and 15-24 of age. Body weight and feed intake were recorded weekly in all replicates. Data were analyzed as a completely randomized design. The results showed that, 1 to 42 days of age, average daily gain (ADG) was significantly higher ($P<0.05$) in AE-75 (65.56 ± 3.97 g/d) and AE-300 (65.01 ± 0.52 g/d) compared with control (60.09 ± 3.18 g/d) and AE-150 (60.10 ± 2.04 g/d. Feed intake (FI) was significantly higher ($P<0.05$) in AE-300 (110.49 ± 11.12 g/d) compared with control (94.10 ± 2.54 g/d) and AE-150 (99.42 ± 5.63 g/d) but similar to AE-75 (102.77 ± 5.10 g/d). However, feed conversion ratio (FCR) did not difference between treatments (1.57 ± 0.09, 1.57 ± 0.04, 1.68 ± 0.10 and 1.63 ± 0.08, respectively). It could be concluded that artichoke extract supplementation in drinking water may improve growth performance of broiler chickens.

Keywords: Artichoke extract, *Cynara scolymus* L., Growth performance, Broiler chickens

Introduction

Antimicrobial additives are often used to suppress or eliminate harmful microorganisms in the intestine and to develop growth and efficiency of feed. It has been shown that the continuous usage of antibiotics as growth promoters encourages the retention of residues in animal tissues and that the human consumption of such animal products would potentially increase processes of antibiotic resistance However, the use of antibiotics as feed additives has been banned in recently years because of the public concern over possible antibiotic residual effects and the development of drug resistant bacteria (JIN et al., 1997; LEESON, 2007; MOHAMMAD et al., 2011). One strategy is being developed to overcome these problems, supplementing the animal with bioactive components such as e.g. herbs, plant extracts and essential oil.

Artichoke (*Cynara scolymus* L.) is a member of the Compositae (daisy) family (NATEGHI et al. 2013). The major bioactive components of artichoke leaves are vitamin C, carotenoids, cynarin, flavonoids, phenolic acids, and caffeic acid (WANG et al., 2003; JOY and HABER, 2007; RADWAN, et al., 2007; FALLAH et al. 2013). Several research have shown that the artichoke extract (AE) had some beneficial effects such as protection the hepatotoxicity in broiler chickens (NATEGHI et al., 2013), reduction of blood urea, cholesterol and triglyceride in broiler chickens (FALLAH et al., 2013), improving the productive, reproductive performance and low cholesterol eggs of Mandarah hens (RADWAN, et al., 2007), modulating the oxidative stability and meat quality of Japanese quail (ABBASI and SAMADI, 2014) and enhancing the immune functions in broiler chickens (TAJODINI et al., 2014). Therefore, the objective of this study was conducted to determine the effect of AE supplementation in drinking water on growth performance of broiler chickens**.**

Material and Methods

Animals and Sample Collection

A total of 272 day-old chicks were randomly distributed in completely randomized experimental design (CRD) into 4 treatments with 4 replicates of 17 birds in each. The experimental treatments consisted of control (without AE), AE-75 (75 ppm AE in drinking water), AE-150 (150 ppm AE in drinking water) and AE-300 (300 ppm AE in drinking water). The dietary period was divided in two phases: starter (1 to 21 days) and grower (21 to 42 days). All chicks were offered

mash feed and drinking water *ab libitum*. AE was provided on day 1-7 and 15-24 of age. Routine medication, vaccination and husbandry practices were administered. Body weight and feed intake were recorded weekly in all replicates. Average daily gain (ADG), Feed Intake (FI) and feed conversion ratio (FCR) were calculated. The data were analyzed using the General Linear Models (GLM) procedure. The means were separated by Least Significant Difference (LSD) when a significant ($P \leq 0.05$) was observed.

Results and Discussion

The result of this study was shown in Table 1. During 1 to 42 days of age, ADG was significantly higher ($P<0.05$) in AE-75 and AE-300 compared with control and AE-150. FI was significantly higher ($P<0.05$) in AE-300 compared with control and AE-150 but similar to AE-75. However, FCR did not difference between treatments. This results were in contrast with previous studies that indicated herbs, plant extracts, essential oil and the main components of essential oil did not affect body weight, feed intake or feed efficiency in broilers (BAMPIDIS et al., 2005; CROSS et al., 2007; MELO and HARKES, 2007; ABBASI and SAMADI, 2014). While, the finding of MELO and HARKES (2007) concluded that supplementing basal diet with Artichoke extract from 1-21 days of age improved feed efficiency of 21 and 42 day-old broilers. These authors suggested that improved feed conversion may be attributed to the lower feed intake reflected in those birds receiving Artichoke extract. In addition, this study has shown that supplementing AE in drinking water improved ADG of broilers. This could be explained by supplementation of Artichoke extract increased antibody levels, resulting in increased activity of the immune system. It has been proposed that immune-stimulation may have adverse effects on growth performance, because more nutrients are repartitioned to synthesize antibodies and develop immune organs, thereby decreasing the amount of nutrients available for growth (HEVENER et al., 1999; TAKAHASHI et al., 2000; TAJODINI et al., 2014).

Table 1. Effect of AE supplementation in drinking water on growth performance (1 to 42 days of age) of broiler chickens (Mean ± SD)

Parameters	**Groups**			
	Control	**AE-75**	**AE-150**	**AE-300**
ADG (g/d)	60.09 ± 3.18[a]	65.56 ± 3.97[b]	60.10 ± 2.04[a]	65.01 ± 0.52[b]
FI (g/d)	94.10 ± 2.54[a]	102.77 ± 5.10[ab]	99.42 ± 5.63[a]	110.49 ± 11.12[b]
FCR	1.57 ± 0.09	1.57 ± 0.04	1.68 ± 0.10	1.63 ± 0.08

[a,b]Means in the same row with different superscripts are significantly different ($P \leq 0.05$)

Conclusions

In conclusion, this finding determined by the results supplementation of 75 ppm artichoke extract in drinking water on day 1-7 and 15-24 of age may improve growth performance of broiler chickens.

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P-042 (ID 234)

EFFECT OF DIETARY SODIUM DIFORMATE ON GROWTH PERFORMANCE, NUTRIENT DIGESTIBILITY, GUT HEALTH AND PROFITABILITY IN BROILERS

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Abstract

Dietary formic acid and its salts act against pathogens, helping to decrease pressure on the immune system and improving nutrient digestibility. The use of the double sodium salt of formic acid, with the same antimicrobial properties as formic acid, but with improved handling properties, has become more prevalent in poultry diets. In a trial conducted at a research farm in Taiwan, sodium diformate (NaDF; 0.3% inclusion rate) was tested in a typical corn-soy diet, against a similar control diet containing no acidifier. 120 day-old broiler chicks (male Arbor Acres) were randomly selected and divided into 2 treatment groups with 3 replicates each. Feed and water were available *ad libitum*. The effects of NaDF on performance (final weight, FCR, broiler index), protein utilization and villi height were examined after 39 days. Data were analysed using the t-test and a confidence level of 95% was defined for these analyses.

Performance was boosted in the birds fed 0.3% NaDF. Treated birds were significantly heavier (2551 g vs. 2316 g; $P<0.05$), while the FCR improved only numerically (1.67 vs. 1.71). Protein utilization was also numerically better (63.3% vs. 61.6%), while the villi height in the jejunum and ileum was significantly increased by between 170 and 450 µm.

This study demonstrates that including NaDF in broiler diets is beneficial to performance, nutrient utilization and gut health and thereby increases the gross profit of broiler operations.

Keywords: broiler, sodium diformate, performance, digestibility

Introduction

Organic acids have a long history of use in animal nutrition, where they are usually added to stabilize compound feed, but also to enhance animal performance. While most early data on this class of additives were collected from studies in pig production (Cole et al., 1968; covering sows, piglets and fatteners), the available literature indicates a later focus on such additives in poultry production. One of the first reports of improved broiler performance when diets were supplemented with a single organic acid was for formic acid (Vogt et al., 1981).

Improvements in broiler performance or their hygienic production conditions with the aid of a variety of organic acids have been reported by many sources, as reviewed by Desai et al. (2007). An important limitation, however, is that organic acids are rapidly metabolised in the fore-gut (crop to gizzard) of birds, which can reduce their impact on growth performance. A newer salt of formic acid, sodium diformate (NaDF), has been demonstrated to be effective against pathogenic bacteria, including salmonella, throughout gastro-intestinal tract (Lückstädt and Theobald, 2009). Reducing the impact of pathogenic bacteria on the broiler, and improving the gut microflora, leads to a state of eubiosis in treated chickens and suggests that the inclusion of diformate in broiler diets can also improve bird performance. This hypothesis was confirmed statistically with a meta-analysis of data from trials carried out in recent years (Lückstädt, 2013). This study analyzed the average impact reported from all studies on the effect of the additive on the performance parameters weight gain, feed efficiency, mortality and productivity. However, these trials focused on weight gain and feed conversion outputs. This study aimed to investigate the effects of sodium diformate on growth performance parameters as well as nutrient digestibility and gut health in broilers.

Material and Methods

The trial was conducted at the research farm in Taiwan. One hundred and twenty day-old male Arbor Acres broilers were randomly allotted into 2 treatment groups, with 3 replications and 20 birds in each pen. The commercially-available corn-soy-based feed in one group contained 0.3% NaDF; this group was compared against a negative control. Feed and water were provided *ad libitum*. Birds were weighed individually after 14 and 39 days; feed consumption was recorded per pen at the end of the trial. The effects of NaDF on performance (final weight, feed conversion ratio, European Broiler Index EBI), protein utilization and villi height were examined after 39 days.
Data were analysed using the t-test and a confidence level of 95% was defined for these analyses.

Results

The inclusion of 0.3% NaDF in the diets of broilers for 39 days exerted significant effects on performance as well as protein digestibility in the birds.

The effect of the additive was already noticeable after only 14 days. At this stage, the body weight of the broilers receiving NaDF had increased significantly (P<0.05), by almost 21% (Table 1). At the end of the trial, treated birds were still significantly heavier (2551 g vs. 2316 g; P<0.05), while the FCR improved numerically (1.67 vs. 1.71). Furthermore, the mortality rate of birds was substantially reduced by 50%. As a result, the European Broiler Index, which includes the three most important productivity parameters in animal production – weight gain, survival and feed efficiency – improved markedly by almost 15%.

Table 1: Performance parameters of broilers fed with or without NaDF at 0.3% for 39 days

	Negative control	0.3% NaDF	Difference [%]
BW-14 days [g]	320^{b}	386^{a}	+20.6
Final weight [kg]	2.316^{b}	2.551^{a}	+10.1
ADG [g]	58^{b}	64^{a}	+10.3
FCR	1.71	1.67	-2.3
Mortality [%]	3.33	1.67	-50
EBI*	329	378	+14.9

Means with a different superscript differ significantly at P<0.05

*EBI = Daily weight gain (g) x Survival (%) / 10 x FCR

Villus height in the jejunum and ileum of birds at 39 days showed a significant increase in birds fed 0.3% NaDF compared to controls (Table 2).

Table 2: Effect of sodium diformate (0.3%) on the morphology of the jejunum and ileum of broilers after 39 days.

	Negative control	0.3% NaDF	Difference [%]
Jejunum - Villus height [µm]	1268^{b}	1721^{a}	+35.7
Ileum - Villus height [µm]	1011^{b}	1184^{a}	+17.1

Means with a different superscript differ significantly at P<0.05

Analysis of the protein utilization of the diets showed a numerical increase from 61.6% to 63.3% in the birds fed diets containing NaDF. This substantial improvement, of almost 3% in total, may be related to the larger intestinal surface area in NaDF fed birds due to the longer villi.

Similar trends (improved performance parameters as well as nutrient utilization) were reported as a result of a holo-analysis of 59 published studies for potassium diformate in pig diets (Rosen, as reported by Lückstädt and Mellor, 2011). In broilers, improved zootechnical performance is thought to arise as a result of improved intestinal microflora, through suppressing pathogenic bacterial species; and improved protein digestion. This was supported by the present study as well as in a previous report under similar conditions (Lückstädt, 2014).

Conclusions

As often seen with other additives, the performance improvements recorded are also influenced in part by the hygienic conditions. The current study was conducted at the research farm of a university. This study shows that even under these rather “clean” conditions, NaDF was able to significantly improve bird performance. Under practical conditions with a higher anticipated “bacterial load”, conditions might weigh even more in favour of the additive effects measured. This has been seen with other trials under commercial conditions. It can therefore be concluded that dietary sodium diformate can play an important role in improving broiler production.

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P-043 (ID 256)

EFFECT OF DIETARY SUPPLEMENTATION OF ENZYMES PRODUCED FROM *TRICHODERMA SP.* AND *ASPERGILLUS NIGER* ON PRODUCTION PERFORMANCE AND EGG PRODUCTION

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Abstract

The aim of the current study was to investigate effect of dietary supplementation of enzymes, mainly cellulase contents, produced from *Trichoderma sp.* and *Aspergillus niger*, containing cellulase activities of 1.60 and 0.65 U/g respectively, on production performance and egg production. A completely randomized design was applied for this study. Four-hundred and eighty laying hens at 17 week old were used for the start of the study until 28 week old of the hens. All studied hens were allocated into 3 dietary treatments of 160 hens for each treatment, equally 4 replications of 40 hens of each. The 3 dietary treatments were group 1 (T1) control (basal) diet with the base contents of broken rice, soybean meal and fish meal, group 2 (T2) the basal diet supplemented with enzymes produced from *Trichoderma sp.* at level of 20 ppm and group 3 (T3) the basal diet supplemented with enzymes produced from *A. niger* at level of 50 ppm. All studied hens were reared in a closed operating barn. Hen body weight, feed intake, egg weight and number of eggs were recorded. The results have shown that the laying hens offered the diet supplemented enzymes produced from *Trichoderma sp.* had better ($P<0.05$) feed efficiency per kg egg mass and higher values of % hen-day egg production of the laying hens when compared with those received enzymes produced from the *A. niger*, but there were no difference from the hens in the control group, offered diet without enzyme addition. Thus, the enzymes produced from *Trichoderma sp.* were more appropriate than those produced from *A. niger* for dietary supplementation for laying hens.

Keywords: laying hens, enzymes, production performance, egg production

Introduction

In the present, enzymes are extensively used as dietary supplementation for improving poultry production. Various enzymes, i.e. phytase, cellulase, xylanase and hemicellulase, were well known and more interested from farmers. One of the most useful enzymes in wide range of industrial applications is cellulase (KUHAD et al., 2011). Although cellulases are usually produced by fungi, bacteria or actinomycetes, the most common producer for industrial application is fungi (ILMÉN et al., 1997; MAKI et al., 2011). Cellulases producing from bacteria are not widely used due to usually lack one of the three cellulase activities, i.e. FPase (SATHYAVRATHAN and KRITHIKA, 2013). However, there are several factors affecting activities of the cellulase. Fungus type is a crucial factor for cellulase activities. Therefore, the aim of the current study was to investigate effect of dietary supplementation of enzymes, mainly cellulase contents, produced from *Trichoderma sp.* and *Aspergillus niger* on production performance and egg production.

Materials and Methods

Four-hundred and eighty laying hens at 17 week old were used for the start of the study until 28 week old of the hens. A completely randomized design was applied for this study. All studied hens were allocated into 3 dietary treatments of 160 hens for each treatment, equally 4 replications of 40 hens of each. The 3 dietary treatments were group 1 (T1) control (basal) diet with the base contents of broken rice, soybean meal and fish meal, group 2 (T2) the basal diet supplemented with enzymes produced from *Trichoderma sp.* (cellulase activities of 1.60 U/g) at level of 20 ppm and group 3 (T3) the basal diet supplemented with enzymes produced from *A. niger* (cellulase activities of 0.65 U/g) at level of 50 ppm. The doses of the enzymes in the T2 and T3 were recommended by the enzyme producers. The laying hens in all treatments were fed *ad libitum* and free to assess drinking water. All studied hens were reared in a closed operating barn. Hen body weight, feed intake, egg weight and number of eggs were recorded weekly. All data were expressed as mean±standard deviation (SD) and analyzed as a completely randomized design (CRD) by ANOVA and then Duncan multiple test were used to detect significant difference between groups at $P\leq0.05$.

Results

The results have shown that there were no differences (P>0.05) for average daily gain, average daily feed intake and % hen-day egg production of the hens among 3 experimental dietary treatments (Table 1.). However, at 18-22 week and 18-28 week of age, the laying hens offered the diet supplemented enzymes produced from *Trichoderma sp.* (T2) had better (P<0.05) feed efficiency per kg egg mass and higher values of % hen-day egg production of the laying hens when compared with those received enzymes produced from the *A. Niger* (T3), but there were no difference from the hens in the control group (T1), offered diet without enzyme addition. In the mean time, the laying hens obtained the dietary treatments in all studied groups had no difference (P>0.05) of feed efficiency per kg egg mass and % hen-day egg production at 23-28 week of age (Table 1.).

Table 1. Production performance of the experimental laying hens

Item	Treatment		
	T1	T2	T3
Average daily gain (g/day)	0.82±0.72	0.36±0.55	0.26±0.21
Average daily feed intake (g/day)	103.17±1.88	103.20±1.42	101.33±2.03
Feed efficiency per kg egg mass			
18-22 wk	4.03±0.58[ab]	3.77±0.51[a]	4.92±0.55[b]
23-28 wk	2.40±0.05	2.34±0.14	2.50±0.16
18-28 wk	3.14±0.26[ab]	2.99±0.30[a]	3.60±0.30[b]
% Hen-day egg production			
18-22 wk	74.37±4.21	75.65±6.16	67.10±8.85
23-28 wk	89.15±1.07	89.41±3.39	83.87±6.51
18-28 wk	76.06±2.25	76.66±3.60	70.18±6.90

[a,b]means in the same row with different superscripts are significant difference (*P*<0.05)

Discussion

The results of the current study have shown that there was not different for growth rate and feed consumption of the laying hens among three experimental groups. The explanation for these results would be the fact that the studied lay hens already have passed growth phase, entering adult age with low growth rate. The experimental laying hens obtained diet supplemented with enzymes producing from *Trichoderma sp*. had better feed efficiency per kg egg mass than those received diet supplemented enzymes produced from the *A. niger*. This result is in accordance with earlier report (SATHYAVRATHAN and KRITHIKA, 2013), which showed that cellulases producing from *Trichoderma sp*. has higher efficient cellulases than that produced from *A. niger*. However, cellulases producing from both *Trichoderma sp*. and *A. niger* had no difference of feed efficiency per kg egg mass when compared with the laying hens in the control group. This would be influence of gastric condition affecting cellulase activities (WARD et al., 2003). For % hen-day egg production, there was no significant difference among 3 dietary treatments, but at 18-22 weeks of age the highest value of % hen-day egg production was found in the laying hens obtained the diet supplemented with enzymes producing from *Trichoderma sp*., which was in agreement with value of feed efficiency per kg egg mass. The same trend was found for the laying hens in the other groups. In addition, all laying hens were reared in a closed operating barn, resulting in less stress obtained from environment. At the period of 23-28 weeks of age, the laying hens in all experiment groups had rather closely values of feed efficiency per kg egg mass and % hen-day egg production. These would be explained by the more enzyme adaptation of the laying hens with age.

Conclusions

Under the condition of the present study, the enzymes produced from *Trichoderma sp.* were more appropriate than those produced from *A. niger* for dietary supplementation for laying hens.

Acknowledgements

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THE ADDITION OF A NUTRITIONAL EMULSIFIER IMPROVES BROILER PERFORMANCE IN AN ENERGY DILUTED DIET

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Abstract

Emulsifiers are used to enhance the fat digestibility and by consequence to improve the feed conversion ratio due to the enhanced dietary MEn content. In a three-phase production trial two emulsifiers, E484 (Excential Energy Plus, 350g/T) and E481 (1000 g/T), were added on top of a negative control (NC) diet. The decrease of energy (0.42 MJ MEn/kg) in the NC diet was induced by exchanging 2.14 % fat for 2.14 % wheat in the positive control (PC) diet. Meanwhile, the addition of the synthetic amino acids was adapted in order to obtain diets with the same content of digestible amino acids. Each treatment consisted of 6 replicates with 30 male Ross 308 broilers. Birds fed the emulsifier diets reached the same final bodyweight (between 2,550 – 2,569 g) as the PC birds but had a significant (P<0.01) higher bodyweight than NC birds (2,417g). Effects on both BWG as FCR were most pronounced in the first 4 weeks of the trial. FCR of both emulsifier supplemented diets was 4.7% better (P<0.05) than those fed the NC-diet. For the overall period (0-39 d), the FCR was significantly (P<0.05) different and amounted 1.553; 1.651; 1.620 and 1.612 for the PC, NC, E484 and E481 emulsifier supplemented diets, respectively. The results demonstrated that the addition of the tested emulsifiers enhanced the performances of broilers but could not fully compensate the energy dilution of 0.42 MJ MEn/kg.

Keywords: broiler, energy, fat, digestibility, emulsifier

Introduction

Energy is a major cost component in broiler diets. Due to its high energy density, fats and oils are important energy sources in feed formulation. Improving the energy efficiency of these raw materials is of high interest from an economical point of view. Nutritional emulsifiers can be used to improve fat digestibility and thus improve the energy efficiency (Maertens et al, 2013). The objective of the trial was to evaluate the effect of 2 emulsifiers on the production performances of broilers. For this goal a negative control (NC) diet (with 0.42 lower energy content than the PC diet) was formulated and supplemented with one of the two test emulsifiers.

Material and Methods

A total of 720 one day old, male broiler chicks (Ross 308) were used in this trial. The birds were housed in the poultry experimental facility of ILVO (Animal Sciences Unit, Section Small Stock Husbandry, Merelbeke, Belgium). A randomised block design was used, with in total 6 blocks of 4 pens and 30 birds per pen. The floor was covered with fresh wood shavings and no additional filling or cleaning of the floor was necessary during the trial.

The experiment consisted of 4 treatments. Treatment 1 was a positive control (PC) diet, formulated with a metabolizable energy content of 11.8, 12.2 and 12.5 MJ/kg for starter, grower and finisher diet, respectively. Treatment 2 was the negative control diet and formulated with 0.42 MJ/kg (100 kcal) lower energy content, compared to PC. In treatment 3, the NC diet was supplemented with emulsifier I (E484, Excential Energy Plus, 350g/T) and in treatment 4 the NC diet was supplemented with emulsifier II (E481, 1000g/T). The chosen inclusion levels were based on practical applied dosages and in accordance with the advice dosage of the supplier. Diets used were wheat-soybean meal based with animal fat as added fat source. Total fat in PC was 7.42%, 8.16% and 8.95% in starter, grower and finisher respectively. The reduction in energy in NC was induced by exchanging 2,14% fat for 2.14% wheat. Meanwhile, the addition of the synthetic amino acids was adapted in order to obtain diets with the same content of digestible amino acids. A three-phase feeding scheme was applied with a starter period of 12 days followed by a grower diet between 12 and 28 days of age and a finisher between 28 and 39 days of age. The diets were prepared at the Institute's feed milling facilities. Feed was provided *ad libitum* as mash and drinking water was provided *ad libitum* by drink belts.

Data were submitted to a two-factorial variance analysis with treatment as factor and block as random factor.

Results

Bodyweight of the birds, body weight gain and feed conversion ratio's for period 0-28 and 0-39 days are presented in Table 2. Already at 12 days of age birds of both emulsifier added diets had a significant higher bodyweight then PC birds (data not shown). At day 28 and at the end of the trial, birds on the emulsifier diets had a significant higher weight than NC birds while at 39 days their weight was equal as birds fed the PC diet.

Table 1: Bodyweight (BW), Body weight gain (BWG) and Feed Conversion ratio (FCR) for period 0-28d and 0-39d

	BW		***BWG***		***FCR***	
	D28	D39	D0-28	D0-39	D0-28	D0-39
Pos Control	1396[ab]	2557[a]	48,3[ab]	64,4[a]	1,456[a]	1,553[a]
Neg Control	1324[b]	2417[b]	45,7[b]	60,8[b]	1,574[b]	1,651[c]
Neg Cont + emulsifier I	1435[a]	2550[a]	49,6[a]	64,2[a]	1,500[a]	1,620[b]
Neg Cont + emulsifier II	1447[a]	2569[a]	50,1[a]	64,7[a]	1,500[a]	1,612[b]

a,b: means in the same column with a different letter are significantly different (P<0.05).

During the grower period as well as in the finisher period, birds fed PC diet had a significant lower FCR compared to NC. However, the addition of both emulsifiers improved the FCR significantly compared with the NC diet. For the grower period (0-28d) the FCR of both emulsfier diets was statistically not different from PC. The overall FCR (d 0-39) of the positive control group was 98 points (P<0.05) lower than the negative control group. The gap in energy between the PC and NC diet was not fully compensated by the addition of the emulsifiers. However, both emulsfiers had a significant positive effect on the overall FCR and induced an average improvement of 2.1%.

Conclusions

The addition of both emulsifiers resulted in a comparable body weight gain (BWG) as observed in PC birds, while BWG was significantly improved (on average 6%) compared to the NC diet. The difference in energy content between PC and NC was clearly demonstrated by the significant different FCR. This gap was partly filled by the addition of the emulsifiers, both having a more favorable (P<0.05) FCR than the NC birds (2,1% improvement of FCR on average). However, the difference with the PC diet still remained significant. This indicates that a positive effect of 100 kcal (0,42 MJ)/Kg due to the addition of the emulsifier is overestimated. Both emulsifiers resulted in the same improvement in BWG and FCR, however emulsifier I (Excential Energy Plus) was more efficient in terms of dosing (350g/T) compared to emulsifier II (1000g/T).

The results indicate that the tested emulsifiers can improve energy efficiency in broiler diets and result in a significant better FCR, especially in the starter and grower period. The estimated energy upgrade under the tested diet composition is less then 100kcal. The practical application of this trial is that it shows a tool to improve feed efficiency that may lead to lower feed costs and more sustainable broiler production.

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P-045 (ID 29)

DIFFERENT LEVELS OF SODIUM BENTONITE AND *SILYBUM MARIANUM* SEEDS IN REDUCING THE ADVERSE EFFECTS OF AFLATOXIN ON BLOOD LIPID LEVELS AND THE RELATIVE WEIGHT OF DIGESTIVE ORGANS OF BROILER CHICKENS

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Abstract

An experiment was conducted to evaluate the ability of different levels of sodium bentonite (SB), *Silybummarianum* seeds (SMS) and their combination for reducing the adverse effects of Aflatoxin B_1 (AFB_1) in broiler chicks. A number of 224 broiler mail chicks (Ross) were used in 8 Treatments: 1) control, 2) diet with 0.5% SB, 3) diet with 0.5% SMS, 4) diet with 0.5% SMS and 0.5% SB, 5) diet with 500ppb AFB_1, 6) diet with 500ppb AFB_1 and 0.5% SMS, 7) diet with 500ppb AFB_1 and 0.5% SB, 8) diet with 500ppb AFB_1 and 0.5% SMS seeds and 0.5% SB, In a completely randomized design arranged with factorial experiment (2 × 2 × 2) with four replications was used from one to 42 days age. The results showed that compared with the control group, diets contaminated with AFB_1 increased the relative weight of gizzard and bile but decreased serum total protein and globulin significantly ($p \leq 0.05$). Broiler chickens fed diets containing SB, SMS and AFB_1 their biochemical indices (globulin and total protein) were improved significantly compare to treatment 5 ($p \leq 0.05$). Treatment 8 in comparison with treatments 6 and 7 to a greater extent reduced relative gizzard weight and increased serum concentrations of total protein ($p \leq 0.05$). The results showed that the combination of the seeds of SMS and SB can effectively eliminate the toxic effects of AFB_1.

Keywords: Aflatoxin B1, globulin, Silybummarianum seeds, sodium bentonite, total protein

Introduction

Chicken meat is an important source of nutrients for human consumption. Broiler feed is exposed to various contaminants during the process of production, transportation and storage. One of the important injurious agents is mycotoxins as it has hepatotoxic, hepatocarcinogenic, mutagenic and teratogenicity effects in many animal species (IARC, 1987). Mycotoxins are metabolites from moulds. Aflatoxins, a group of closely related biologically active mycotoxins, are produced by strains of *Aspergillus flavus* and *Aspergillus parasiticus* (Tedesco et al., 2004). Numerous strategies for detoxification or inactivation of mycotoxin in contaminated feedstuff have been used. Most of these techniques are impractical or ineffective (Piva et al., 1995). One approach to the detoxification of mycotoxin is the use of nonnutritive absorptive materials in the diet to reduce the absorption of mycotoxins from the gastrointestinal tract (Thaxton et al., 1974). Layered alumino silicates, such as sodium bentonite, have been found effective in counteracting mycotoxins (Eraslan et al., 2005). The effects of *silymarin* or various products made from the seed of the plant *Silybum marianum* containing silymarin have also been tested on farm animals. The hepatoprotective activities were assessed on the basis of selected biochemical blood indicators in chicken broilers that had been exposed to the effects of aflatoxins (Tedesco et al., 2004). The purpose of this study was to evaluate the changes of blood lipid levels and the relative weight of digestive organs of broiler chickens when different levels of sodium bentonite and *Silybum marianum* seeds were used as a feed additive in their diet.

Material and Methods

Two hundred and twenty four of day old male Ross 308 broiler chickens obtained from a local hatchery were randomly distributed among eight treatments with four replicate groups per treatment and 7 chickens per replicate. the nutritional requirement of the chicks was based on Ross 308 company for starter (0-10 days) and grower (11-24 days) diets (Table I). Birds were distributed into the following eight treatments: 1) control 2)diet with (%) 500ppb aflatoxin B_1, 3) diet with 500ppb Aflatoxin B_1 and 0.5% *Silybummarinum* seeds, 4) diet with 500ppb aflatoxin B_1 and 0.5% Sodium Bentonite, 5)diet with 500ppb Aflatoxin B_1 and 0.5% *Silybummarinum* seeds and 0.5% Sodium Bentonite, 6)diet with 0.5% *Silybummarinum* seeds, 7) diet with 0.5% Sodium Bentonite, 8) diet with 0.5% *Silybummarinum* seeds and 0.5% So-

dium Bentonite. At 24 day of age, two broilers per pen were randomly selected and 2 mL of blood samples were obtained by wing puncture and centrifuged at 3000 rpm for 20 min to separate serum. Serum total protein(TP), Glubulin and relative weight of digestive organs were analyzed using the general linear model procedure of SAS.

Results

The effect of experimental treatmets on different parameters in Table 1 show that compared with the control group, diets contaminated with AFB_1 increased the relative weight of gizzard but decreased serum total protein and globulin significantly ($p \leq 0.05$). AFB has been shown to cause inhibition of protein synthesis (16). total protein levels in the serum proved to be sensitive indicators of aflatoxicosis in broilers. Use of absorbents in broiler diet will cause the subsequent absorption of toxin in their digestive tract and this prevents the reduction of blood serum albumin, globulin and protein in the case of using contaminating diet (7). However, in our case, the biochemical parameters for broilers fed diets containing SB + AF did not completely return to normal values, showing an inhibition of protein synthesis. Broiler chickens fed diets containing SB, SMS and AFB_1 their biochemical indices (globulin and total protein) were improved significantly compare to treatment 5 ($p \leq 0.05$). Treatment 8 in comparison with treatments 6 and 7 to a greater extent reduced relative gizzard weight and increased serum concentrations of total protein ($p \leq 0.05$).

Conclusions

In conclusion Dietary inclusion of sodium bentonite had not any adverse effects on serum biochemical characteristics and it can also improve the values of TP in broiler chickens also results of the present study showed that consumption of level of SMS from 0.5 % in poultry diets can reduce the toxicity of AFB_1 in broiler chickens. But results showed that the combination of SB and the SMS are more beneficial as separate application of them for decreased toxic effect of Aflatoxin B_1 in diets.

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Table 1. effect of different treatment on TP and Glubulin (U/l) and digestive organ (gr/kg live weight).

				TP	Glubulin	Liver	Gizzard	Proventriculus
Main effects	Aflatoxin (ppm)	0		2.44[a]	1.44[a]	1.80	1.46[b]	0.45
		500		2.01[b]	1.25[b]	1.84	1.69[a]	0.44
	p-value			0.00	0.00	0.41	0.00	0.95
	Silybum (%)	0		1.98[b]	1.19[b]	1.82	1.69[a]	0.45
		0.5		2.47[a]	1.50[a]	1.82	1.45[b]	0.44
	p-value			0.00	0.00	0.98	0.00	0.39
	Bentonite (%)	0		2.09[b]	1.29	1.82	1.68[a]	0.43[b]
		0.5		2.36[a]	1.40	1.82	1.47[b]	0.46[a]
	p-value			0.00	0.09	0.97	0.00	0.04
	SEM			0.05	0.04	0.03	0.03	0.00
Dual interaction effects	Aflatoxin * silybum							
	0	0		2.21	1.28	1.79	1.50[b]	0.46
	0	0.5		2.66	1.61	1.80	1.42[b]	0.43
	0	500		1.75	1.11	1.85	1.89[a]	0.44
	0.5	500		2.27	1.39	1.83	1.49[b]	0.45
	p-value			0.65	0.68	0.72	0.00	0.13
	Aflatoxin * ben							
	0	0		2.36	1.40	1.67	1.47[b]	0.41[b]
	0	0.5		2.52	1.48	1.83	1.44[b]	0.48[a]
	0	500		1.83	1.18	1.87	1.89[a]	0.45[ab]
	0.5	500		2.19	1.32	1.81	1.49[b]	0.44[ab]
	p-value			0.19	0.68	0.24	0.00	0.04
	Silybum * ben							
	0	0		1.84	1.07	1.79	1.93[a]	0.44
	0	0.5		2.13	1.32	1.84	1.46[b]	0.46
	0	500		2.35	1.51	1.84	1.43[b]	0.43
	0.5	500		2.58	1.48	1.79	1.48[b]	0.45
	p-value			0.70	0.05	0.38	0.00	0.39
	SEM			0.07	0.06	0.05	0.14	0.01
Treatments	AFB * silybum * Ben							
	0	0	0	2.03[bcd]	1.07[b]	1.70	1.58[b]	0.43
	0	0	0.5	2.40[abc]	1.49[ab]	1.87	1.42[b]	0.50
	0	0.5	0	2.70[a]	1.73[a]	1.82	1.36[b]	0.40
	0	0.5	0.5	2.63[a]	1.48[ab]	1.78	1.47[b]	0.46
	500	0	0	1.65[d]	1.07[b]	1.89	2.28[a]	0.45
	500	0	0.5	1.85[d]	1.15[b]	1.81	1.50[b]	0.43
	500	0.5	0	2.01[cd]	1.29[b]	1.85	1.49[b]	0.46
	0.5	0.5	500	2.54[ab]	1.49[ab]	1.80	1.48[b]	0.44
	p-value			0.02	0.00	0.27	0.01	0.95
	SEM			0.11	0.09	0.07	0.06	0.01

Means within a column that do not have a common superscript are significantly different ($P<0.05$).

SEM= Standard error of means. AFB= Aflatoxin B1. Ben= Bentonite.

P-046 (ID 122)

EFFECT OF LIGNOCELLULOSE ON EGG PRODUCTION AND EGG QUALITY

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Abstract

Fibre has no direct nutritious benefit in poultry nutrition and has until now been regarded as `useles roughage´. However, increase of the content of raw fibers in the diets for poultry showed positive effects especially in broiler breeder production. In recent years, a lot of research results reported a positive effect on egg production in laying hens, as well as on the egg quality. Higher content of lignocellulose in diet have a positive impact on fecal consistency, litter quality and a reduced percentage of dirty eggs.

The experiment was conducted on 480 ISA Brown layers which were 37 weeks of age at the start of the experiment. Hens were divided in two groups: Control group with standard diet and Experimental group with standard diet+1% lignocellulose (Arbocel, JRS) "on top". The basic production parameters and egg quality characteristics were determined during the 12 consecutive weeks. The results showed that Arbocel had no significant effect on egg production or egg weight, but had a positive influence on feed consumption. A significant ($P<0.05$) positive effect of Arbocel on egg shell cleanness was established in the trial. Based on the results of this study it can be concluded that lignocellulose has a positive impact on feed consumption and egg shell cleanness.

Keywords: laying hens, lignocellulose, production, egg quality

Introduction

Fibre has no direct nutritious benefit in poultry nutrition and has until now been regarded as `useles roughage´. However, increase of the content of raw fibers in the diets for poultry showed positive effects especially in broiler breeder production. In recent years, a lot of research results reported a positive effect on egg production in laying hens, as well as on the egg quality. Higher content of lignocellulose in diet have a positive impact on fecal consistency, litter quality and a reduced percentage of dirty eggs.

The term lignocellulose includes the mixture of cellulose, hemicellulose and lignin. The advantage of these products is standardized in its composition (the content of crude fiber content 50% -65%, depending on product), the absence of mycotoxins and the heat treatment which provides a microbiological quality (POTTGÜTER, 2008). Research on the effect of sodium in the diets of the laying hens showed that it has a positive effect on reducing the incidence of cannibalism, which is especially important in free range laying hens (VAN KRIMPEN et al, 2005; HARLANDER-MATAUSCHEK, 2007; VAN KRIMPEN 2008). ESMAIL (1997) found a direct relationship between the cellulose content in the diet and the occurrence of cannibalism and mortality in laying hens. In general, cannibalism is reduced with the increase of fiber content in the diet, especially the increase of insoluble fiber. Authors HARTINI et al. (2003) demonstrated that a higher concentration of sodium in the diet affects the performance of laying hens. The hens that were fed diets with a higher content of cellulose had improved production and had less mortality rates compared to that of hens fed diets with a low content of cellulose. It has been found that the portions with a high cellulose content improves the consistency of the feces, resulting in a dryer litter and decreasing the content of ammonia in the air (REZAEI et al., 2008).

Material and Methods

The trial was carried out on the experimental farm of the Department of Animal Science of the Faculty of Agriculture in Novi Sad. Investigation was conducted on 480 ISA Brown layers which were 27 weeks of age at the start of the experiment. Hens were divided in two groups (two treatments) with eight replicates each. One replicate consists 6 cages with 30 hens. The first group (Control) was fed a standard diet and the second (experimental) group was fed with a standard diet+1% lignocellulose (Arbocel, JRS) "on top". The standard diet containing 17% crude protein, 11,35 ME MJ/kg, 3.5% Ca, 0.35% available P and ad libitum water was supplied by nipple drinkers. Layers were beak trimmed. The basic production parameters were determined during the 12 consecutive weeks. The following parameters were used: Egg production parameter: laying percentage per housed hen, laying percentage per average hen and feed intake. Examination

of external and internal egg quality characteristics was carried out four time in four-week periods, on a random sample of 96 eggs per group (12 eggs from each replicate). External and internal egg quality characteristics: egg weight, shell color, shell cleanness, shell breaking force, yolk color and albumen height. Egg weight was measured by electronic digital balance. Shell cleanness was evaluated by a scoring system on a scale from 1 (very dirty shell) to 5 (completely clean). Shell color was visually evaluated with points 1 (weight) – 5 (dark). Egg breaking force was measured by an Egg Force Reader (Orka Food Technology Ltd, Israel). Egg yolk color was determined according to the Roche yolk colour fan. Albumen height was measured using a tripod micrometer. Data were analyzed by ANOVA and means were separated by Duncan's post hoc test using StatSoft computer package (STATISTICA 11, 2012).

Results

The results showed that there were no statistically significant differences in egg production parameters between the control and arbocel group, although the arbocel group had a greater laying percentage than the control group. From the results in Table 2 it can be seen that the increase in cellulose content had a positive impact on increasing the weight of the eggs as well as the cleanliness of the shell. The difference in shell cleanliness was statistically significant. This is confirmed by the fact that higher cellulose content in food has an impact on the digestive tract and fecal consistency, which resulted in soiled eggs, which was lower in the arbocel group. A significant difference was observed in the yolk color in the group with increased cellulose content showing 0.17 points lower on the Roche scale.

Table 1. Egg production parameters

Week	Laying percentage, per housed hen (%)		Laying percentage, per average hen (%)		Feed intake (g)	
	Arbocel	Control	Arbocel	Control	Arbocel	Control
28	94,55	93,87	94,55	93,87	126	124
29	94,64	93,94	94,64	93,94	138	135
30	95,49	94,48	95,49	94,7	131	126
31	94,93	94,61	94,93	94,61	131	126
32	93,93	94,18	94,33	94,39	125	115
33	94,88	94,9	94,88	94,9	125	126
34	94,32	94,96	94,32	94,96	134	132
35	94,21	93,94	94,21	93,94	129	127
36	93,71	93,35	93,71	93,35	144	144
37	93,21	93,35	93,21	93,43	125	123
38	94,08	94,28	94,08	94,28	128	125
Average	**94,36**	**94,18**	**94,4**	**94,21**	**131**	**128**
p – value						
Treatments	**0,782**		**0,791**		**0,141**	
Week	**0,051**		**0,033**		**0**	
Interaction	**0,937**		**0,964**		**0,443**	

Table 2. External and internal egg quality characteristics

Week	Egg weight (g)		Shell color (points)		Shell cleanness (points)		Albumen height (mm)		Yolk color (Roche)		Shell breaking force (kg)	
	A	C	A	C	A	C	A	C	A	C	A	C
30	66	65	2,21	2,43	1,33	1,16	8,41	8,1	14,26	14,4	4,09	4,1
32	67,5	66,5	2,41	2,46	1,21	1,5	8,2	8,24	14,26	14,21	4,24	4,15
34	67	67,1	2,52	2,37	1,19	1,23	6,91	7,18	14,39	14,59	4,06	3,89
36	66	65,7	2,45	2,51	1,66	2,22	7,94	7,55	14,08	14,48	4,09	4,06
Average	**67**	**66**	**2,4**	**2,44**	**1,17**	**1,28**	**7,86**	**7,77**	**14,25**	**14,42**	**4,12**	**4,05**
p – value												
Treatments	**0,137**		**0,524**		**0,002***		**0,161**		**0,023***		**0,326**	**0,334**
Week	**0,005***		**0,162**		**0,013***		**0,000***		**0,027***		**0,44**	**0,364**
Interaction	**0,416**		**0,103**		**0,066**		**0,014***		**0,074**		**0,447**	**0,403**

A – Arbocel, C-Control,* $p<0.05$

Conclusions

It can be concluded that lignocellulose has a positive impact on feed consumption and egg shell cleanness and therefore it is necessary to continue the research on this subject, preferably during the whole production cycle.

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P-047 (ID 226)

EFFECT OF DIETARY SUPPLEMENTATION OF *BOESENBERGIA PUNDURATA* ON SMALL INTESTINAL MORPHOLOGY AND IMMUNOLOGICAL RESPONSE OF BROILER CHICKENS

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Abstract

The aim of the current study was to investigate the effect of dry powders of *Boesenbergia rotunda* (BR) on small intestinal morphology and immunological response of broiler chickens against Newcastle disease virus (NDV). Ninety-six chickens (1 day-old) were randomly allocated into four experimental groups equally and housed in 16 cages with 6 chickens each (4 cages per treatment). The chickens in group 1, 2, 3 and 4 were fed basal diet supplemented with 0.00% (T1), 0.05% (T2), 0.10% (T3) and 0.15% (T4) of dry powders of BR, respectively, lasting for 6 weeks. One chicken was randomly chosen from each cage at the age of 3 and 6 weeks in order to collect small intestines for measuring villous height (VH) and crypt depth (CD). All experimental chickens were vaccinated against NDV at 2 and 14 days old and the blood samples of the chickens were collected to measure the levels of heamagglutination inhibition (HI) titers against NDV at 2, 14 and 42 days old. The results have illustrated no significant difference (P>0.05) was seen for the VH, CD and VH:CD of small intestine. The HI titers against NDV at the day 14 of the chickens in T3 (3.00±1.07 $\log_2$) was significant lower ($P \leq 0.05$) than those in T1 (4.25±0.71 $\log_2$), while the HI titers against NDV of the chickens at day 2 and 42 was no significant differences among four experimental groups (P>0.05). Thus, the dietary supplementation of dry powders of BR for broiler chickens had no influence on small intestinal morphology, but the impact of the supplementation on immunological response to NDV was varied for broiler chickens.

Keywords: *Boesenbergia pundurata*, small intestinal morphology, immunological response, broiler chicken

Introduction

There were tremendous interest for the use of local herbs for improving broiler chicken production and health, in order to alleviate residues from anti-microbial agents, especially in Asian countries. Several kinds of herbs were investigated for many aspects. One of a promising herb is *Boesenbergia rotunda* (BR) found many activities for control diseases. The main proposed active ingredient in BR was flavonoid compound containing important activities of anti-microbial and anti-inflammation (CHONG et al., 2012). There was very effective for inhibition of pathogenic *Escherichia coli* under *in vitro* condition (MIKSUSANTI et al., 2008). Both anti-microbial and anti-inflammation would be beneficial for health of broilers chickens, which have higher chance to get gastro-intestinal infection from bacteria, especially during nutrition stress from diet formula change. This would then lead to lower immunity responses to several and key infectious diseases. Therefore, the current study was aimed to investigate effect of dry powders of BR supplementation in diet with various doses on small intestinal morphology and immunological response of broiler chickens against Newcastle disease virus (NDV).

Materials and Methods

A total of 96 male day-old broiler chickens were randomly allocated into four studied groups containing four replicates of 6 chickens in each. The chickens of group T1 (Control), T2, T3 and T4 were offered with the corn-soybean diet supplemented with dry *Boesenbergia pundurata* powders at 0.00%, 0.05%, 0.10% and 0.15%, respectively lasting for 6 weeks. All chicks were fed *ad libitum* and free to assess drinking water. One chicken was randomly chosen from each cage at the age of 3 and 6 weeks in order to collect small intestines at jejunum part for measuring villous height (VH), crypt depth (CD) and ratio of VH:CD. All experimental chickens were vaccinated against NDV at 2 and 14 days old and the blood samples of the chickens were collected to measure the levels of heamagglutination inhibition (HI) titers against NDV at 2, 14 and 42 days old. All data were expressed as mean ± standard deviation (SD) and analyzed as a

completely randomized design (CRD) by ANOVA and then Duncan multiple test were used to detect significant difference between groups at P≤0.05.

Results

There were no significant difference (*P*>0.05) detected for VH, CD and VH:CD ratio of jejunum intestine at week 3 and 6 among four dietary teatments (Table 1.). The HI titers against NDV at the day 2 and 42 of the chickens were no significant difference (*P*>0.05) among four experimental groups. However, the HI titers against NDV at the day 14 of the chickens in T3 (3.00±1.07 log_2) was significant lower (*P*≤0.05) than those in T1 (4.25±0.71 log_2) while the HI titers of the chickens at day 14 in the other groups were no significant difference (*P*>0.05) as illustrated in the Table 2.

Table 1. Small intestinal morphology of the chickens offered the dietary treatments

Item	Dietary treatment			
	0.00 %	**0.05 %**	**0.10 %**	**0.15 %**
Villous height (μm)				
Wk3	1,020.56±97.40	1,009.45±108.31	917.50±126.92	942.46±50.86
Wk6	1,204.83±150.28	1,126.66±90.39	1,175.66±49.80	1,130.06±32.32
Crypt depth (μm)				
Wk3	81.61±9.00	88.34±17.36	90.75±21.21	79.06±26.28
Wk6	106.14±20.33	113.57±8.00	102.96±3.27	116.20±25.15
Villous height : Crypt depth				
Wk3	12.57±1.24	11.76±2.59	10.85±4.30	12.72±3.27
Wk6	11.56±1.77	9.97±1.17	11.44±0.80	10.08±2.25

Table 2. Heamagglutination inhibition titer against the ND viruses of the chickens offered the dietary treatments

Item	Dietary treatment			
	0.00 %	**0.05 %**	**0.10 %**	**0.15 %**
Heamagglutination inhibition titer (log_2)				
Day2	6.38±1.06	7.25±0.87	7.00±1.41	6.75±0.71
Day14	$4.25±0.71^{a}$	$3.25±1.04^{ab}$	$3.00±1.07^{b}$	$3.88±1.46^{ab}$
Day42	1.75±1.04	0.75±0.89	1.13±1.73	1.13±1.00

^{a,b} means in the same row with different superscripts are significant difference (*P*<0.05)

Discussion

From the results of the current study, there was not found effect of the dietary BR powders supplementation on VH, CD and ratio of VH:CD at jejunum of the broiler chickens. This would be the fact that no pathogenic *E. coli* challenge in the current study, resulting no severity of villous damage by *E. coli* in small intestine, mainly normal flora and less pathogenic bacteria. This results is in agreement with earlier study (DUARTE et al., 2014) reported that very small effect of flavonoid in crude propolis on small intestinal morphology. For immunological responses to NDV vaccination, in general all chickens had gradually decrease of the HI titers to NDV after vaccination, implying lower maternally derived antibodies (MDA) with chicken age. The pattern of immunological responses of the chickens in each studied group is rather similar each other. However, the chickens obtained the dry powders of BR at 0.10% had lower HI titer than those obtained on BR powders in diet at 14 day after vaccination, which is the day of building high level of immunity for protection. This result might be the variation of immunological responses and MDA of individual chickens (JALOB et al., 2011). In the clinical point of view, a HI titer of 3 log_2 is generally accepted as positive for specific immunity against ND (ALLAN and GOUGH, 1974). This would be interpreted that the HI titers of most studied chickens in the current study had protective immunities up to around 2 weeks of age, but had a risk of getting NDV infection somewhere between 4-6 weeks of age. Thus, there was hardly to make a conclusion for the influence of dietary BR supplementation on immunological responses. In general, the further study should test the effects of dietary BR supplementation with pathogenic *E. coli* challenge and measure the HI titer weekly to make more clear influence on immunological responses.

Conclusions

The dietary supplementation of dry powders of BR for broiler chickens had no influence on small intestinal morphology, but the impact of the supplementation on immunological response to NDV was varied for broiler chickens.

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P-048 (ID 232)

A NOVEL *IN VITRO* POULTRY MODEL TO COMPARE RELEASE KINETICS OF (UN)PROTECTED BUTYRATE

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Abstract

The gastrointestinal (GIT) segment wherein dietary additives, such as butyrate, are released may influence their observed efficacy. Release kinetics are influenced by pH, gastric and enteric motility, retention time in the different GIT segments and enzymes concentration. Unfortunately, none of the available in vitro poultry model mimics accurately these conditions. As a consequence, a novel in vitro poultry GIT model was developed to compare the release kinetics of commercially available butyrate formulations. Four fat-embedded butyrate matrices originating from three suppliers as well as tributyrin, the triglyceride of butyrate, were evaluated in a three-step (pre-gastric, gastric and enteric) in vitro digestion procedure. Aliquots were drawn at 5 stages of digestion representing the end of the crop, proventriculus-gizzard, duodenum, jejunum and ileum. The percentage of the initial butyrate dose solubilized was calculated for each stage. Significant differences were found among formulations for each digestion stage ($P < 0.001$). Half of the fat-embedded butyrate matrices showed a sustained release profile, while the other released all their content in the pre-enteric regions. Tributyrin had a release profile targeting enteric segments. Results of this study indicate that despite almost identical visual appearance, release kinetics can vary significantly among butyrate derivatives. Such variations can possibly explain the inconsistency of the bird's growth response to dietary butyrate supplementation as shown in literature.

Keywords: *in vitro* model, release kinetics, feed additives, butyrate

Introduction

In poultry nutrition, butyrate is used as a feed additive. This short chain fatty acid can be fed as an unprotected salt or in the form of protected derivatives such as butyrate glycerides or butyrate-loaded matrices. Butyrate elicits stimulatory, albeit inconsistent, effects on growth performance of broiler chickens. The GIT segment wherein butyrate is released may modulate its effects given the diversity of cell types and pH conditions encountered throughout the avian GIT, and the differences in microbiota composition in the different gut segments. Release properties of protected derivatives can therefore contribute to the understanding of the mode of action of butyrate. Release kinetics can be determined *in vivo* by measuring directly the butyrate content in the chyme or by measuring the oxidation of labelled butyrate in respiratory chambers. The first technique doesn't allow to discriminate between the dietary or fermentative origin of butyrate, while the prohibitive costs of the latter prevents its use when screening numerous formulations. *In vitro* models are interesting alternatives to the aforementioned techniques as they are quick, cost-effective, and the results are a not confounded by fermentation end-products. Release kinetics are being influenced by environmental factors e.g. pH, temperature, enzymatic activity or retention time. Specific conditions encountered in the avian GIT are described in the literature (Long, 1967; Philips and Fuller, 1983; Zyla et al., 1999; Dendow, 2015), but not in *in vitro* poultry models. The present work was undertaken to develop a novel *in vitro* poultry model, using realistic digestion parameters, to compare release kinetics of commercially available butyrate derivatives.

Material and Methods

Tributyrin (T8626, Sigma-Aldrich, Saint-Louis, USA) and 4 commercially available fat-protected butyrate derivatives: Adimix 30 C (Nutri-ad International NV, Belgium), Ding Su (Xiamen Fujian Co., China), Sodium Butyrate 300 and 500 (Vega Pharma Limited, Hangzhou, China) were used in triplicate in the *in vitro* incubation procedure. The procedure consisted of pre-gastric, gastric and enteric steps. The enteric step had three different durations, resulting in a total of five incubation stages. Approximately 0.25 g of sample was incubated for 35 mn in 20 ml of a 1 N tris-HCl buffer solution (pH 5.8) containing 1 g/l NaCl and 0.5 g/l of α-amylase from porcine pancreas (10 U/mg solid; A3176, Sigma-Aldrich). When only the pre-gastric digestion was considered, the procedure was stopped by transferring the tube to ice. Otherwise, incubation proceeded with addition of 2 ml of a 0.085 N HCl solution containing 11 g/l of pepsin from porcine

gastric mucosa (250 U/mg solid; P7000, Sigma-Aldrich). If necessary, pH was adjusted to 2.7 ± 0.05 with drops of 6 N HCl or 6 N NaOH. The second stage of incubation then proceed for 35 mn. The procedure was stopped when only the gastric digestion was considered by transferring the tubes to ice. Otherwise, 4 ml of a 1 N tris-buffer solution (pH 6.5) containing 1.875 g/l pancreatin from porcine pancreas (8 x USP specifications; P7545, Sigma-Aldrich) and 0.7 g/l bile salts (48305, Sigma-Aldrich) was added to the tube. If necessary, pH was adjusted to 6.5 ± 0.05 with drops of 6 N HCl or 6 N NaOH. The incubation was stopped by transferring the tubes to ice 10 mn, 80 mn or 170 mn after addition of pancreatin when duodenal, jejunal or ileal digestion were considered, respectively. A temperature of 40 ± 0.1°C and a stirring rate of 360 rpm were maintained throughout the different stages of incubation. Aliquot were drawn at the end of each digestion stage. Butyrate content was determined for each derivative by incubating ground samples in 20 ml of 1 M HCl solution during 24 hours. Aliquots were analysed for butyrate concentration by gas chromatography. Butyrate yield was expressed as the percentage of the total incubated butyrate dose that was solubilized at a certain time point. Butyrate yields were analysed for each incubation stage with the PROC GLM procedure of SAS® version 9.2 (SAS Institute Inc., Cary, NC, USA), using a significance level *P* of 0.05. LSD test was carried as a post-hoc test.

Results

Table 1 presents the cumulative butyrate release per digestion stage of the assayed butyrate derivatives. For each digestion stage, significant differences were found among the derivatives ($P < 0.001$). Throughout the enteric phases of incubation, pH dropped gradually when tributyrin was incubated, resulting ultimately in a pH significantly lower than the one observed with fat-embedded butyrate matrices ($P < 0.001$).

Table 4. Cumulative butyrate release (%) per incubation stage for different butyrate formulations

Butyrate derivative	Incubation stage[1]				
	Crop	Proventriculus -gizzard	Duodenum	Jejunum	Ileum
Tributyrin	$13.4^{d} \pm 1.5$	$13.9^{c} \pm 2.4$	$20.1^{d} \pm 2.2$	$34.0^{c} \pm 4.4$	$41.2^{c} \pm 2.8$
Adimix 30	$57.2^{c} \pm 3.3$	$77.8^{b} \pm 5.2$	$82.3^{c} \pm 3.3$	$89.7^{b} \pm 20$	$95.6^{b} \pm 6.1$
Ding Su	$88.3^{b} \pm 7.4$	$97.7^{a} \pm 4.1$	$105.2^{a} \pm 5.6$	$99.1^{ab} \pm 2.1$	$99.6^{b} \pm 0.2$
Vega 30	$66.8^{c} \pm 8.6$	$87.0^{b} \pm 9.1$	$100.0^{ab} \pm 5.9$	$107.7^{a} \pm 1.6$	$112.1^{a} \pm 9.9$
Vega 50	$99.2^{a} \pm 2.2$	$97.8^{a} \pm 1.7$	$96.5^{b} \pm 2.2$	$97.8^{ab} \pm 1.6$	$97.1^{b} \pm 0.4$
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Values represent the mean ± standard deviation of three observations per time point. Means with different letters within a column differ significantly at $p < 0.05$. [1]Digestion stage 1: 35 min, 2: 70 min, 3: 80 min, 4: 160 min, 5: 250 min.

Conclusion

Despite almost identical visual appearance, *in vitro* release profiles can differ significantly among fat-embedded butyrate matrices. In the present study two matrices showed a sustained release profile, with a gradual release of butyrate throughout all the digestion stages. Conversely, two other matrices showed no protection, with a complete release of their content in the first stage of digestion. Differences in release profiles may, in addition to the already described dose, health status and ages factors, be an underlying mechanism explaining the observed discrepancies in the literature concerning the of use fat-embedded butyrate matrices. Tributyrin showed a release profile targeting the enteric region, with a total cumulative yield being lower than the theoretical maximum. This could be due to an inhibitory effect of the butyric acid-induced drop of pH on pancreatic lipase during the enteric steps.

The present *in vitro* model allows to compare release profiles of different butyrate derivatives in quick and inexpensive way, thus meeting the goal of the study. There are, however, a few limitations related to the use of such a model. Avian-specific motility, e.g. gizzard contractions and anti-peristaltic movements of the chyme, are not included in the model. Yields greater than 100% indicates that the butyrate content determination needs to be refined. Also, pH stability can be compromised despite the use of a concentrated buffer when acidic compounds are assessed.

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EFFECTS OF ACIDIFIED AND ALKALINIZED DIETS ON GROWTH PERFORMANCE AND INTESTINAL MORPHOMETRY OF BROILER CHICKENS

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Abstract

The purpose of this experiment was to study growth performance and intestinal morphology in broilers fed on acidified and alkalinized diets. Two hundred and fifty Ross 308 male broiler chicks were randomly allotted to 5 dietary treatments consisting a nutritionally balanced basal diet supplemented with 10, 20 and 30 g/kg citric acid (CA) or Ca $(OH)_2$ (ALK; 8.9 g/kg for growth period and 8.6 g/kg for finisher period) from day 7 up to day 42 of age. Inclusion of 30 g/kg CA significantly increased body weight, average daily weight gain, average daily feed intake and improved feed efficiency. Addition of 30 g/kg CA to the diets increased villus length in duodenum, jejunum and ileum, crypt depth and villus length to crypt depth ratio in the jejunum but reduced epithelial thickness in the duodenum and jejunum segments ($P<0.05$). Alkalinized diet significantly reduced average daily feed intake, whereas had no significantly effect on morphometrical parameters concerned. In conclusion, this study demonstrated that supplementation of broiler diets with 30 g/kg of CA improved performance and morphometric indices. Furthermore, change of acidity of gut toward alkaline by feeding an alkalinized diet resulted in a reduced growth performance.

Keywords: citric acid, calcium hydroxide, broiler chicken, intestinal morphology

Introduction

Acidified diets further benefits to birds by activating the functionality of proteolytic enzymes, promoting feed intake, reducing ammonia and other growth-depressing microbial metabolites, favouring mineral absorption, and lowering the incidence of subclinical infections (CHAVEERACH et al. 2004). Citric acid (CA) appears to have a positive impact on the small intestine morphometry, thereby facilitating nutrient absorption and growth performance in broiler chicken (NOURMOHAMMADI and AFZALI, 2013). However, the effectiveness of acidified diets in broiler chicken may also depend upon composition of diet and its buffering capacity. The mode of action for dietary organic acids, including CA, is mainly believed to rely on their ability to acidify the diet and ultimately the contents of the digestive tract (NOURMOHAMMADI et al., 2012). These compounds are primarily used to prevent pathogen contamination such as Salmonella through sanitising the feed. However, experimental results showed that CA acts to improve nutrient digestion and absorption, mucosal immunity and exert topical effects on the intestinal brush border in broiler chicken (NOURMOHAMMADI et al., 2012). To the best of our knowledge, the supplemental effects of alkalinisation (ALK) diets in broilers are largely unrevealed. Therefore, the present study was conducted to compare the effects of acidified or alkalinized diets on growth performance and intestinal morphology in broiler chicken.

Material and Methods

A total number of 250 male Ross 308 broiler chicks were obtained from a commercial hatchery and randomly distributed into 25 floor pens in an environmentally controlled house. Each of the five treatments was replicated five times, with 10 birds per pen. The experimental period was divided into grower (7 to 21 d) and finisher phases (22 to 42 d). The basal diet was supplemented with 10, 20 or 30 g/kg CA to provide three of the experimental diets. An experimental diet was also provided by addition of Ca $(OH)_2$ in the form of mile of lime (8.9 g/kg for growth period and 8.6 g/kg for finisher period). The pH value for the alkaline solution was set as 12.3. Considering pen as experimental unit, body weight (BW) and feed intake (FI) were recorded weekly and data were used to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed to gain ratio (FCR) for 7- to 42 d period of age. Fragments of approximately 5 cm were obtained from each of the following segments of the small intestine: the duodenum and the jejunum to evaluate the intestinal morphology. The fragments from each segment were then placed on polystyrene sheets, opened longitudinally, washed in saline solution, fixed in 10% formaldehyde solution, dehydrated and embedded in paraffin. The measurements of villus length and crypt depth were taken using a light microscope and a system that analyses computerised images, and the data were used to calculate the villus length to crypt depth ratio. The height of 30 villi and the depth of

30 crypts were measured from each segment and replicate, and the means were utilized for statistical analysis. Data were analyzed using GLM procedure (SAS, Institute) in a completely randomized design. All of the treatment means were compared using Tukey-Kramer's test. For the different statistical tests, significance was declared at $P < 0.05$.

Results

Inclusion of 30 g/kg CA increased BW, ADG, and ADFI, and decreased FCR compared to the control birds from 7 to 42 days of age (Table 1). Alkalinized diet (ALK) significantly decreased ADFI of the birds in comparison with the birds received control diet (P <0.01). Dietary CA at 30 g/kg significantly increased villus length in duodenum, jejunum and ileum epithelium compared to the control birds (Table 2; P <0.05). The acidified diets at 30 g/kg level also significantly increased the jejunal crypt depth and villi length: crypt depth ratio and reduced epithelial thickness in the duodenum and jejunum segments (P <0.05).

Conclusions

Results of the current study for increased ADFI and ADG due to dietary CA (30 g/ kg) through day 42 of age are consistent with those reported by NOURMOHAMMADI et al. (2012). In the present study, all dietary treatments were isocaloric; therefore, the increased performance with 30 g/kg of CA at day 42 of age may be attributed in part to the positive effect of CA on improved gut health in favour of nutrients availability. It was shown that dietary acidification increases gastric proteolysis and protein/amino acid digestibility by enhancing digestive enzyme activities (LANGHOUT, 2000). The negative response or lack of response in most of the variables to alkalinized diet in the current research is unknown. However, ADFI was significantly reduced for ALK diet, a phenomenon which may be reasoned by impaired plasma acid-base balance or reduced palatability of the diet.

NOURMOHAMMADI and AFZALI (2013) reported that CA as natural growth promoters increased villus lengths in duodenum and jejunum through decreasing the intestinal colonization and infectious processes resulting in inflammatory reactions at the intestinal mucosa. The increased villus length represents an attempt to increase intestinal surface area to maximize absorption. Moreover, increased jejuna villus length could be hypothetically caused by reduced cell turnover and migration, resulting in the retention of epithelial cells on the villi (NOURMOHAMMADI and AFZALI, 2013). The positive influence of CA-added diets at 30 g/kg level on villus length and crypt depth in this study suggest that CA may enhance rate of nutrient absorption and a reduce rate of enterocyte cell migration from the crypt to the villus. The villus length to crypt depth ratio is an indicator of the likely digestive capacity of the small intestine. An increases ratio corresponds to an increased digestion and absorption. In conclusion, this study demonstrated that supplementation of broiler diets with 30 g/kg of CA improved performance and morphometric indices. Furthermore, change of acidity of gut toward alkaline by feeding an ALK diet resulted in a reduced growth performance in broiler chickens.

Table 1. Effects of citric acid (CA) and alkaline diet (ALK) on productive performance of broilers

Parameter	Control	Dietary acidification (g CA/kg)			ALK	SEM	*P*-value
		10	20	30			
BW at 42 d, g	1815^{b}	1856^{b}	1850^{b}	1993^{a}	1781^{b}	15.7	0.003
ADG, g/b/d							
7 to 42 d	48.2^{b}	49.6^{b}	49.3^{b}	53.8^{a}	47.6^{b}	1.35	0.007
ADFI, g/b/d							
7 to 42 d	91.8^{ab}	94.6^{a}	90.9^{b}	93.9^{a}	86.4^{c}	1.61	0.002
FCR, g/g							
7 to 42 d	1.91^{a}	1.90^{a}	1.84^{ab}	1.74^{b}	1.82^{ab}	0.05	0.041

$^{a\text{-}c}$ Means within a row with different superscripts are significantly different (P <0.05).

SEM, standard error over general mean

Table 2. Effects of citric acid (CA) and alkaline diet (ALK) on intestinal morphology of broilers

Item	Control	Dietary acidification (g CA/kg)			ALK	SEM	*P*-value
		10	20	30			
Duodenum							
Villus length, µm	1726[b]	1727[b]	1733[b]	1782[a]	1738[b]	6	0.001
Crypt depth, µm	148	149	148	151	150	1	0.147
Ratio	11.7	11.6	11.7	11.8	11.6	0.1	0.253
Epithelial thickness, µm	52.3[a]	52.2[a]	50.8[ab]	50.0[b]	51.3[ab]	0.6	0.007
Jejunum							
Villus length, µm	1224[b]	1270[b]	1260[b]	1423[a]	1261[b]	4	0.001
Crypt depth, µm	132[b]	132[b]	133[b]	136[a]	133[b]	1	0.004
Ratio	9.3[b]	9.6[b]	9.5[b]	10.5[a]	9.5[b]	0.2	0.034
Epithelial thickness, µm	34.0[a]	33.6[ab]	33.5[ab]	32.4[b]	33.8[ab]	0.4	0.041
Ileum							
Villus length, µm	876[b]	869[b]	896[ab]	934[a]	873[b]	3	0.028
Crypt depth, µm	114	116	115	118	116	1	0.373
Ratio	7.7	7.5	7.8	7.9	7.5	0.1	0.084
Epithelial thickness, µm	33.2	32.4	32.7	32.1	32.2	0.5	0.472

[a-c] Means within a row with different superscripts are significantly different ($P < 0.05$).

SEM, standard error over general mean

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EFFECT OF DIETARY ACIDIFICATION AND ALKALINIZATION ON ILEAL DIGESTIBILITY AND TIBIA PARAMETERS OF BROILER CHICKENS

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Abstract

The objective of this study was to determine the effects of dietary acidification and alkalinization on ileal nutrients digestibility, serum enzyme activities and bone mineralization in broiler chickens. The birds were allotted to 5 dietary treatments for 35 d, each of which was replicated 5 times with 10 chicks per replicate. The dietary treatments consisting a nutritionally balanced basal diet supplemented with 10, 20 and 30 g/kg citric acid (CA) or Ca $(OH)_2$ (ALK; 8.9 g/kg for growth period and 8.6 g/kg for finisher period) from day 7 up to day 42 of age. Inclusion of 30 g/kg CA significantly increased tibia ash, tibia Ca content, ileal digestibility of crude protein and apparent metabolizable energy corrected to zero nitrogen retention at day 42 of age ($P<0.05$). Lactate dehydrogenase activity in serum was significantly elevated in the birds fed on the 30 g/kg CA-treated diets, whereas alkaline phosphatase activity was decreased ($P<0.05$). The results indicated that ALK diet significantly reduced tibia ash, tibial P and Ca contents. In conclusion, these findings demonstrate that inclusion of 30 g/kg CA bring about great economical benefits as nutrients digestibility and bone mineralization improvements. Also, the prominent impact of alkalinized diet was reduction of nutrients absorption.

Keywords: citric acid, calcium hydroxide, broiler chicken, serum enzyme activity, nutrient digestibility

Introduction

Inclusion of citric acid (CA) in poultry diet creates an acidic condition (pH 3.5 to 4.0) in gut favouring development of lactobacilli and other beneficial bacteria. Dietary acidification increases the activities of gastric enzymes, leading to improvements in digestion and absorption of protein and fibre (MARTIN et al., 2011). The CA appears to have a positive impact on histology of the small intestine, thereby facilitating nutrient absorption and growth performance in broiler chicken (NOURMOHAMMADI and AFZALI, 2013). On the other side, NOURMOHAMMADI et al. (2012) found that severe reduction pH in gut due to high dose dietary acidification brought about decreased digestion and absorption of nutrients, disrupted liver and kidney function in broiler chicks.

Despite of extensive body of research findings demonstrating favorite effects of acidified diets on performance, health and welfare of broiler chicken, effect of dietary alkalinization on poultry are not fully investigated. OWEN et al. (1994) reported that pH alteration of gut from the normal acid-base balance towards alkalinization affects health, welfare and production of broiler chicken. Therefore, the present study was conducted to evaluate the effects of acidified or alkalinized diets on ileal digestibility, intestinal histomorphology, bone mineralization, and serum enzyme activities in broiler chicken.

Material and Methods

A total number of 250 male Ross 308 broiler chicks were obtained from a commercial hatchery and randomly distributed into 25 floor pens in an environmentally controlled house. Each of the five treatments was replicated five times, with 10 birds per pen. The experimental period was divided into grower (7 to 21 d) and finisher phases (22 to 42 d). The basal diet was supplemented with 10, 20 or 30 g/kg CA to provide three of the experimental diets. An experimental diet was also provided by addition of Ca $(OH)_2$ in the form of mile of lime (8.9 g/kg for growth period and 8.6 g/kg for finisher period). The pH value for the alkaline solution was set as 12.3. At day 42 of age, blood were collected from two killed birds in non heparinised tubes and kept on ice slashes until they were subjected to serum collection by centrifuging the whole blood sample at 2,500 g for 10 min. The serum samples were analyzed for the activity of alkaline phosphatase (ALP) alanine aminotransferase (ALT) aspartate aminotransferase (AST) and lactate dehydrogenase (LDH). At day 30 of age, titanium oxide (1 g/kg of diet) was added to all the diets for five days and was used as an analytical marker to determine the effect of treatments on digestibility of crude protein (CP), apparent metabolizable energy corrected to zero nitrogen retention (AMEn), calcium (Ca) and phosphorous (P). Tibia ash was determined by removing

the adhering tissue, drying the bone at 110 °C for 12 h and extraction of fat with ether. The dry fat-free bones were ashed in a muffle furnace at 550°C for 3 h. Ash weight was calculated as a percentage of dry fat-free bone weight. The tibia contents of Ca and P were measured using dry-ashed bone samples following the same procedure of minerals estimation in feed and excreta. Data were analyzed using GLM procedure (SAS, Institute) in a completely randomized design. All of the treatment means were compared using Tukey-Kramer's test. For the different statistical tests, significance was declared at $P < 0.05$.

Results

The effect of CA-containing and ALK diets on ileal digestibility and bone mineralization are summarized in Table 1. Inclusion of 30 g/kg CA in diet significantly increased ileal digestibility of CP ($P < 0.05$) and AMEn ($P < 0.01$). Tibia ash and Ca content were greater in the birds fed 30 g/kg CA than those received ALK and CON diets ($P <0.05$). Alkalinized diet decreased tibia ash, Ca and P contents ($P<0.05$) compared to the acidified and CON diets. Inclusion of 30 g/ kg CA in diet significantly reduced ALP activity ($P <0.01$) and increased LDH activity ($P <0.01$) compared to the control birds at day 42 of age (Table 2).

Conclusions

In this study, all of the chicks fed on alkalinized diet showed severe leg paralysis at 24 d of age. It seems that the same birds were suffered from calcium tetany characterized by muscle weakness or paralysis and as a result of hypocalcemia (MARTIN et al., 2011). The risk factors associated in the development of calcium tetany in broilers are reported as poor flock uniformity and high calcium feed (> 1.2% Ca) (MARTIN et al., 2011). Calcium tetany may also develop when the normal response to a decline in blood Ca level becomes disrupted, for example by metabolic disturbances that increase blood pH and consequently cause blood Ca becoming unavailable. Metabolic disturbances that increase blood pH, called respiratory or metabolic alkalosis depending on the underlying cause, shift the plasma calcium flux from the ionized (active) form to the protein bound (inactive) form (MARTIN et al. 2011). It has been recognized that CA improve protein and energy digestibility by reducing the microbial competition with the host for nutrients, endogenous nitrogen losses and ammonia production in addition to their other beneficial effects for broilers (NOURMOHAMMADI et al., 2012).

The organic acids have shown positive impact on histology of the small intestine, thereby facilitating nutrient absorption and growth performance in broiler chicken (NOURMOHAMMADI and AFZALI, 2013). The decrease in serum ALP activity associated with the diets supplemented with 30 g/kg of CA might reflect the down regulation of this enzyme caused by increased availability of phosphorus (Huff et al. 1998). The increase in LDH activity that we observed in the birds fed on 30 g/kg CA may be related to normal function of liver, because this enzyme decreases sharply as the disease progresses.

Table 1. Effects of citric acid (CA) and alkaline diet (ALK) on ileal nutrients digestibility and bone mineralization of broilers

Item	Control	Dietary acidification (g CA/kg)			ALK	SEM	*p*-value
		10	20	30			
CP, %	80.7^{c}	81.6^{bc}	83.1^{ab}	83.8^{a}	81.7^{bc}	0.4	0.038
AMEn, kcal kg^{-1}	2713^{b}	2709^{b}	2722^{b}	2775^{a}	2708^{b}	4	0.004
Ca, %	58.5	58.5	58.9	59.0	58.6	0.3	0.129
P, %	38.7	38.4	38.6	38.9	38.0	0.3	0.146
Tibia ash, %	46.0^{b}	46.5^{b}	46.1^{b}	48.6^{a}	43.1^{c}	0.3	<0.001
Ca, %	37.0^{b}	38.0^{b}	37.8^{b}	38.9^{a}	31.6^{c}	0.3	<0.001
P, %	19.17^{a}	19.21^{a}	19.20^{a}	19.45^{a}	18.63^{b}	0.08	0.037

$^{a-c}$ Means within a row with different superscripts are significantly different ($P <0.05$).

SEM, standard error over general mean

Table 2. Effects of citric acid (CA) and alkaline diet (ALK) on serum enzyme activities (U/L) of broilers

Item	Control	Dietary acidification (g CA/kg)			ALK	SEM	*p*-value
		10	20	30			
ALP	14302[a]	14315[a]	14321[a]	14107[b]	14394[a]	165	0.002
ALT	1.5	1.8	1.7	1.6	1.7	0.1	0.086
AST	239	238	237	238	241	3	0.073
LDH	714[b]	726[b]	717[b]	749[a]	718[b]	7	0.007

[a-b] Means within a row with different superscripts are significantly different (P <0.05).

SEM, standard error over general mean

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P-055 (ID 221)

EFFECT OF ARTICHOKE (*CYNARASCOLYMUS* L.) EXTRACT SUPPLEMENTATION ON BLOOD CHEMISTRY OF BROILER CHICKENS

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Abstract

This study was carried out to investigate the effects of different levels of artichoke (*Cynarascolymus* L.) extract (AE) supplementation in drinking water on liver enzyme and blood chemistry of broiler chickens. A total of 272 one-day-old broiler chicks (Ross, 308) were randomly allotted to 4 treatment groups, each with 4 replicate cages containing 17 chicks for a period of 42 days. The treatment groups consisted of: control (C) group (without AE added in drinking water) and AE added in drinking water at the level of 75 ppm (T1), 150 ppm (T2) and 300 ppm (T3). Blood samples were collected at 0, 14, 28 and 42 days of age and then analyzed for the liver enzyme; Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), serum plasma protein (TP) and serum albumin (AL). The results have illustrated that there was significantly ($P<0.05$) higher of the AST at 42 days of the chicks in T1, T2 and T3 than that in the C. The ALT at 42 days of T2 was significantly higher ($P<0.05$) than that in the C groups, but no difference from the T1 and T3. The TP at 42 days of the chicks in the T3 were significantly lower ($P<0.05$) than the other groups and gradually increased ($P<0.05$) with lower level AE. The AL of the chicks in the T3 had lowest value, whereas the AL levels in other groups were varied without systematic pattern. It was concluded that AE supplementation in drinking water would improve liver function, while unclear effects on AL level in broiler chickens.

Keywords: artichoke extract, blood chemistry, liver, broiler chickens

Introduction

Poultry industry produces animal proteins for human consumption most effectively and economically within the shortest possible time (JAVED et al., 2013). The current difficulties facing poultry industry is the occurrence of hepatotoxicity and oxidative stress condition which directly effects and damages the chicken liver and the health and quality of the meat of the chicken. Artichoke extract has been used since ancient times as a remedy for indigestion and liver problem. The major bioactive component of artichoke leave are cynarin, flavonoids, phenolic acid and caffeic acid (WANG et al., 2003; JOY and HABER, 2007).Obtaining the cynarin substance from artichoke extract and its usage in the medical science as hepatoprotective triggered the area of utilization particularly in the poultry farming. The motivation of keeping the liver function within the intended level in the broiler, where intensive farming is dealt has on the other hand increased the importance of such supplements (UZATICI and CELIK, 2014). Therefore, the objective of this study was to determine the effects of different levels of artichoke (*Cynarascolymus* L.) extract (AE) supplementation in drinking water on blood chemistry of broiler chickens.

Material and Methods

Animal and Sample collection

A total of 272 one-day-old broiler chicks (Ross, 308) mixed sex, day-old broiler chickens were housed in 6 x 10 m curtain-sided, open house system with rice husk as litter material. There were randomly allotted to 4 treatment groups, each with 4 replicate cages containing 17 chicks for a period of 42 days. The treatment groups consisted of: control (C) group (without AE added in drinking water) and AE added in drinking water at the level of 75 ppm (T1), 150 ppm (T2) and 300 ppm (T3). All chicks were offered mash feed and drinking water *ab libitum*. AE was provided on day 1-7 and 15-24 of age. Blood samples were collected at 0, 14, 28 and 42 days of age and then analyzed for the liver enzyme; Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), serum plasma protein (TP) and serum albumin (AL).

Data analysis

Data from all experiments were analysed using the ANOVA procedure of SAS® software (SAS Institute, 2005) for completely randomized designs. A probability level of P<0.05 was used to determine statistical significance of differences among the treatments.

Results

The results of the current study was shown in Table1. The results have illustrated that there was significantly (P<0.05) higher of the AST at 42 days of the chicks in T1, T2 and T3 than that in the C group. The ALT at 42 days of T2 was significantly higher (P<0.05) than that in the C groups, but no difference from the T1 and T3. The TP at 42 days of the chicks in the T3 were significantly lower (P<0.05) than the other groups and gradually increased (P<0.05) with lower level AE. The AL of the chicks in the T3 had lowest value, whereas the AL levels in other groups were varied without systematic pattern.

Table1: Effect of artichoke (*Cynarascolymus* L.) extract supplementation on blood chemistry of broiler chickens (Mean ± SD)

Variable	Group			
	Control	T1 (AE 75 ppm)	T2 (AE 150 ppm)	T3 (AE 300 ppm)
AST(IU/L)				
0 day	147.2 ± 77.73	147.71 ± 65.4	156.5 ± 86.43	107.14 ± 91.28
14 day	157.38 ± 38.77	168.25 ± 26.27	167 ± 35.64	190.75 ± 14.95
28 day	163.75 ± 54.14	188.13 ± 34.95	161.75 ± 62.49	174.13 ± 34.15
42 day	57.5 ± 39.96^{a}	254.75 ± 131.45^{b}	238.75 ± 119.43^{b}	171 ± 60.64^{b}
ALT (IU/L)				
0 day	7 ± 4.9	5.88 ± 4.09	7.5 ± 4.04	8.43 ± 2.3
14 day	0.13 ± 0.35	2.12 ± 3.94	2.38 ± 2.2	1.25 ± 1.16
28 day	4.33 ± 2.66	4 ± 2.5	2.75 ± 2.49	2.25 ± 0.5
42 day	1.8 ± 0.84^{a}	5 ± 1.77^{ab}	6.13 ± 4.29^{b}	4.67 ± 2.88^{ab}
TP (g/dL)				
0 day	3.27 ± 0.69	3.7 ± 0.97	3.82 ± 0.8	3.26 ± 0.49
14 day	2.74 ± 0.84^{a}	3.28 ± 0.34^{b}	3.08 ± 0.24^{ab}	3.28 ± 0.24^{b}
28 day	3.45 ± 0.54^{ab}	3.9 ± 0.73^{b}	3.75 ± 0.54^{ab}	3.15 ± 0.77^{a}
42 day	4.29 ± 0.83^{c}	3.85 ± 0.48^{bc}	3.55 ± 0.3^{b}	2.83 ± 0.53^{a}
AL (g/dL)				
0 day	0.7 ± 0.18^{a}	0.93 ± 0.19^{ab}	0.95 ± 0.28^{b}	0.87 ± 0.16^{ab}
14 day	1.49 ± 0.78	1.31 ± 0.18	1.24 ± 0.2	1.24 ± 0.17
28 day	1.23 ± 0.16^{ab}	1.20 ± 0.19^{a}	1.40 ± 0.16^{b}	1.13 ± 0.21^{a}
42 day	1.53 ± 0.3^{ab}	1.88 ± 1.17^{b}	1.31 ± 0.12^{ab}	1.09 ± 0.33^{a}

^{a,b}means in the same row with differentsuperscripts are significant difference (*P*<0.05)

All variations of AST, ALT, TP and AL were in the normal range of healthy chicks, indicating no hepatotoxic substances, such as CCl_4, toxin, which can damage the liver. In addition, the AST, ALT, TP and AL in this study agree with earlier report (ABDO et al., 2007). This would imply for beneficial influence of the AE supplementation to broiler chickens.

Conclusions

In conclusion, the artichoke (*Cynarascolymus* L.) extract supplementation in drinking water would improve liver function, while unclear effects on albumin level in broiler chickens.

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P-057 (ID 53)

EFFECTS OF ENHANCED ACIDIFIERS ON THE REDUCTION OF *E. COLI* IN CHALLENGED TURKEYS

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Abstract

A combination of synergistically acting organic acids and a permeabilizing substance in water application can reduce the microbial contamination in turkeys. An experiment was conducted to confirm these effects using water application with a blend of formic, acetic and propionic acid and a permeabilizing substance (Biotronic® Top liquid, BIOMIN, Austria). 60 day-old female turkey poults were randomly assigned to three groups with 20 turkeys each. A control group (NC) received a commercial diet with no antibiotic or NGP, trial group (NGP) received water supplemented with Biotronic® Top liquid 1.25 ml/l of water and positive control group (PC) received water supplemented with Enrofloxacin 0.5 ml/l of water. On day 10 the animals in all groups were challenged with *E. coli* O78. The trial lasted for 30 days. Bacterial counts of the intestinal tract were recorded on day 20 and 30. On day 20, *E. coli* counts of NGP group ($5.42^a \pm 1.07$) were significantly ($p<0.05$) lower than NC ($7.42^b \pm 0.78$) and PC group ($7.24^b \pm 1.18$). On day 30, *E. coli* counts of NGP group ($5.12^a \pm 0.44$) were as well significantly ($p<0.05$) lower compared to NC ($8.09^b \pm 0.21$) and PC group ($7.89^b \pm 0.31$). The results of the NGP group showed a significant ($p<0.05$) reduction of *E. coli* counts in the intestinal tract compared to NC and PC group.

Introduction

Antibiotic growth promoters (AGP) are antibiotics which are added to animal feed or water at subtherapeutic levels to improve the growth performance of the animals and to decrease the incidence of diseases (Khachatourians, 1998). AGP‘s have been used in animal production worldwide since 1946 (Moore et al., 1946). Nowadays antibiotic resistance has become a hot topic, as the use of antibiotics over time in animal health and growth promotion has resulted in bacterial resistance to antibiotics (Smith et al., 2010, Van de Bogaard and STOBBERRING, 1999). The problem of bacterial resistance has resulted in banning and/or regulating the use of AGP for growth promoting issues by a number of countries (Council regulation 98/2821/CEE, 1998; WHO, 2001). This leaded to the development of alternative products, which can be used instead of AGP’s for growth promoting issues of the animals.

Organic acids (OA) as alternatvies to AGP have increasingly and successfully been supplemented in poultry feed. It is generally known that acidifiers improve poultry performance. Acids reduce the microbial load, aid the buffering capacity and reduce the pH in the feed and in the gastrointestinal tract, which enhances protein digestion (Eidelsburger and Kirchgessner, 1994, Overland et al., 2000). With the reduction of gastric pH, pepsinogen is activated into pepsine and besides that, the pH of the stomach is also brought closer to the optimum for pepsin activity (Mroz, 2000).

Despite the fact that natural replacements for AGPs are known, fighting Gram-negative bacteria is still a challenge. The outer membrane of Gram-negative bacteria acts as a barrier that prevents antimicrobial compounds from entering the cell and destroying vital functions (Canovas et al., 2005). This outer membrane can be destabilized by a permeabilizing substance (PS), which makes the bacterial cell more susceptible to antimicrobial compounds (Alakomi, 2007). The Biomin® Permabilizing Complex, is a PS, which boosts the anitmicrobial effectivity of the organic acids.

Material and Methods

The trial was carried out at IZSLER, Italy, using 60 1-day old female turkeys (Big 6 Aviagen®), which were housed in 3 isolators. Turkeys were assigned to 3 treatments. The negative control group received a standard diet with no natural or antibiotic growth promoter, positive control group was fed a standard diet and water was supplemented with Enrofloxacin at 0.5 ml/l of water from day 11 to 20 and trial group was fed a standard diet and water supplemented with Biotronic® Top liquid at 1.25 ml/l of water (pH 4.5) from day 0 to 30.

The duration of the trial was 30 days. On day 10 the birds in all groups were orally challenged with *E. coli* O78 (1.38×10^8 CFU/ml). 10 birds from each isolator were sacrificed 10 days post infection (dpi) and the rest of the birds

were sacrificed 20 dpi at the end of the trial. Bacterial counts (*E. coli*) of the intestinal tract posterior to duodenal loop were recorded on day 20 (10 dpi) and 30 (20 dpi) of the trial.

Bacteriological analysis: The samples were diluted 10-fold in Buffered Peptone Water (BPW). All samples were serially diluted to appropriate levels for plating. An aliquot from each sample (100 µL) was spiral plated on the following: Tryptone Bile X-Glucuronide (TBX) agar for the isolation and counting of *E. coli*. Any TBX plate containing isolated *E. coli* colonies were subcultured and sent to IZSLER headquarter laboratories for "O" serotyping to see if the isolated serotype matched the *E. coli* serotype used in the challenge.

Results

The results of the present study showed that water supplementation with Biotronic® Top liquid reduced *E. coli* counts in turkeys' intestinal tract. *E. coli* counts in the intestinal tract on day 20 and 30 of the experiment, in the trial group were significantly ($p<0.05$) reduced compared to NC and PC. On day 20, *E. coli* counts of the trial group were 27.0 % lower than NC and 25.1 % lower than PC. On day 30, *E. coli* counts of trial group were decreased by 36.7 % and 35.1 % in comparison to NC and PC.

Table 1. Bacterial counts intestinal tract of control and trial groups at day 20 and 30 of the experiment (log CFU/g)

Parameter	NC	PC	NGP
No of animals	20	20	20
E. coli count day 20	$7.42^{b} \pm 0.78$	$7.24^{b} \pm 1.18$	$5.42^{a} \pm 1.07$
E. coli count day 30	$8.09^{b} \pm 0.21$	$7.89^{b} \pm 0.31$	$5.12^{a} \pm 0.44$

Conclusions

Supplementation of water with Biotronic® Top liquid reduced the number of *E. coli* in the intestinal tract of challenged turkeys. Based on this result it might be assumed that a healthy gut of the animals from first day of life is maintained, which protects against intestinal infections (Goren et al., 1988, Goren et al., 1984, Nurmi and Rantala, 1973). Potentially harmful bacteria, like *E. coli* can cause infections (Jeurissen et al., 2002), compete with the host for nutrients, stimulate the turnover of epithelial cells, secrete toxic compounds and increase the inflammatory processes in the animal. In this case the animals lose energy to the microflora and growth performance is reduced (Drew et al., 2003, Lan et al., 2005, Richards et al., 2005).

Based on the results of this experiment, the trial group outperformed the PC group, showing that Biotronic® Top liquid was more effective than Enrofloxacin in terms of antimicrobial efficacy, which confirms that NGP's can easily be replaced by AGP's.

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P-061 (ID 152)

SECONDARY PLANT COMPOUNDS AND THEIR ANTIMICROBIAL PROPERTIES TO INHIBIT THE GROWTH OF ANTIBIOTIC RESISTANT BACTERIA

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Abstract

After the European ban of antibiotics as growth promoters in animal production, there is higher interest in naturally derived and generally recognised as safe substances as an appropriate replacement. The emergence of antibiotic resistant pathogens makes the demand for alternatives even more urgent.

In contrast to plant extracts, that can vary considerably in the content of bioactive compounds and thus also in their efficacy, purified secondary plant compounds allow the production of a standardised blend and thus a reproducible mode of action. Appropriate mixtures should be composed with a broad efficacy against major gram negative and positive pathogens.

In an attempt to determine the antimicrobial efficacy of Activo® Liquid, a water soluble mixture of standardized secondary plant compounds, we conducted in vitro experiments on major poultry pathogens by Agar Diffusion Tests. Here, the blend of secondary plant compounds showed an antimicrobial effect on all tested bacteria occurring in farm animals. The degree of growth inhibition positively correlated with the concentration of Activo® Liquid.

To further determine the antimicrobial efficacy in liquid medium, we tested Activo® Liquid against ESBL producing E.coli and Methicillin resistant S. aureus field isolates compared to their corresponding reference strains in a MIC assay.

The efficacy of Activo® Liquid against the tested strains could be demonstrated in a concentration dependent manner with antimicrobial impact at higher concentrations and bacteriostatic effects in dilutions of up to 0,1% (ESBL) and 0,2% (MRSA).

These encouraging results give reason for further investigations with Activo® Liquid in animal production.

Keywords: Antibiotic Resistance, Secondary Plant Compounds, ESBL, MRSA

Introduction

Antibiotics were and still remain the method of choice against bacterial diseases. Due to their additional positive effect on growth performance they increasingly have been used in animal husbandry. But every use of antibiotics is a selection of resistant bacteria and especially this prophylactic use at low dosage gives bacteria a better chance to adapt. For this very prophylactic and metaphylactic use secondary plant compounds could be an alternative: they are generally recognized as safe and a lot of them show antimicrobial characteristics, e.g. Carvacrol and Cinnamon aldehyde. They effectively act against *Salmonella, E. coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Entero-* and *Staphylococcus* and *Candida albicans* (Baiju and Modak, 2007; Khan et a., 2009; Yossa et al., 2011) but also against multi drug resistant strains of *Escherichia coli*, *Klebsiella pneumoniae* and *Candida albicans* (Khan et al., 2009). Some compounds influence digestion and others act as antioxidants. An optimal combination has both - positive influence on health and performance. As secondary plant compounds act via several modes, the question was if they can also have an effect against antibiotic resistant bacteria like ESBL producing *E. coli* or MRSA.

Material and Methods

Trial I: in so called “agar diffusion tests” (method available on request), the sensitivities of reference strains – representative for the different species of pathogens - were evaluated with different concentrations of a specific blend of secondary plant compounds. The effectiveness of the active substances was determined by the extent to which they prevent the development of bacterial overgrowth. The diameter of the bacteria free zone around an applied component can be translated into the antimicrobial efficacy against a densely grown bacterial population on a petri dish - the bigger the bacteria free zone, the higher the antimicrobial effect.

Trial II: Farm isolates of four ESBL producing *E. coli* and two Methicillin resistant *Staphylococcus aureus* strains were compared to nonresistant reference strains of the same species with respect to their sensitivity against Activo® Liquid. In a Minimal Inhibitory Concentration Assay (MIC) under approved experimental conditions (Vaxxinova Diagnostic, Muenster, Germany) the antimicrobial efficacy of Activo® Liquid in different concentrations was evaluated.

Results

Trial I: In this trial, the blend of secondary plant compounds and organic acids (Activo® Liquid) showed an antimicrobial effect on all tested bacteria occurring in farm animals. The degree of growth inhibition positively correlated with the concentration of Activo® Liquid (see table 1).

Table 1: Inhibition of field isolated standard pathogens by different concentrations of Activo® Liquid

	Secondary Plant Compounds (Activo® Liquid)		
Central Poultry Diagnostic Laboratory, Kondapur, Hyderabad (India)	**10%**	**2%**	**1%**
E. coli (reference strains)	++	+	+
Proteus vulgaris (reference strains)	+	+	+
Pseudomonas fluorescens	++	+	-
Salmonella pulmorum	++	++	+
Salmonella gallinarum	++	++	+
Staphylococcus aureus (reference strains)	+++	++	++

+++ 22 – 29 mm ZOI (zone of inhibition)
++ 15 – 21 mm ZOI
+ 10 – 14 mm ZOI
- < 10 mm ZOI.

Trial II: The efficacy of Activo® Liquid against the tested strains could be demonstrated in a concentration dependent manner with antimicrobial impact at higher concentrations and bacteriostatic efficacy in dilutions up to 0,1% (ESBL) and 0,2% (MRSA)(see table 2).

Table 2: Efficacy of secondary plant compounds (Activo® Liquid) against ESBL producing *E. coli* and MRSA

	Secondary Plant Compounds (Activo® Liquid)			
Laboratory: Vaxxinova, Münster, Germany	**0.1%**	**0.2%**	**0.4%**	**1%**
E.coli Reference ATCC25922	+	++	++	++
ESBL 1 (Pig)	-	++	++	++
ESBL 2 (Pig)	+	++	++	++
ESBL 3 (Poultry)	+	++	++	++
ESBL 4 (Poultry)	-	++	++	++
S. aureus Reference ATCC29213	-	+	++	++
MRSA 1 (Pig)	-	+	++	++
MRSA 2 (Pig)	-	+	+	++
- no effect + growth inhibiting ++ bactericide				

Conclusions

In order to contain the emergence and spread of newly formed resistance mechanisms it is of vital importance to reduce the use of antibiotics. A general rethinking is necessary to rise to the challenge and give new approaches a chance. These approaches however, will only be successful in combination with good management practices. Antibiotics must not be used for growth promotion or metaphylactic treatment, but only as a pure curative instrument. In in vitro-trials the liquid blend of secondary plant compounds and organic acids (Activo® Liquid) showed very antimicrobial effects against prevalent livestock pathogens. This gives reason for further investigations with secondary plant compounds in animal production in order to use them for pro- and metaphylaxis and to reduce the use of antibiotics with their help in the future. The positive influence on performance parameters, as shown in many other trials, is an additional incentive for farmers to use these flavoring substances in the feed.

The high efficacy of secondary plant compounds against ESBL Producing *Escherichia coli* and Methicillin resistant *Staphylococcus aureus* (MRSA) could be a further step towards the reduction of antibiotic use. Due to the specific mode of action of antibiotics, resistance mechanisms are more likely to emerge. To support antibiotic treatment, Activo® Liquid, a blend of natural compounds with broad spectrum efficacy probably can be a safe supplement for the control of pathogenic organisms.

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P-062 (ID 92)

1, 25 $(OH)_2D_3$-GLYCOSIDE ENHANCES GROWTH PERFORMANCE AND BONE MINERALIZATION OF BROILERS FED DIETS CONTAINING LOW AVAILABLE PHOSPHORUS IN THE PRESENCE OF PHYTASE

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Abstract

Vitamin D and its metabolites have been reported to improve phosphorus utilization. In commercial broiler diets, phytase is practically included to release phosphorus. To study the effectiveness of 1, 25 $(OH)_2$ D_3-glycoside when supplement to diets containing low available phosphorus (avP) in the presence of phytase, 1,400 one-day-old male broiler chicks (Ross 308) were divided into 7 dietary treatments. The diets were a positive control (PC, 0.5 and 0.45% avP in starter and grower diets, respectively), moderate avP reduction (0.3 and 0.25% avP in starter and grower diets, respectively), and high avP reduction (0.2 and 0.15% avP in starter and grower diets, respectively) supplemented with 500 U/kg phytase in combination with 100 and 200 g/ton of 1, 25 $(OH)_2$ D_3-glycoside (Panbonis®). Ca:avP ratio in all diets was maintained at 2:1. Each treatment consisted of 10 replications with 20 birds each. Growth performances of broilers decreased with a reduction of avP ($P<0.05$). The supplementation of 100 g/ton 1, 25 $(OH)_2$ D_3-glycoside improved broiler performances at both levels of avP reduction to the similar level of PC group ($P<0.05$). The supplement of 1, 25 $(OH)_2$ D_3-glycoside at 200 g/ton showed a superior effect on growth performances of broiler fed moderate but not high avP reduction diets ($P<0.05$). Similarly, bone breaking strength and bone mineralization were improved with 1, 25 $(OH)_2$ D_3-glycoside supplementation ($P<0.05$). The supplementation of 1, 25 $(OH)_2$ D_3-glycoside at higher level in the presence of phytase, therefore, elicits better broiler performances.

Keyword: Broiler, 1, 25 $(OH)_2$ D_3-glycoside, bone mineralization, tibia ash, Blood Ca and P

Introduction

Fast growing broilers are susceptible to bone abnormalities. Therefore, a good management to ensure good development of structural bone is critical for their growth performances. Phosphorus (P) is an important element for bone development. Supplementation of commercial phytase in broiler diets has commonly known to improve P digestibility and growth performance. In practical, phytase normally used in broiler diet with low available P (avP) to minimize feed cost while maintaining growth performance of broilers. However, superior effect of improving P utilization was observed when supplemented vitamin D in the presence of phytase since vitamin D helps to improve P absorption. This study was conducted to evaluate the effect 1, 25 $(OH)_2$ D_3-glycoside in diet containing phytase with different levels of low avP phosphorus on growth performance and bone characteristics of broiler.

Material and Methods

One thousand and four hundred, one day old, male broiler chicks of Ross 308 were divided into 7 dietary treatments (Table 1). Each treatment consisted of 10 replications with 20 birds per replication. All diets were formulated to be isocaloric and isonitrogenous. Phytase was supplemented at 500 FTU/. Body weight and average feed intake of all the birds were recorded at 1, 17 and 35 days of age for the calculation of feed conversion ratio. At the end of the study (35 DOA): 2 tibias (left) from 2 chicks per pen were for collected for bone breaking strength and % tibia ash and minerals content (Ca and P) analysis.

Table 1. Description of treatments

Treatment	Description
T1	Positive control with 0.50% avP (starter) and 0.45% avP (grower)
T2	Negative control 1 with 0.30 % avP (starter) and 0.25% avP (grower)
T3	T2 + Phytase + 1, 25 (OH)2 D3-glycoside 100 g/ton
T4	T2 + Phytase + 1, 25 (OH)2 D3-glycoside 200 g/ton
T5	Negative control 2 with 0.20 % avP (starter) and 0.15% avP (grower)
T6	T5 + Phytase + 1, 25 (OH)2 D3-glycoside 100 g/ton
T7	T5 + Phytase + 1, 25 (OH)2 D3-glycoside 200 g/ton

Results

Growth performances

The reduction of avP at both levels showed negative effect on growth performance of broiler. The worst performance was observed in bird fed high avP reduction (P<0.01). The supplementation 1, 25 $(OH)_2$ D_3-glycoside both at 100 and 200 g/ton diet in the present of phytase improved broiler performance fed diet containing both moderate and high reduction avP. However, only growth performance of broiler fed moderate avP reduction could be recovered to the same level of the control group (Table 2). These results were in agreement with ABURTO et al. (1998) that 1, 25 $(OH)_2$ D_3 supplementation improved broiler performance.

Table 2. Effect of 1, 25 $(OH)_2$ D_3-glycoside supplementation on growth performance of broilers (1-35 days of age)

Treatment	Body weight (g)	Weight gain (g)	Feed intake (g)	FCR	Mortality (%)
T1	$2243.16^{b,c}$	$2197.61^{b,c}$	3368.68^{a}	1.533^{b}	1.00^{b}
T2	1749.67^{e}	1704.51^{e}	2694.16^{c}	1.581^{b}	0.50^{b}
T3	$2292.90^{a,b}$	$2248.16^{a,b}$	3434.54^{a}	1.530^{b}	1.50^{b}
T4	2426.77^{a}	2381.76^{a}	3454.86^{a}	1.471^{b}	1.50^{b}
T5	811.07^{f}	765.72^{f}	1451.45^{d}	1.912^{a}	52.50^{a}
T6	$2126.54^{c,d}$	$2081.18^{c,d}$	3222.45^{b}	1.550^{b}	1.00^{b}
T7	2097.95^{d}	2052.80^{d}	3157.25^{b}	1.538^{b}	0.00^{b}
P-value	<.0001	<.0001	<.0001	<.0001	<.0001
SEM	18.2793	18.2851	14.5075	0.0181	0.5548

^{a,b,c,d,e,f} Means in the same column with different superscripts are significantly different (P<0.05)

Bone breaking strength and mineralization

The reduction of avP at both levels caused a decrease in tibia breaking strength, tibia ash, Ca and P of broiler. The worst effect was observed in bird fed high avP reduction (P<0.01). In the presence of phytase, the supplementation 1, 25 $(OH)_2$ D_3-glycoside both at 100 and 200 g/ton diet improved tibia breaking strength tibia ash, Ca and P to be equivalent to that of the control group. Similarly, BACHMANN et al. (2013) report that the supplementation of 1, 25 $(OH)_2$ D_3 improved tibia breaking strength of broiler. In addition, tibia Ca and P of broiler fed high avP reduction were significant higher than that of the control group when fed with diet containg 200 g 1, 25 $(OH)_2$ D_3-glycoside (Table 3).

Table 3. Effect of 1, 25 $(OH)_2$ D_3-glycoside supplementation on bone breaking strength and bone mineralization of broilers

Treatment	Tibia breaking strength (N)	Tibia ash (%DM)	Tibia calcium (%DM)	Tibia phosphorus (%DM)
T1	271.67[a]	53.14[a]	19.67[b]	8.79[b]
T2	188.38[b]	47.99[b]	18.30[c]	7.51[c]
T3	277.87[a]	52.97[a]	20.23[ab]	8.47[bc]
T4	298.46[a]	53.39[a]	20.34[ab]	8.11[b,c]
T5	87.48[c]	39.83[c]	15.70[d]	6.47[d]
T6	267.16[a]	51.76[a]	20.55[a]	9.20[ab]
T7	294.82[a]	52.45[a]	20.68[a]	9.94[a]
P-value	<.0001	<.0001	<.0001	<.0001
SEM	4.2888	0.2093	0.0956	0.1360

[a,b,c,d] Means in the same column with different superscripts are significantly different (P<0.05)

Conclusions

The supplementation of 1, 25 $(OH)_2$ D_3-glycoside improved growth performances, bone breaking strength and bone mineralization of broiler fed low avP diets. Superior effect on growth performances was observed when supplemented 200g of 1, 25 $(OH)_2$ D_3-glycoside in broiler fed moderate avP reduction. The supplementation of 1, 25 $(OH)_2$ D_3-glycoside at higher level in the presence of phytase, therefore, elicits better broiler performances.

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P-063 (ID 93)

EFFECT OF QUATERNARY BENZOPHENANTRIDINE AND PROTOPINE ALKALOIDS ON GROWTH PERFORMANCE AND INTESTINAL MORPHOLOGY OF BROILERS

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Abstract

Current research has been focusing on the effects of plant extracts on poultry growth performance. It is hypothesised that potential improvements in performance parameters are related to changes in intestinal morphology. The aim of this experiment was to investigate the effects of a standardized formulation of quaternary benzophenanthridine and protopine alkaloids (QBPA and PA) from one selected plant of the *Papaveraceae* family on growth performance, villus height and crypt depth of broilers. 1600 one-day-old male broiler chicks (Ross 308) were assigned to 4 dietary treatments representing 0, 30, 60 and 100 mg/kg of QBA and PA (Sangrovit® Extra, Phytobiotics GmbH, Eltville, Germany). Each treatment consisted of 8 replicates with 50 birds each. Birds were vaccinated for coccidiosis at 4 days of age. Birds were fed with diets based on corn and soybean meal. Feed and water were provided *ad libitum*. Overall body weight gain did not differ between treatments (P>0.05). However, overall feed intake and feed conversion ratio were decreased (P<0.05) with increasing QBA and PA levels in the diet. Furthermore, QBA and PA reduced (P<0.05) mortality. Mortality was lowest when birds were fed 100 mg/kg QBA and PA. Supplementation of QBA and PA increased (P<0.05) villus height, villus width, villus height:crypt depth ratio in the jejunum and villus surface area in the jejunum, especially in the highest inclusion rate. In conclusion, feeding QBA and PA has potential to improve growth performance and gut morphology of broiler chickens.

Keywords: Broiler, quaternary benzophenantridine, protopine alkaloids, intestinal morphology

Introduction

Plant extracts are increasingly being used in animal production industry as an alternative to antibiotic growth promotor. Sanguinarine and chelerythriene are the major alkaloids found in leaves of *Macleaya cordata*. These substances belong to quaternary benzophenantridine and protopine alkaloids group. These alkaloids possess antimicrobial and anti-inflammatory effects (LENFELD et al., 1981). They also are known to improve endogenous digestive enzyme secretion, activate of the immune system and improve protein retention of farm animals (MELLOR, 2001). In the present study, benzophenanthridine and protopine alkaloids (QBPA and PA mixture) was supplemented in the diets to improve growth performance and intestinal morphology of broiler under hot climate management.

Material and Methods

One thousand six hundred, one day old, male broiler chicks of Ross 308 were divided into 4 dietary treatments including, corn-soy basal diet supplemented with 0, 30, 60 and 100 mg/kg of QBA and PA mixture (QBA 1.20 % and PA 0.23%). Each treatment consisted of 8 replications with 50 birds per replication. All birds were vaccinated for coccidiosis at 4 days of age. All diets were formulated to be isocaloric and isonitrogenous. Feed and water were provided *ad libitum*. The average environment temperature throughout the experimental period was 31.5°C (25.5-38.5°C) and the average relative humidity was 82% (50-99%). Body weight and average feed intake of all the birds were recorded at 1, 17 and 35 days of age for the calculation of feed conversion ratio. At 35 DOA, 2 birds from each replication were sacrificed and villus height, villus width, crypt depth, villus height/crypt depth and villus surface area of jejunum and ileum section were assayed (BRUNNSGAARD, 1998)

Results

Growth performance

There was no significant effect of QBA and PA mixture supplementation on body weight gain of broiler throughout the experimental period. However, feed intake and feed conversion ratio were significantly decreased with an increase of QBA and PA mixture levels in the diets (P<0.05). Similarly, LARISSA *et al.* (2013) indicated that chicks fed diet contain-

ing sanguinarine at 50 and 25 ppm had improved FCR. Broiler fed diet containing QBA and PA mixture had a significant reduction in mortality rate. The lowest mortality was observed in birds fed 100 ppm QBA and PA mixture (P<0.05). These results indicated that dietary QBA and PA mixture may exert growth-promoting effects on broiler chicks.

Table 1. Effects of QBA and PA mixture supplementation on growth performance of broilers

Treatment	QBA and PA mixture level (ppm)				P-value	SEM
	0	30	60	100		
Initial BW. (g)	42.08	41.85	41.94	42.03	0.6747	0.0690
BW gain (g)	512.81	505.76	511.30	517.89	0.3412	2.3131
FI (g)	723.10	729.55	710.08	703.45	0.1809	4.5093
FCR	1.410[ab]	1.443[a]	1.389[ab]	1.359[b]	0.0267	0.0094
Mortality (%)	0.75	0.25	0.50	0.50	0.8243	0.1860
Grower						
BW gain (g)	1,612.63	1,605.18	1,598.09	1,614.80	0.9001	8.6467
FI (g)	2,598.92[a]	2,525.95[b]	2,462.05[c]	2,459.80[c]	0.001	8.6687
FCR	1.613[a]	1.575[ab]	1.541[b]	1.523[b]	0.0158	0.0097
Mortality (%)	2.50[a]	1.25[ab]	1.00[ab]	0.25[b]	0.1582	0.3423
All peroid						
BW gain (g)	2,083.36	2,069.09	2,067.45	2,090.66	0.7339	8.5846
FI (g)	3,322.02[a]	3,255.50[b]	3,172.13[c]	3,163.25[c]	0.001	10.0187
FCR	1.595[a]	1.574[ab]	1.534[bc]	1.514[c]	0.0011	0.0069
Mortality (%)	3.25[a]	1.50[ab]	1.50[ab]	0.75[b]	0.1487	0.3824

[a,b.c] Mean values in a same row with no common superscripts are significantly different (P<0)

Gut morphology

The supplementation of QBA and PA mixture significantly improved gut health by increasing villi height, villi width, villi height:crypt depth ratio and surface area of jejunum, especially, when supplement at 100 ppm in the diet (P<0.05) and significant increased villi height:crypt depth ratio of ileum (P<0.05). This result is partly in agreement with CHANG (2008) that the supplementation of QBA and PA mixture positively influenced villi height, villi:crypt depth ratio and villi surface area. This improvement could enhance rate of nutrient digestion, absorption and utilization which led to improve growth performance.

Table 2. Effects of QBA and PA mixture supplementation on jejunum and ileum morphology of broiler chicken at 35 days of age

Treatment	QBA and PA mixture level (ppm)				P-value	SEM
	0	30	60	100		
Jejunum						
Crypt depth (µm)	135.27	120.27	116.13	112.06	0.1894	3.9534
Villi height (µm)	1,182.97[b]	1,376.30[ab]	1,293.86[ab]	1,385.70[a]	0.1181	32.9307
Villi width (µm)	203.06[b]	177.95[b]	265.72[a]	289.16[a]	0.0002	9.3445
Villi height : Crypt depth ratio	9.55[b]	11.56[a]	10.70[ab]	12.45[a]	0.0219	0.3322
Villi surface area (mm^2)	0.2407[b]	0.2463[b]	0.3686[a]	0.4043[a]	0.0001	0.0148
Ileum						
Crypt depth (µm)	121.39	116.44	102.83	105.47	0.2279	3.6227
Villi height (µm)	1,101.69	1,199.33	1,251.63	1,251.45	0.3545	33.6171
Villi width (µm)	248.88[a]	252.59[a]	195.19[b]	201.59[b]	0.0126	7.6612
Villi height : Crypt depth ratio	8.83[b]	10.48[ab]	10.64[ab]	11.94[a]	0.0312	0.3593
Villi surface area (mm^2)	0.2936	0.3008	0.2937	0.2537	0.2933	0.0104

[a,b] Mean values in a same row with no common superscripts are significantly different (P<0.05).

Conclusion

Under hot climate management, the supplementation 60 ppm of QBA and PA mixture without the addition of growth promoter has a potential to improved growth performance of broiler. The superior effect on improving gut health was obtained with higher supplementation of QBA and PA mixture in the diet.

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P-064 (ID 91)

THE SUPPLEMENTATION OF 1, 25 $(OH)_2$ D_3-GLYCOSIDE IN COMBINATION WITH DIFFERENT PHOSPHORUS LEVELS ON GROWTH PERFORMANCES AND BLOOD CHEMICALS OF BROILERS

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Abstract

An improvement of Ca and P utilization could enhance bone quality during rapid growth of commercial broiler. 2100 one-day-old male broiler chicks (Ross 308) were used to study the effect of using 1, 25 $(OH)_2$ D_3-glycoside (Panbonis®) in combination with 3 patterns of dietary Ca and P on broiler performances and blood chemicals. During starter period, all birds were fed with diet containing equal level of Ca and available P (avP) and then divided into 6 dietary treatments including, normal avP, reduced avP 0.10% and 0.2% with and without 1, 25-$(OH)_2$ D_3-glycoside supplementation during grower and finisher periods. Ca:avP ratio in all diets was maintained at 2:1. Each treatment consisted of 7 replicates with 50 birds each (25 males and 25 females). All birds were fed with diets based on corn and soybean meal. Feed and water were provided Ad-*libitum*. There was no significant interaction and significant main effect on growth performances and carcass yields of broilers (P>0.05). However, an interaction effect was observed on serum Ca and P, bone breaking strength, tibia ash, tibia Ca and P (P<0.01). The supplementation of 1, 25 $(OH)_2$ D_3 glycoside increased serum Ca and P in all diets. The highest serum Ca and P was observed in birds fed normal diet (P<0.01). Bone breaking strength, tibia ash, tibia Ca and P of birds fed diets containing low avP were improved with 1, 25 $(OH)_2$ D_3-glycoside supplementation. Therefore, 1, 25 $(OH)_2$ D_3-glycoside could improve bone quality of broiler fed low avP diets.

Keywords: Broiler, tibia ash, bone breaking strength, 1, 25 $(OH)_2$ D_3-glycoside, Blood Ca and P

Introduction

Modern broiler chicken has a very rapid growth due to the extreme genetic improvement which could cause bone problem. Calcium and phosphorus are considered very important minerals for proper bone growth and development. It has been demonstrated that supplementation of vitamin D in the diet containing normal level of calcium appeared to be superior to calcium alone in maintaining optimal bone health and reducing bone fracture (NEWMAN and LEESON, 1999). A natural form of vitamin D metabolite, 1, 25 $(OH)_2$ D_3-glycoside, derived from *Solanum glaucophyllum* (SG) has been reported to improve bone breaking strength of broiler by improving Ca and P absorption (BACHMANN et al., 2013). In the present study, 1, 25 $(OH)_2$ D_3-glycoside was supplemented to broiler diet containing various levels of Ca and P to investigate the effect on growth performance and bone characteristics.

Material and Methods

The experiment was conducted using 2 x 3 Factorial in CRD. There were 2 levels of 1, 25 $(OH)_2$ D_3 supplementation and 3 levels of Ca: available P (avP) in the diet. A total of one thousand, on hundred and fifty two, one day old, broiler chicks of Ross 308 were fed with corn-soy basal diet for 39 days (Table 1). During starter period, all birds were fed with diet containing equal level of Ca:avP without and with 1, 25-$(OH)_2$ D_3-glycoside supplementation (100 ppm)and then divided into 6 dietary treatments including, including, normal avP, reduced avP 0.10% and 0.2% with and without 1, 25-$(OH)_2$ D_3-glycoside supplementation during grower and finisher periods (50 ppm). All experimental diets were corn-soy bean meal diet containing vitamin D_3 3000 IU. Each treatment consisted of 7 replications with 50 birds per replication (25 males and 25 females). Body weight and average feed intake of all the birds were recorded at 1, 17 and 39 days of age for the calculation of feed conversion ratio. At the end of the study, 5 tibias (left) from 5 chicks per replication were collected for bone breaking strength, tibia ash and minerals content (Ca and P) analysis. Blood sample was collected from 2 birds per replication for serum Ca and P analysis.

Table 1. Dietary treatment

Treatment[1]	1, 25 $(OH)_2$ D_3[2] Supplementation	Ca : avP level		
		Starter	Grower	Finisher
T1	-	1.05:0.5	0.90:0.45	0.85:0.42
T2	+	1.05:0.5	0.90:0.45	0.85:0.42
T3	-	1.05:0.5	0.75:0.35	0.60:0.30
T4	+	1.05:0.5	0.75:0.35	0.60:0.30
T5	-	1.05:0.5	0.60:0.30	0.45:0.23
T6	+	1.05:0.5	0.60:0.30	0.45:0.23

Results

Growth performance

There was no significant interaction and significant main effect on growth performances and carcass yields of broilers (P>0.05). The reduction in Ca: avP level during grower and starter period had no effect on broiler performance (data not shown). The result clearly indicated that if the proper Ca:avP level was offered to broiler during early age, there will be no adverse effect when Ca:avP level was reduced in the later part of life. Moreover, all experimental diets had vitamin D3 3000 IU which, could help to maintain Ca and P utilization of bird fed low Ca:avP diets.

Bone breaking strength and bone mineralization

The supplementation of 1, 25 $(OH)_2$ D_3-glycoside improved bone breaking strength, tibia ash, tibia Ca and P of birds fed diets containing low Ca:avP level to be equivalent to that of the control group (Table 2). This possibly due to 1, 25 $(OH)_2$ D_3-glycoside increased Ca and P absorption which led to higher deposition in the bone (VRIES et al., 2010)

Serum Ca and P

There was a significant interaction effect of 1, 25 $(OH)_2$ D_3-glycoside supplementation and Ca:avP level on serum Ca and P of broilers (Table 3, P<0.05). The supplementation of 1, 25 $(OH)_2$ D_3 glycoside increased serum Ca (P=0.0148) and P (P=0.0686) in all diets. The highest serum Ca and P was observed in birds fed normal diet with 1, 25 $(OH)_2$ D_3-glycoside supplementation. Similar effect was reported by CHENG et al., 2004).

Conclusion

Feeding low Ca:avP level to broiler received normal Ca:P during early age had no effect on growth performances. The supplementation of 1, 25 $(OH)_2$ D_3-glycoside to broiler fed diet containing vitamin D3 showed no beneficial effect on growth performance. However, the supplementation of 1, 25 $(OH)_2$ D_3-glycoside improved bone breaking strength, bone mineralization and serum Ca and P in broiler fed low Ca:avP level.

Table 2. Effects of 1, 25-(OH)2 D3-glycoside in combination with dietary calcium and phosphorus level on bone breaking strength and bone mineralization of broilers

Treatment[1]	1, 25-$(OH)_2$ D_3	Ca:avP ratio	Tibia breaking strength (kg force)	Ash (%)	Ca (%)	P (%)
1	-	Normal	277.82[c]	41.81[a]	14.91[a]	9.50[a]
2	+	Normal	342.53[a]	42.18[a]	15.02[a]	9.60[a]
3	-	- avP 0.1%	287.60[bc]	39.32[b]	13.95[b]	8.41[b]
4	+	-avP 0.1%	327.85[ab]	43.02[a]	15.51[a]	9.58[a]
5	-	-avP 0.2%	271.00[c]	38.96[b]	13.41[b]	8.38[b]
6	+	-avP 0.2%	295.99[bc]	43.05[a]	15.38[a]	9.20[a]
Source of variation				P-value		
1, 25-$(OH)_2$ D_3 (A)			0.6529	0.9739	0.3752	0.3599
Level Ca : avP (B)			0.0509	0.1517	0.1503	<0.0001
A*B			0.0034	<0.0001	<0.0001	<0.0001
SEM			48.6404	1.7095	0.8172	0.4255

[a,b,c]Means in the same column with different superscripts are significantly different (P<0.05)

Table 3. Effects of 1, 25-(OH)2 D3-glycoside in combination with dietary calcium and phosphorus level on serum Ca and P of broilers at 39 days of age

Treatment[1]	1, 25-$(OH)_2$ D_3	Ca:avP Ratio	Ca (mg%)	P (mg%)
1	-	Normal	4.33[a]	6.22
2	+	Normal	4.56[a]	6.79
3	-	- avP 0.1%	3.10[b]	5.89
4	+	-avP 0.1%	4.44[a]	6.30
5	-	-avP 0.2%	2.69[b]	5.61
6	+	-avP 0.2%	3.07[b]	6.37
Source of variation			P-value	
1, 25-$(OH)_2$ D_3 (A)			Normal	0.2550
Level Ca : avP (B)			0.0003	0.1235
A*B			0.0148	0.0686
SEM			0.8123	0.7069

[a,b,c]Means in the same column with different superscripts are significantly different (P<0.05)

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P-065 (ID 194)

EFFECT OF SUPPLEMENTING VIA DRINKING WATER A BLEND OF MONO-GLYCERIDES, ALONE OR IN COMBINATION WITH ORGANIC ACIDS, ON *CAMPYLOBACTER* COLONIZATION IN BROILERS

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Abstract

An experiment was conducted within the EU-FP7 project CAMPYBRO in order to evaluate the effect of a blend of short and medium chain fatty acids (C3+C4+C6+C10+C12) mono-glycerides (MG) and a mixture (60:40 %) of the same mono-glycerides with organic acids (MGOA) added to the drinking water on *Campylobacter* counts in broilers. There were three treatments applied from 1 to 42 d of age, T1: Positive controls (*Campylobacter*, no additives), T2: T1 + MG at 4ml/l from 0-21d and at 2ml/l at 21-42d and T3: T1 + MGOA at 9ml/l from 0-21d and at 4.5ml/l at 21-42d. A total of 126 one-day-old Ross 308 broilers (half male and half female) were divided into the experimental treatments. At 14 d of age, all broilers were orally gavaged with 100 µl of a solution containing 1 x 10^5 CFU/ml of ST-45 *C. jejuni* strain. On days 21, 35 and 42, ceca from 12 birds per treatment were collected and *Campylobacter* counts determined (ISO 10272). Data expressed as log_{10} CFU/g caecal content were analysed by the nonparametric test of Kruskall-Wallis (SPSS v.19.0). Although treatments, especially T3, decreased the *Campylobacter* counts 0.9 to 2.3-log, no significant differences were detected between the two products tested and the control at any of the times evaluated (21, 35 and 42 d of age). It is concluded that the two mono-glyceride combinations tested did not significantly reduce *Campylobacter jejuni* colonization under the experimental model assayed.

Keywords: mono-glycerides, organic acids, *Campylobacter jejuni*, broiler

Introduction

Campylobacter is the major cause of bacterial gastroenteritis in humans worldwide. The number of confirmed cases of campylobacteriosis in the European Union has followed a significant increasing trend in the last years, along with a clear seasonal trend. In 2013, it was the most commonly reported zoonosis with 214,779 confirmed human cases (EFSA, 2015). *In vitro* studies have demonstrated that organic acids, medium chain fatty acids, or their monoglycerides have a strong bactericidal effect on *Campylobacter spp.* (CHAVEERACH et al., 2002; HERMANS et al., 2010). However, inconsistent results have been reported in *in vivo* trials (VAN DEUN et al., 2008; SOLIS DE LOS SANTOS et al., 2008, 2010; MOLATOVÁ et al., 2011; HERMANS et al. 2012). Therefore, it is clear that there exist differences in the results *in vitro* and *in vivo*. The objective of this work was to evaluate the effect of a blend of short and medium chain fatty acids (C3+C4+C6+C10+C12) mono-glycerides (MG) and a mixture (60:40 %) of the same mono-glycerides with organic acids (MGOA) added to the drinking water on *Campylobacter* counts in broilers.

Material and Methods

A total of 126 Ross 308 broilers (50% male and 50% female) from 1 to 42 days of age were used and allocated to the experimental treatments. There were three treatments applied to the drinking water from from 1 to 42 d of age, T1: Positive controls (*Campylobacter*, no additives), T2: T1 + MG at 4ml/l from 0-21d and at 2ml/l from 21-42d and T3: T1 + MGOA at 9ml/l from 0-21d and at 4.5ml/l from 21-42d. The trial was carried out in an Animal Biosafety Level 2 Unit for broilers. Broilers were housed in cages in groups of three. The individual bird was the experimental unit. Mash (non-pelleted) feeds were fed *ad libitum* with no added coccidiostat, growth promoter or veterinary antibiotics. Diets were cereal based diets similar to those used commercially. Prior to the infection of the animals, caecal samples of 9 birds randomly selected were collected to verify that chicks were all *Campylobacter*-free. At 14 d of age, all broilers were orally gavaged with 100 µl of a solution containing 1 x 10^5 CFU/ml of ST-45 *C. jejuni* strain. On days 21, 35 and 42, ceca were collected from 12 birds per treatment and *Campylobacter* counts determined (ISO 10272). Detection limit was 2 logs, and animals below limit of detection were assigned 2 logs for statistical analysis. Data, expressed as log_{10}

CFU/g caecal content, were analysed by the nonparametric test of Kruskall-Wallis (SPSS v.19.0). Statistical significance is declared at $P \leq 0.05$, with $0.05 < P \leq 0.10$ considered as a near-significant trend.

Results

Both treatments showed numerically lower *Campylobacter* counts than control, with reductions of 0.60, 0.44 and 0.66-log for T2 and 0.90, 2.3 and 1.73-log for T3 at 21, 35 and 42d, respectively (Figure 1). However, the high variability observed mainly due to the broilers below detection level observed in T3 (8 and 42% and 17 and 33% in T2 and T3 at 35 and 42 d, respectively) caused that no significant differences in the *Campylobacter* counts were observed between the two products tested and the control treatment at any of the times evaluated (21, 35 and 42 d of age).

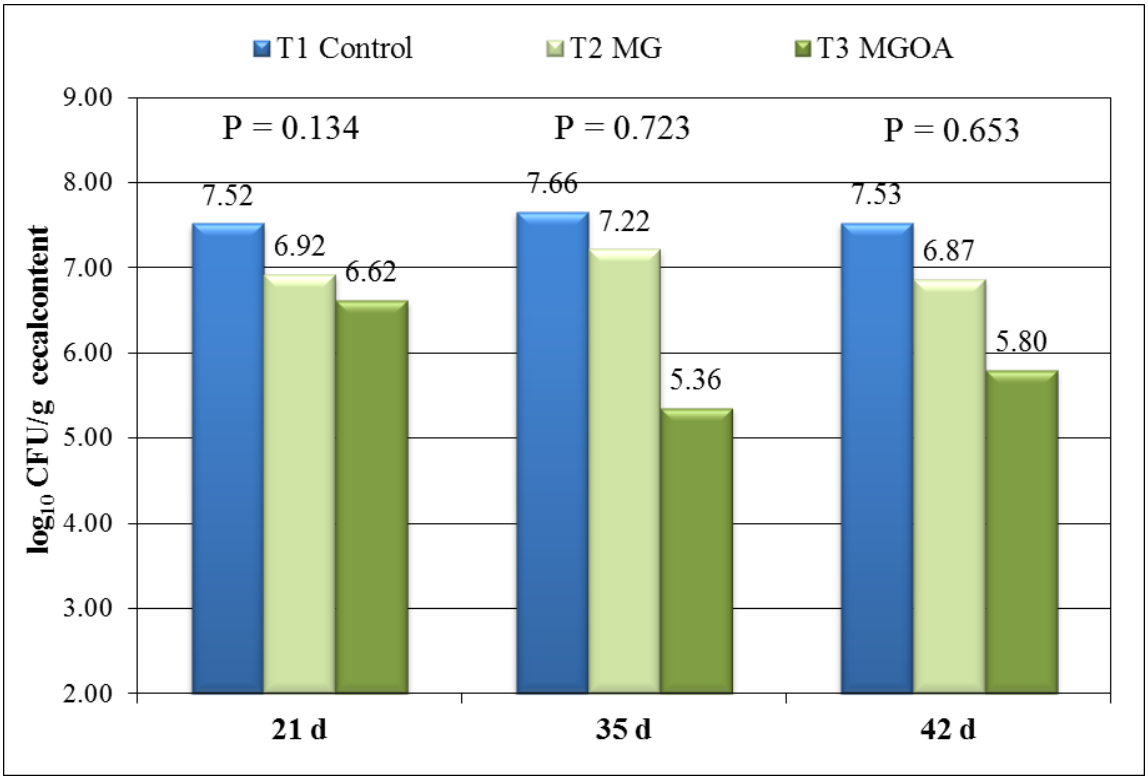

Figure 1. Effect of dietary treatment (T1: Control, T2: MG at 4ml/l from 0-21d and at 2ml/l from 21-42d and T3: MGOA at 9ml/l from 0-21d and at 4.5ml/l from 21-42d) on *Campylobacter* counts (log_{10} CFU/g) in the caeca of broilers at 21, 35 and 42 days of age.

Conclusions

Under our experimental conditions, it is concluded that the two mono-glyceride combinations tested did not significantly reduce *Campylobacter jejuni* colonization under the experimental model assayed

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P-069 (ID 32)

EFFECT OF TWO MANNANASES ON PERFORMANCE OF BROILERS FED GALACTOMANNAN ENRICHED DIETS

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Summary

The objective of this study was to evaluate the effect of two mannanases on the performance of broilers fed galactomannan-enriched diets. For the negative control (NC), a maize-soybean meal-based diet with 1.5% guar gum was formulated to exceed dietary recommendations by about 5%. This NC diet was fed as is or supplemented with four levels of a Trichoderma-ß-mannanase (TMan) (40, 70, 120 or 220 U/kg feed, respectively) or a commercially available Bacillus-ß-mannanase product (BMan) at the highest recommended dosage (240 U/kg feed). A positive control diet (PC) was formulated by using maize starch instead of guar gum in the NC. 672 one-day-old Cobb 500 male broiler chicks were given pelleted experimental diets for 35 days. Inclusion of guar gum in the corn-soybean meal-based diet (NC) significantly decreased ($P < 0.05$) daily BWG and increased feed:gain ratio compared with the PC. The supplementation of BMan and 40 U of TMan to the NC had a positive effect on broiler performance ($P < 0.05$), but birds were not able to achieve the level of performance of the PC. Increasing TMan supplementation levels up to 70, 120 and 220 U/kg feed resulted in performance values similar to the PC without a dose-response relationship. It was concluded that TMan was more effective to hydrolyze water-soluble galactomannan as compared with BMan.

Keywords: mannan, mannanase, performance, broilers

Introduction

Mannan polysaccharides are anti-nutritional components present in the majority of poultry diets. In plant cells mannans act as a storage compound or as a building block of the plant cell wall. Protein meals and some other by-products used in poultry nutrition can contain substantial amounts of ß-mannan.

In general, the presence of ß-mannans in poultry diets may lead to reduced nutrient digestibility and growth performance. The reasons for the latter might be an increase in intestinal viscosity, adverse effects on innate immune response, and microbial proliferation in the gut (Shastak et al., 2015b). The negative effects of ß-mannan can however be overcome by the addition of ß-mannanases to the feed of domestic fowl.

Guar gum (GG) and guar meal predominantly contain water-soluble galactomannan with more than 5% by weight of D-galactose residues (Aspinall, 1959). GG, the endosperm of guar seeds, has often been used to mimic inclusion of feed ingredients high in galactomannan to practical diets (Daskiran et al., 2004).

The objective of this study was to evaluate the effect of two mannanases on the performance of broilers fed galactomannan-enriched diets.

Material and Methods

In a previous study we evaluated a fungal ß-mannanase at supplementation levels equal to or higher than 400 U/kg feed (Shastak et al., 2015a). The lowest supplementation level of the fungal ß-mannanase (400 U/kg) to the negative control diet (containing guar gum) fully restored broiler performance to the level of birds fed a positive control diet (no guar gum). An increase of the ß-mannanase supplementation level (up to 1600/U kg feed) led to no further improvement of broiler performance. Thus, the present study used lower dosages of the same ß-mannanase source to determine the optimal supplementation level of the enzyme.

For the negative control (NC), a maize-soybean meal-based diet with 1.5% guar gum was formulated to exceed dietary recommendations (CVB, 2008) by about 5% (22% CP; 13.1 MJ ME/kg). This NC diet was fed as is or supplemented with four levels of a Trichoderma-ß-mannanase (TMan) (40, 70, 120 or 220 U/kg feed, respectively) or a commercially available Bacillus-ß-mannanase product (BMan) at the highest recommended dosage (240 U/kg feed). One mannanase unit (U) was defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one µmol mannose from mannan per minute under the assay conditions. A positive control diet (PC) was

formulated by using maize starch instead of guar gum in the NC. 672 one-day-old Cobb 500 male broiler chicks were given pelleted experimental diets for 35 days. Each of the seven diets was fed to eight replicate pens of 12 birds. Birds had continuous access to feed and water. Body weight for each pen was recorded on days 1 and 35 post hatch. Feed intake was measured on a pen basis. Total BW gain and mortality-corrected feed conversion ratio were calculated. Data were analyzed by one-factorial ANOVA, treatment differences were assessed by Tukey test.

Results

Treatment effects were not significant for mortality (P > 0.05) (Table). Inclusion of guar gum in the maize-soybean meal-based diet (NC) significantly decreased (P < 0.01) daily BWG, FI and increased feed:gain ratio compared with the PC. The supplementation of BMan (240 U/kg) and 40 U/kg of TMan to the NC had a positive effect on broiler performance (P < 0.01), but birds were not able to achieve the level of performance of the PC and treatments NC+70, NC+120 or NC+220 U/kg feed of TMan (P < 0.05). Increasing TMan supplementation levels up to 70, 120 and 220 U/kg feed resulted in performance values similar to the PC without a dose-response relationship. Performance for all NC+70, NC+120 or NC+220 U/kg feed of TMan treatments was significantly increased (P < 0.05) as compared to the NC, NC+BMan or NC+40 U/kg TMan treatments (Table).

Table 1: BW Gain (BWG), Feed Intake (FI) and Mortality-Corrected Feed Conversion Ratio (FCR).

Diet	BWG g/d	FI g/d	FCR feed/gain	Mortality, %
Positive control diet	67.4[c]	104.5[b]	1.55[a]	8.3
Negative control diet (NC)	32.6[a]	74.9[a]	2.31[c]	11.9
NC+240 U BMan	57.2[b]	103.6[b]	1.81[b]	4.2
NC+40 U TMan	58.4[b]	104.3[b]	1.79[b]	3.1
NC+70 U TMan	63.9[c]	106.8[bc]	1.67[a]	13.5
NC+120 U TMan	67.6[c]	110.3[c]	1.63[a]	10.4
NC+220 U TMan	67.8[c]	108.6[bc]	1.59[a]	7.3

*[a-c]Means within columns without common superscript differ significantly (*P* < 0.05)

Discussion

ß-Mannans from guar gum (galactomannans, water-soluble) in the NC diet had a negative effect on broiler performance (Table). Supplementation of both ß-mannanases to the NC improved daily BWG and feed:gain ratio compared to the NC. However, the results showed that two ß-mannanase sources had different abilities to hydrolyze water-soluble galactomannan, with higher inclusion levels necessary for BMan (240 U/kg feed) to degradate the same amount of galactomannnan. Only 40 U/kg feed of TMan was required to achieve the same broiler performance level as compared with BMan. The reason for this might have been differences in resistance against proteolytic enzyme activity, pH optimum of enzyme, substrate specificity, etc. The three highest supplementation levels of TMan (70, 120 and 220 U/kg feed) fully restored broiler performance to the level of birds fed the PC diet.

Conclusions

It was concluded that TMan was more effective in hydrolyzing water-soluble galactomannan as compared with BMan. 70 U/kg feed of TMan fully restored broiler performance to the level of birds fed the PC diet. Further studies are required to evaluate both enzymes on water-insoluble linear ß-mannan.

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P-072 (ID 19)

THE EFFECTS OF DIETARY PLANT ALKALOIDS SUPPLEMENTATION WITH VARIOUS LEVELS OF DIGESTIBLE LYSINE-METHIONINE AND CALCIUM-NON PHYTIC PHOSPHORUS RATIO ON PRODUCTIVE PERFORMANCES AND EGG QUALITY OF LAYING HENS IN THE LATE PHASE OF PRODUCTION

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Abstract

This study was conducted to evaluate the effect of plant alkaloids (Sangrovit® Extra), digestible lysine-methionine (Lys-Met) and calcium – non phytic phosphorus (Ca-NPP) ratio on productive performances and egg quality of laying hens in the late phase of production. A 2× 2 × 2 factorial arrangement of treatments comprising of 2 dietary Lys-Met ratio (0.81: 0.44 and 0.72:0.39%) and 2 levels of Ca-NPP ratio (3.75 : 0.45 and 3.38:0.41%) with and without Sangrovit® Extra (non and 60 mg/kg feed) was used. Isa brown hens (n=360,50 weeks of age) were randomly divided into 8 dietary treatments (5 replicates of 9 birds per treatment). The experiment was recorded for 12 weeks (50 to 62 weeks of age) and productive performances and egg quality traits were randomly every 4 week and shell quality traits were determined at 62 weeks of age. Dietary Lys-Met ratio and dietary Ca- NPP ratio had no effect (p>0.05) on egg production (EP), feed intake (FI), feed conversion ratio (FCR), egg weight (EW), egg mass (EM) and weight gain (WG).However, Sangrovit® Extra supplementation throughout the experimental period was significant lowered (P<0.05) mortality rate (MR) than without supplementation group. The supplementation with Sangrovit® Extra had no significant effect (P>0.05) on yolk color (YC), shell weight (SW), shell thickness (ST); however, feeding Sangrovit® Extra was significantly improved (P<0.05) % albumen (AP). It was also found that dietary Lys-Met and Ca- NPP ratio had no adverse effect (P>0.05) on egg quality. It was concluded, based on the data, that laying hens in the late phase of production required approximately 0.72 and 0.39% of digestible Lys-Met and required 3.38 and 0.41% of Ca- NPP, respectively. The supplementation of Sangrovit® Extra could improved survival rate and egg quality in term of albumen egg.

Keywords: digestible lysine-methionine, calcium- non phytic phosphorus, plant alkaloids andlaying hens

Introduction

In the recent years, the potential of the emphasis on alternative feed additive such as phytobiotics in reducing the excretion of N and P by farm animals to protect the environment, have received interest in the use of low-protein, amino acid-supplemented diets for various kinds of poultry (Keshavarz and Austic, 2004). The objective of this study was to evaluate the effect of plant alkaloid (Sangrovit® Extra) in form of quarternary benzophenanthridine alkaloid (QBA) and protopine alkaloid (PA) from Phytobiotics (Thailand) Co., Ltd. supplementation with various levels of digestible lysine-methionine and calcium- non phytic phosphorus ratio on productive performances and egg quality of laying hens in the late phase of production.

Material and Methods

Three hundred and sixty Isa brown laying hens of 50 weeks of age in five replicates per treatment (9 birds/replication) were used. Eight experimental diets which contained of 2 levels of digestible lysine-methionine ratio (0.81:0.44, control and 0.72 : 0.39 %) with 2 levels of calcium- non phytic phosphorus ratio (3.75:0.45%,control and 3.38:0.41 %) and supplemental with plant alkaloid (Sangrovit® Extra) in the levels of 0 and 60 mg/kg feed of isoenergetic diets on laying hens performances and egg quality. The reduced-digestible lysine-methionine and calcium- non phytic phosphorus ratio had approximately 10 percentage units lower than their control counterparts while another nutrient contents met the minimum requirements by the recommendation of NRC (1994). Birds were raised in laying cage and received 16-h constant lighting regime throughout the experimental period from 50 to 62 weeks of age and received feed in mash form with feed and water were provided for *ad liblium* consumption. The number of total dirty, broken, shell-less egg and mortality were recorded daily by replicate. Feed intake was recorded by replicate every 28d and in each period of experiment, egg production (%), feed intake (g/hen/day), FCR (kg feed / kg egg) were calculated of each 28-day period and overall (12 weeks). On the last 3 consecutive days of each period of experiment, egg quality was evaluated by the breaking method to measure egg weight, albumen weight, yolk weight, shell weight, yolk color and shell thickness. Data were

analyzed by ANOVA and compared the difference between dietary treatment means by Duncan's New Multiple Range Test (DMRT) by the recommendation of Steel and Torrie (1980) for a factorial completely randomized design. A significance level of P<0.05 was used.

Results

There were no interaction between main treatments and between experimental periods, only main treatments are presented (Table 1). It was found that MR and AP of laying hens fed diets supplemented with Sangrovit® Extra throughout the experimental period significantly improved (P<0.05) when compared with control groups. These results were consistented with the literature of Keshavarz and Austic (2004) and Rama Rao et al., (2011) who reported that feeding diets formulated without crude protein minimum while supplemented with essential first limiting amino acid resulted in the similar of production performance and egg quality when compared with diets formulated base on crude protein minimum. However, MR was significantly improved (P<0.05) in birds fed diets supplemented with Sangrovit® Extra. It was supported by the report of Rangpanit (2014) who reported that diets supplemented with plant alkaloids can reduce serum cortisol hormone (stress hormone) when compared with the control group, so that it may be influenced birds to have high immunity to against the stress and disease, particularly when reared in high ambient temperature. It was also found that laying hens fed diets supplemented with Sangrovit® Extra had significantly improved (P<0.05) AP when compared with control group. These results were agreemented with the reported of Rangpanit (2014) who reported that laying hens when received diets supplemented with plant alkaloid will get an improvement in the gastrointestinal functions, particularly improved in villus height which could lead to a better absorption and utilization of nutrient such as essential amino acids and ultimately improved egg composition in term of % albumen.

Table 1 Influence of dietary plant alkaloids (Sangrovit® Extra) supplementation with various levels of digestible lysine-methionine and calcium-non phytic phosphorus ratio on productive performances and egg quality of laying hens in the late phase of production.

Factors	Parameter							
	HD (%)	EW (g)	FCR (kg/kg)	FI (g/d)	MR (%)1/	AP (%)1/	YP (%)	SP (%)
Sangrovit® Extra (mg/kg feed)								
0	84.17	58.49	2.19	115.70	7.94a	61.97b	23.20	12.68
60	83.76	58.17	2.17	116.50	6.07b	63.28a	23.70	12.75
Digestible Lys:Met (%)								
0.81:0.44%	83.45	57.96	2.15	116.10	6.99ab	62.47ab	23.71	12.51
0.72:0.39%	83.67	57.24	2.13	116.30	6.81ab	62.61ab	23.52	12.52
Cal-NPP (%)								
3.75:0.45	83.12	58.13	2.17	115.90	6.77ab	62.43ab	23.51	12.40
3.38:0.41	83.24	58.26	2.14	115.70	6.67ab	62.18ab	23.47	12.42
Pooled SEM	1.98	0.46	0.07	0.87	0.12	0.32	0.21	0.09

1/ a-b Mean within a column and under each main effect with no common superscripts differ significant (P<0.05)

Conclusions

The present study has shown that it can be used diets composed of 0.72 and 0.39 % of digestible lysine-methionine and 3.38 and 0.41 % of calcium– non phytic phosphorus ratio for optimum egg production performances and egg quality of laying hens in the late phase of production. It was also found that the supplementation of Sangrovit® Extra in the levels of 60 mg/kg feed could improved survival rate and egg quality in term of albumen egg.

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P-073 (ID 47)

EVALUATION OF THE EFFECT OF TWO COMMERCIAL BUTYRATE ADDITIVES ON THE PERFORMANCE OF BROILERS

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Abstract

The objective of the study was to assess the effect of including tributyrin and a coated butyrate product at iso-butyric levels on the performance of broilers. A total of 714 male day old Ross broilers were used for the study. To better mimic commercial conditions the bedding was 'spiked' with dirty litter from a commercial unit. There were three treatment diets with 12 pens allocated to each treatment with 19/20 birds allocated to each pen at study start. The three treatment diets were control industry standard diet (T1), the control diet with tributyrin (T2) and the control diet with coated butyrate, (T3). The diets were fed for the first 9 days as phase 1 diets, from day 9 to 25 as phase 2 diets, and the final phase 3 diets were fed from day 25 to 35. Feed intake, body weight, FCR, FCE and % mortality were calculated for each phase and for the overall period (days 0 to 35).The results of the study indicate that the inclusion of tributyrin and coated butyric acid in standard broiler diets significantly improve bird live weight at days 25 and 35 as well as FCR and FCE corrected for mortality between days 9 to 25 and day 0 to 35 compared to birds offered standard industry diets. There were no overall significant differences in bird performance between the additions of tributyrin compared to coated butyrate at iso-butyric levels in this study. Both butyric acid products showed significant improvements in bird performance. This is thought to be through improved gut health

Keywords: Gut Health, Butyrate, Broilers, tributyrin, FCR improvement

Introduction

The objective of the study was to assess the effect of including Tributyrin, a glycerol ester of butyric acid, and a coated sodium-butyrate product on the performance of broilers fed wheat based diets at iso-butyric levels. Performance was assessed through measuring average daily feed intakes, live weight, feed conversion ratio (FCR - feed/gain), feed conversion efficiency (FCE - gain/feed) and mortality. To better mimic commercial conditions the bedding was 'spiked' with dirty litter from a commercial unit to disease challenge the birds.

Material and Methods

A total of 714 male, day old Ross broilers were used for the study. They were housed in three rooms in 36 pens. There were three treatment diets, with 12 pens allocated to each treatment, with 19/20 birds allocated to each pen at study start. The three treatment diets were

- control industry standard diet (T1);
- the control diet with Tributyrin (T2) ;
- the control diet with sodium butyrate, coated (T3)

The diets were fed for the first 9 days as phase 1 diets, from day 9 to 25 as phase 2 diets, and the final phase 3 diets were fed from day 25 to 35.

Food and water were provided *ad libitum* throughout the study. Birds were weighed on days 0, 9, 25 and 35 of the study. The amount of feed offered to each pen was weighed when added and refusals were weighed back on days 9, 25 and 35. Feed intake, body weight, FCR, FCE and % mortality were calculated for each feeding phase and for the overall period (days 0 to 35).

Results

The results of the study indicate that the inclusion of Tributyrin in standard broiler diets significantly improve bird live weight at days 25 and 35 and FCR and FCE corrected for mortality between days 9 to 25 and day 0 to 35 compared to birds offered standard industry diets.

The inclusion of sodium butyrate coated product in standard broiler diets significantly improved bird live weights at day 25 and 35 and the FCR and FCE in phase 2 and the overall study compared to birds fed the control diet.

Table 1. Average live weight per bird (kg) for days 0, 9, 25 and 35 for all treatments

Treatment	Day 0	Day 9	Day 25	Day 35
T1 (Control)	0.039	0.262	1.511[a]	2.511[a]
T2 (Tributyrin)	0.039	0.268	1.569[b]	2.627[b]
T3 (Sodium Butyrate coated)	0.039	0.265	1.554[b]	2.606[b]
Significance Level	0.875	0.077	***0.017***	***0.033***

There were no overall significant differences in bird performance between the addition of Tributyrin compared to addition of sodium butyrate coated in a commercial broiler diet during phase 2, 3 and the overall study. There were significant differences in bird FCR and FCE corrected for mortality during phase 1 with birds offered the diet with the addition of Tributyrin performing better than the birds offered the diet containing the sodium-butyrate coated.

Table 2. Average FCR (corrected for mortality) for phase 1, 2, 3 and the overall study period for all treatments

Treatment	Phase 1 (Day 0 – 9)	Phase 2 (Day 9 – 25)	Phase 3 (Day 25 – 35)	Overall (Day 0 – 35)
(Control)	1.093	1.403[b]	1.756	1.515[b]
(Tributyrin)	1.079	1.353[a]	1.713	1.472[a]
(Sodium Butyrate coated)	1.105	1.339[a]	1.691	1.460[a]
Significance Level	0.310	***<0.001***	0.228	***<0.001***

Conclusions

Both butyric acid products (Tributyrin and sodium butyrate coated) included in the diets of broiler chickens produced significant improvements in bird performance thought to be due to their gut stimulation, growth promotion and / or antibacterial properties.

There were no detrimental effects of inclusion of Tributyrin and sodium-butyrate coated in broiler diets on bird health, welfare or performance.

P-075 (ID 235)

MILK SERUM: WASTE OR RECOURSE FOR BROILER DIET?

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Abstract

Whey is the milk by-product resulting from the production of cheese or curd cheese from milk. The nutritional value of whey as animal feed has been appreciated for a long time and it is no longer considered as a waste material. However, the absence of lactase in the digestive tract of poultry restricts the incorporation of high quantities in their diets. The objective of the present study was to investigate the effect of whey in various concentrations in poultry diets on the performance and the physico-chemical conditions of intestinal ecosystem of broiler chicks.

One hundred and twenty 1-day-old broiler chicks were randomly allocated to four treatment groups: **group A**, negative control, **group B**, supplemented with 1% of whey in the diets of birds, **group C**, 2% of whey and **group D**, with 5% of whey, respectively. The following parameters were measured: body weight, pH and viscosity of intestinal digesta.

According to the results of this study, the supplementation of poultry diet with low concentration of whey (1-2%) increased significantly ($P \leq 0.05$) the body weight of birds and the pH of jejunum digesta, while reduced significantly ($P \leq 0.05$) the pH of caecum digesta and viscosity of jejunum and ileum digesta. Contrarily, the supplementation of poultry diet with high concentration of whey (5%) caused a significant reduction ($P \leq 0.05$) in the body weight, in the pH of caecum digesta and in the viscosity of jejunum digesta, while increased significantly ($P \leq 0.05$) the pH of duodenum and jejunum digesta as well as the viscosity of ileum digesta.

In conclusion, whey may constitute a valuable ingredient of poultry diet improving the performance and the gut health parameters of chicks. Contrarily, high concentrations of whey in poultry diets can cause growth retardation and impairment of gut health.

Keywords: whey, broiler chicks, performance, gut health

Introduction

Whey is the milk by-product resulting from the production of cheese or curd cheese from milk. More than 50% of its production is used by the feed industry on the basis of its excellent nutritional and functional properties. Moreover, the use of whey in animal feed is not only economically sensible, but it also solves the otherwise difficult problem of its disposal as waste. However, the absence of lactase in the digestive tract of poultry restricts the incorporation of high quantities in their diets (GULSEN et al., 2002; SHARIATMADARI et al., 2005). Therefore, few attempts have been made to evaluate the effect of whey usage on broiler performance. The objective of the present study was to investigate the effect of whey in various concentrations in poultry diets on the performance and on physico-chemical conditions of intestinal ecosystem of broiler chicks.

Material and Methods

One hundred and twenty 1-day old broiler chicks were randomly allocated into four treatment groups of thirty (30) chicks each. The stocking density for each experimental group was 15 birds/m^2. Broilers in all groups fed a three phase ration (starter, grower and finisher), which was based on wheat, corn and soya. The addition of whey powder was done on grower and finisher rations. No antibiotic growth promoters, organic acids and phytobiotics were used. Feed and water were available *ad libitum* throughout the trial. The body weight was measured on the 13th, 23rd and 37th day of age, while mortality was recorded daily. After euthanasia on 37th day of age, the digesta of the duodenum, jejunum, ileum and cecum from each bird were immediately collected in separate tubes and vortexed. The pH of the duodenum and cecum from each bird was measured using a digital pH-meter. The content of the jejunum and the ileum, from each

bird, were centrifuged at 3,000 g for 45 minutes. Supernatants from each tube were taken and the pH as well as the viscosity were measured using a digital viscometer. Two readings were taken from each sample.

One-way analysis of variance was performed, to statistically evaluate possible effects of treatment on chicks' body weight, as well as on the pH and viscosity values of intestinal digesta. Shapiro-Wilk and Lilliefors tests were used to test for normality and the Levene's test for testing the homogeneity of variances. Differences between means of specific treatments were evaluated using Duncan's new multiple range test. Where assumptions about either variability or normality were seriously violated, the Kruskal-Wallis npar test was applied to evaluate treatment dependent differences, while the npar Wilcoxon rank sum test was used to determine whether the medians score values of specific treatments were different enough. All analyses were conducted using the statistical software program SPSS for Windows (v. 19.0).

Results and Discussion

According to the results presented in Table 1, the supplementation of poultry diet with low concentrations of whey (1-2%) significantly increased ($P \leq 0.05$) the body weight of birds and the pH of jejunum digesta, while significantly reduced ($P \leq 0.05$) the pH of caecum digesta and viscosity of jejunum and ileum digesta. Thus, addition of whey to broiler diets by less than 2%, it may function as a natural growth promoter, since it contains lactose, proteins, minerals and yet unidentified growth factors (SHARIATMADARI et al., 2005). Moreover, whey increases the absorptive surface of intestinal mucosa and consequently, enhances effective utilisation of nutrients (GULSEN et al., 2002). In addition, lactose, the main sugar of whey, can be used as a readily available substrate for fermentation by intestinal lactic acid producing bacteria. Lactic acid influences the pH in the intestine, enhances the performance of birds and could suppress the growth of pathogenic bacteria (CORRIER et al., 1990; MCREYNOLDS et al., 2007).

However, the use of high concentrations of whey in poultry diets is compromised by its high lactose and sodium contents. The absence of lactase in the digestive tract of poultry indicates that incorporation of high concentrations of whey in the diet is likely to cause osmotic diarrhoea, with negative effects on broiler performance and gut health related problems (BILGILI and MORAN, 1990; MCREYNOLDS et al., 2007). As it is shown on the Table 1, the supplementation of poultry diet with high concentration of whey (5%) caused a significant reduction ($P \leq 0.05$) in the body weight, in the pH of caecum digesta and in the viscosity of jejunum digesta, while it simultaneously increased significantly ($P \leq 0.05$) the pH of duodenum and jejunum digesta as well as the viscosity of ileum digesta.

The mechanisms underlying on the effect of viscosity on nutrient digestion and absorption are rather complex. It is assumed that increased intestinal viscosity depresses nutrient digestibility by interference with the diffusion of digestive enzymes to their substrates and with the movement of digesta across the intestinal lumen. In addition, a higher intestinal viscosity increases the average retention time of the digesta and creates a favorable environment for bacterial activity, because the flow of digesta is reduced and the amount of undigested material in the intestinal tract is increased. This gives more time to the microbes to colonize the small intestine and results in greater competition with the host for nutrients (TSIOURIS et al., 2013).

Table 1. Effect of whey in various concentrations in poultry diets on the performance, pH and viscosity of intestinal digesta of broiler chicks ($\bar{x}$ ±SEM).

	EXPERIMENTAL GROUPS			
	A **0 % whey**	**B** **1 % whey**	**C** **2 % whey**	**D** **5 % whey**
	***Body weight* (Kg)**			
13th day	0.435 ± 0.004 [a]	0.429 ± 0.004 [a]	0.431 ± 0.004 [a]	0.432 ± 0.004 [a]
23rd day	1.168 ± 0.014 [a]	1.165 ± 0.016 [a]	1.180 ± 0.014 [a]	1.137 ± 0.019 [a]
37th day	2.438 ± 0.065 [a]	2.586 ± 0.056 [b]	2.607 ± 0.037 [b]	2.239 ± 0.077 [c]
	pH			
Duodenum	5.96 ± 0.05 [a]	6.04 ± 0.04 [a]	6.03 ± 0.03 [a]	6.16 ± 0.04 [b]
Jejunum	5.19 ± 0.12 [a]	5.58 ± 0.10 [b]	5.62 ± 0.07 [b]	5.54 ± 0.11 [b]
Ileum	5.40 ± 0.18 [a]	5.54 ± 0.21 [a]	5.59 ± 0.19 [a]	5.58 ± 0.19 [a]
Caecum	6.53 ± 0.10 [a]	6.10 ± 0.08 [b]	6.05 ± 0.10 [b]	6.01 ± 0.09 [b]
	Viscosity (Centipoise)			
Jejunum	2.23 ± 0.09 [a]	1.51 ± 0.05 [b]	1.88 ± 0.05 [c]	1.82 ± 0.08 [c]
Ileum	2.42 ± 0.08 [a]	2.14 ± 0.09 [b]	2.62 ± 0.09 [ac]	2.85 ± 0.12 [c]

[a, b, c] Mean values in the same row with a different superscript differ significantly ($P \leq 0.05$).

Conclusions

The use of whey, in well tolerated concentrations, in poultry diets promotes the performance of birds and may suppress the growth of pathogenic bacteria, comprising an attractive feed additive in poultry diets. Further studies are required in order to elucidate the mechanisms underlying these actions as well as the effect of whey against intestinal pathogens.

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P-078 (ID 166)

EVALUATION OF PERFORMANCE AND LITTER QUALITY OF BROILERS FED SUPPLEMENTED NADMG IN DIETS WITH VARYING ENERGY AND PROTEIN LEVELS

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Abstract

The effect of NaDMG (Taminizer® D, Eastman) on performance of broilers fed diets differing in energy and protein content was evaluated in a performance trial. A total of 450 male chicks (Ross 308) were divided over 30 pens. The trial was set up as a 3×2 factorial design with 3 diets differing in protein and energy content and 2 levels of NaDMG supplementation (with or without). The broilers were fed a 3 phase feeding program, with wheat-soybean meal-corn-rapeseed meal based diets and nutrient levels according to CVB recommendations. Birds were housed at commercial density and had *ad libitum* access to feed and water. Average pen weight and feed intake were recorded at 1, 14 and 38 days of age. Feed conversion ratio (FCR), average daily growth (ADG) and average daily feed intake (ADFI) were calculated for each period and the total period. Litter quality and foot pad lesions were scored visually at day 21, 28 and 38. All data were analyzed by GLM procedure. Birds fed the low energy-low protein diets showed lower ADG *($P < 0.05$)*. Both in the starter period and the total period, FCR was improved significantly (-/- 0.04) by NaDMG supplementation *($P < 0.05$)*. None of the treatments affected ADFI of the broilers. In this experiment no interactions between energy/protein level and NaDMG supplementation were observed. Mortality was numerically lower for the NaDMG supplemented groups (-/- 1.8%). Litter quality and foot pad lesion scores were not affected by one of the treatments.

Keywords: broiler, energy, lysine, dimethylglycine, NaDMG, litter quality, foot pad

Introduction

Dimethylglycine (DMG) is a tertiary amino acid involved in a variety of biological processes, because it is an intermediary metabolite in the cellular metabolism of choline and betaine. DMG can be uses as a source of glycine for glutathionine synthesis (FRIESEN et al., 2007). Besides, DMG has beneficial effects on nutrient digestibility probably due to surfactant properties of DMG esters (CLAPÉS and INFANTE, 2002). KALMAR et al. (2010) reported a significant improvement of apparent digestibility of both carbohydrates and protein when a control diet was supplemented with 167 ppm *N,N*-dimethylglycine sodium salt (NaDMG). In the same study, the authors also reported that the antioxidant properties of NaDMG could attenuate the progression towards pulmonary hypertension. The positive effects of NaDMG (1000 ppm) on nutrient digestibility have been confirmed by PROLA et al. (2013).

The incidence of food pad lesions is one of the main concerns in the broiler industry. One of the main risk factors is wet litter. Besides an increase in the incidence of food pad lesions also bird performance is frequently impaired by wet litter. It is well published that a surplus of (indigestible) protein either or not in combination with an increased intestinal viscosity, is harmful for the intestinal health of broiler chicks. There is a clear relationship between protein digestibility and litter quality (VELDKAMP, 2010).

The main objective of the present trial was to investigate the effect of NaDMG on the performance of broiler fed diets with varying energy and protein contents. In addition, litter quality and foot pad lesions were monitored.

Material and methods

A broiler experiment was conducted to evaluate NaDMG (Taminizer® D, Eastman) in diets differing in energy and protein content. One-day old male broiler chicks (Ross 308) were divided in 6 groups (treatments) of 5 replicates (0.8 m^2 floor pens). The birds were housed in the poultry experimental facility of Zootechnical Centre (Catholic University of Leuven, Belgium). For the present trial, 30 pens were used. Birds were housed at a high density (15 broilers per pen). The floor was covered with fresh wood shavings; no additional filling or cleaning was executed during the trial.

A 3-phase feeding scheme was applied with a starter period of 14 days followed by a grower diet between 15 and 28 days of age and a finisher diet between 29 and 38 days of age. All diets were prepared in the feed plant of Research Diet Services (Wijk-bij-Duurstede, the Netherlands) and delivered in bags to ZTC. Feed was provided *ad libitum* to the birds as a crumble (starter phase) or small pellets (grower and finisher phase). The trial was set up as a 3×2 factorial design with 3 diets differing in protein and energy content and 2 levels of NaDMG supplementation (0 or 1 g/kg NaDMG in the starter, 0 or 0.5 g/kg in the grower, and 0 or 0.3 g/kg in the finisher diet). Birds were fed one of the following treatments: (I) a wheat-soybean meal-corn-rapeseed meal based control diet (starter: 12.0 MJ/kg $AME_{broiler}$, 20.5% CP and 1.10% dig. Lys; grower: 12.45 MJ/kg $AME_{broiler}$, 19.5% CP and 1.03% dig. Lys; finisher: 12.66 MJ/kg $AME_{broiler}$, 19.0% CP and 0.96% dig. Lys); (II) as (I) with 2% lower levels of CP and digestible amino acids; (III) as (I) with 2% lower $AME_{broiler}$ and 4% lower digestible amino acids and retainable P; (IV) as (I) with NaDMG; (V) as (II) with NaDMG; (VI) as (III) with NaDMG. All experimental diets contained NSP-enzymes, whereas no phytase and coccidiostats were included.

Average pen weight was recorded at 1, 14 and 38 days of age. Feed intake was recorded in the respective periods: 1-14 and 14-38 days. Feed conversion ratios (FCR), daily growth rate (ADG), bird-days and daily feed intake (ADFI) per bird were calculated for 1-14, and 14-38 days and also for the cumulative period 1-38 days. Litter quality was scored visually at day 21, 28 and 38 on a scale 0 to 3. A score 3 means very wet litter with a light (orange brown) colour; a score 0 implies very dry litter with a dark colour. On day 38 the foot pads of the present broilers per pen were scored (FPD scores). The scoring classes were on a scale of 0 (no or very mild lesions) to 3 (very severe lesions). All performance parameters were subjected to the analysis of variance (GLM procedure in SPSS).

Results

The experiment was successfully conducted without any problems. Overall mortality was 4.0%. Both in the starter period (day 1-14) and the whole period (day 1-38) ADG was significantly affected by energy and protein level ($P < 0.05$), but not by NaDMG supplementation. FCR was in the starter period affected by both energy-protein level and NaDMG supplementation ($P < 0.05$). NaDMG supplementation significantly improved FCR ($P < 0.05$) in the whole period (-/- 0.04). Final body weight of the broilers fed NaDMG was numerically higher (+ 30 g). None of the treatments showed an effect on ADFI in the starter period and the whole period. The adjusted energy-protein levels and NaDMG supplementation did not improve litter scores and FPD scores. In this experiment, no interactions between energy-protein levels and NaDMG supplementation on the response parameters have been observed. Probably, it was not possible to prove significant interactions in this trial because of the very good experimental conditions and the limited amount of replicates. A summary of the main treatment effects on broiler performance and FPD score is represented in Table 1.

Table 1: Bird performance and FPD score (means ± SD) as affected by dietary treatment (day 0-38): main effects

	AME/CP level			NaDMG		Effect of:		
	control	low AA	low AME/ low AA	no	yes	AME/ CP	NaDMG	AME/ CP x NaDMG
BW day 38 (g)	2765a (± 97)	2701ab (± 99)	2625b (± 146)	2682 (± 138)	2712 (± 117)	*P < 0.05*	*NS*	*NS*
ADG (g/d)	71.6a (± 2.6)	69.9ab (± 2.6)	67.9b (± 3.8)	69.4 (± 3.6)	70.2 (± 3.0)	*P < 0.05*	*NS*	*NS*
ADFI (g/d)	111.5 (± 4.8)	109.3 (± 5.3)	107.9 (± 4.6)	110.4 (± 4.8)	108.8 (± 5.1)	*NS*	*NS*	*NS*
FCR	1.56 (± 0.03)	1.56 (± 0.04)	1.59 (± 0.06)	1.59x (± 0.05)	1.55y (± 0.03)	*NS*	*P < 0.05*	*NS*
FCR 1500 g	1.06b (± 0.05)	1.10ab (± 0.05)	1.16a (± 0.11)	1.12x (± 0.10)	1.07y (± 0.06)	*P < 0.05*	*P < 0.05*	*NS*
Mortality (%)	4.7 (± 4.5)	5.3 (± 5.3)	2.0 (± 3.2)	4.9 (± 4.7)	3.1 (± 4.3)	*NS*	*NS*	*NS*
FPD score	0.7 (± 0.8)	0.3 (± 0.6)	0.5 (± 1.1)	0.6 (± 1.0)	0.4 (± 0.8)	*NS*	*NS*	*NS*

ab, xy: significant differences ($P < 0.05$)

Conclusions

The results of this experiment suggest that NaDMG supplementation improves broiler performance parameters of which FCR was improved significantly. Although no significant interactions have been observed, the positive effect of NaDMG on FCR seems to be most pronounced in the diets with reduced energy and/or protein levels.

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P-079 (ID 229)

EFFECTS OF ß-MANNANASE IN BROILERS ON LOW ENERGY DIETS

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Abstract

ß-Mannans are compounds present in e.g. soybean meal, with a structure that resembles pathogens. They may thus induce an immune response, which costs energy that cannot be used for production processes anymore. Therefore, enzymes breaking down ß-mannans may have an energy sparing effect. The aim was to test if dietary supplementation with ß-mannanase (Hemicell) could improve broiler performance in a practical-scale experiment. Four treatments were tested with eight replicate pens (820 Ross broilers per pen). For the starter, grower, and finisher phase a positive control (PC) with a standard energy level and a low energy control (LE = PC minus 120 kcal/kg AMEn) were formulated. One half of the LE diet was supplemented with ß-mannanase. Also a medium energy diet (ME = PC minus 60 kcal/kg AMEn) with ß-mannanase was formulated. The diets were maize/wheat/soybean meal based. The enzyme was dosed at 110 mL per tonne of feed. Diets were pelleted and freely available. Results from 0-38 days were analyzed with ANOVA. All treatments with reduced AMEn had a significantly higher feed intake than PC. Addition of ß-mannanase to LE significantly improved body weight gain (81 g) and FCR (3.5 points). The supplemented LE treatment had a somewhat lower performance than PC, whereas the supplemented ME treatment performed similar to PC. Conclusion: dietary supplementation of ß-mannanase improved broiler performance in low energy diets, suggesting an energy sparing effect.

Keywords: poultry, enzyme, ß-mannanase, energy, production performance

Introduction

ß-Mannans are fibers present in e.g. soybean meal, sunflower meal, palm kernel cake, guar meal, and rapeseed meal. Mannose sugar molecules, the main component of β-mannans are Pathogen-Associated Molecular Patterns (PAMPs). ß-Mannans can thus be mistaken for invading pathogens and elicit an immune response. Energy needed for immune responses cannot be used for production processes. Therefore, enzymes breaking down ß-mannans may have an energy-sparing effect. The aim of this experiment was to test if supplementation of ß-mannanase (Hemicell®) to low energy diets could improve broiler performance under practical-scale conditions.

Material and Methods

The experiment had a randomized complete block design with four treatments and eight replicate pens per treatment. Each pen had a surface area of 47.5 m^2 with fresh wood shavings, and contained 820 broilers (Ross 308, straight run). The trial had a starter phase from D0-10; a grower I phase from D10-21; a grower II phase from D21-31; and a finisher phase from D31-38.

The diets were maize/wheat/soybean meal based. For each feeding phase a positive control (PC) with a standard energy level and a low energy control (LE = PC minus 120 kcal/kg AMEn) were formulated. One half of the LE diet was supplemented with ß-mannanase. Also a medium energy diet (ME = PC minus 60 kcal/kg AMEn) with ß-mannanase was formulated. The treatments are summarized in Table 1.

Table 1. Energy content (kcal/kg) of the diets in each feeding phase

Treatment	Description	Starter	Grower I	Grower II	Finisher
		D0-10	D10-21	D21-31	D31-38
1	Positive control (PC)	2820	2850	2900	2950
2	Low energy (LE)	2700	2730	2780	2830
3	LE + ß-mannanase	2700	2730	2780	2830
4	ME+ ß-mannanase	2760	2790	2840	2890

ß-Mannanase was dosed at 110 mL per tonne of feed in a post-pelleting application. All diets contained xylanase and phytase, and were formulated to have the same amino acid content within a feeding phase. The starter diets were fed as crumbles, whereas the grower and finisher diets were fed as pellets.

Body weight, feed intake, and mortality were recorded per pen on a daily basis throughout the experimental period. Body weight gain and FCR were corrected for mortality in each feeding phase. The European Poultry Efficiency Factor (EPEF) was based on overall production figures and calculated as [live weight (kg) * survival (%)] / [age * FCR] * 100. The data were analyzed with ANOVA, using Genstat statistical software. Values with P≤0.05 were considered statistically significant. Statistics were based on two-sided tests. Treatment means were compared by least significant differences (LSD) after confirmation of significant treatment effects.

Results

The birds performed well during the experiment, especially given the low dietary energy levels. Mean final body weight on day 38 was 2,356 g. The performance results are presented in Table 2. Reduction of AME by 120 kcal/kg and 60 kcal/kg significantly increased feed intake compared to PC. The addition of ß-mannanase to LE did not significantly affect feed intake compared to the non-supplemented LE treatment. Broilers eat to their nutritional requirements, and when dietary energy is reduced, feed intake will increase in order to meet energy requirements. If ß-mannanase would have a direct energy-releasing mode of action, then the inclusion of the enzyme in the low energy treatment would be expected to reduce feed intake compared to the low energy treatment without ß-mannanase. However, this experiment showed that this was not the case: feed intake in the low energy diet was numerically higher with ß-mannanase than without. This indicates that the mode of action of the ß-mannanase was not attributable to a direct energy-releasing effect, which supports the hypothesis of an energy-sparing effect due to lower costs spent on an immune response. However, actual effects on immune parameters were not measured.

The overall weight gain in LE was numerically lower than PC. The weight gain in the ß-mannanase supplemented treatments were numerically higher than PC without being statistically different. Adding ß-mannanase to the LE diet significantly increased body weight gain compared to the non-supplemented LE control.

The feed conversion ratio was significantly lower in the PC and the β-mannanase supplemented ME diet than in both LE diets. However, adding ß-mannanase to the LE diet significantly improved FCR compared to the non-supplemented LE diet.

To account for differences in overall body weight gain, FCR was corrected to a standard body weight gain of 2,250 g, assuming 0.03 points FCR for each 100 g of body weight gain ($FCR_{2250} = FCR_{D0-38} – 0.0003*(\text{body weight gain}_{D0-38} – 250\text{ g})$). The results show that FCR_{2250} was significantly higher in the non-supplemented LE treatment than in the other treatments, which were not significantly different from each other.

Table 2. Body weight gain (g), feed intake (g), feed conversion ratio (FCR; g/g), feed conversion ratio corrected to 2250 g body weight gain (FCR_{2250}), and mortality (%) in broilers from 0 to 38 days of age, as affected by treatment. PC = positive control; LE = low energy; LEH = low energy + Hemicell; MEH = medium energy + Hemicell.

Treatment	**1**		**2**		**3**		**4**				
Description	**PC**		**LE**		**LEH**		**MEH**		**LSD**		**P**
Body weight gain	2308 [ab]		2278 [a]		2359 [b]		2344 [b]		56.0		0.03
Feed intake	3690 [a]		3822 [bc]		3875 [c]		3788 [b]		60.9		< 0.001
FCR	1.599 [a]		1.678 [c]		1.643 [b]		1.616 [a]		0.0216		< 0.001
FCR_{2250}	1.581 [a]		1.670 [b]		1.610 [a]		1.588 [a]		0.0361		< 0.001
Mortality	2.74		3.20		2.41		2.71		0.619		0.10

[a,b,c] Values without a common superscript within a row differ significantly (P≤ 0.05).

The EPEF results are presented in Figure 1. It shows that the non-supplemented LE treatment had a significantly lower EPEF than the other treatments. Supplementation of ß-mannanase in the LE diet significantly improved EPEF over the non-supplemented LE diet, resulting in similar EPEF as in PC.

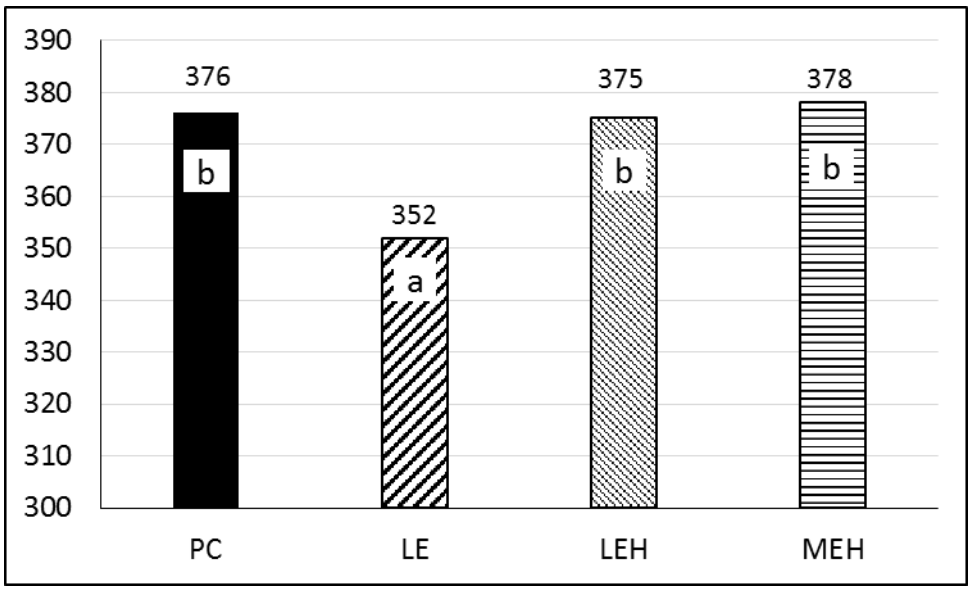

Figure 1. European Poultry Efficiency Factor as affected by treatment. PC = positive control; LE = low energy; LEH = low energy + Hemicell; MEH = medium energy + Hemicell

Conclusion

It is concluded that dietary supplementation of ß-mannanase in a low energy diet (120 kcal/kg below a standard energy level) significantly improved broiler performance over the same low energy diet without ß-mannanase, which suggests an energy sparing effect of the enzyme. Supplementation of ß-mannanase in a diet with 60 kcal/kg lower energy content resulted in similar performance as a non-supplemented positive control with a standard energy level.

P-080 (ID 1)

THE EFFECT ON BROILER PERFORMANCE OF A CORN AND SOYBEAN MEAL DIET SUPPLEMENTATION WITH A SYNERGISTIC BUTYRATE BASED PRODUCT

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Abstract

The objective of this trial was to determine the effect on performance of a synergistic butyrate based supplementation of a corn and soybean meal broiler diet. A trial was carried out at the Experimental Broiler House of Impextraco Latin America, Brazil. Eight hundred day-old male Cobb® broilers were divided into 2 treatment groups, randomly distributed into 20 pens. Three high standard corn and soybean meal based diets were formulated according to bird's age. Treatments consisted of a negative control group (C) with standard feed and a group supplemented with a product based on coated calcium butyrate, calcium lactate and natural extracts (B) incorporated at 750g/ton in starter period and 500g/ton in grower and finisher period. Body weight (BW) and feed consumption were controlled weekly to provide data on feed conversion rate (FCR). A multivariate analysis of variance (MANOVA) was performed. At the end of the trial, the synergistic butyrate based supplementation of broiler feed improved FCR significantly and final body weight numerically. It may be concluded that, feeding a synergistic calcium butyrate based product on top of a gut health promoting corn based diet, still had beneficial effects on performance results of broilers.

Keywords: butyrate, broiler, performance

Introduction

Butyrate is a natural substance present in mammals, with a biological role, as it is naturally produced by the gastrointestinal microbiota. Butyrate is the main energy source of colonocytes (Roediger, 1982). In physiological conditions, the positive effect on performance could be explained by the increased nutrient digestibility, the stimulation of the digestive enzyme secretions (Harada et al., 1983), the modification of intestinal microbiota (Van der Wielen et al., 2000), the improvement of the epithelial integrity (Sakata et al., 1983) and defense systems (Segain et. Al., 1999). Butyrate has also been implicated in down-regulation of bacterial virulence, both by direct effects on gene expression (Gantois et al., 2006) and by acting on cell proliferation of the host cells. In animal production, butyrate is a helpful feed additive, as it enhances performance (Hautekiet et al., 2011) and controls gut health disorders caused by bacterial pathogens.

Material and Methods

The present trial was carried out at the Experimental Broiler House of Impextraco Latin America, located in Irati, Brazil. Eight hundred day-old male Cobb® broiler chicks, vaccinated against Infectious Bronchitis and Marek Disease at hatch, were individually weighed and divided into 2 groups, composed by 10 replicates of 40 animals each, and randomly distributed into pens (2 x 2m) covered with wood shavings. Two corn and soybean meal based diets were divided into three phases according to birds' age: starter (1 to 21 days of age), grower (22 to 35 days of age) and finisher feed (36 to 42 days of age). Treatments consisted of a negative control group (C) with standard feed and a group supplemented with a product based on coated calcium butyrate, calcium lactate and natural extracts (B) (BUTIFOUR® F) incorporated at 750g/ton in starter period and 500g/ton in grower and finisher period. Ad libitum access to water and feed was provided throughout the entire experiment, according to the CCAC guidelines (1993). Body weight (BW) and feed consumption were controlled weekly to provide data on feed conversion ratio (FCR). Weight of all dead animals was registered in order to correct FCR. The variables average FCR and average BW were analyzed using the software JPM® (SAS Institute Inc.). A multivariate analysis of variance (MANOVA) was performed, considering $p \leq 0,05$.

Table 1. Composition of feeds

	FEED		
INGREDIENTS (%)	**Starter**	**Grower**	**Finisher**
Corn	57,3383	59,4107	61,7346
Soybean meal	36,3133	33,7422	31,1283
Soybean oil	2,3821	3,0860	3,7061
Limestone	1,3824	1,3774	1,1974
Dicalciumphosphate	1,6541	1,4622	1,3216
Salt	0,4399	0,4421	0,4442
L-Lysine	0,0390	0,0493	0,0514
DL-Methionine	0,2352	0,2301	0,2096
Threonine	0,0157	0,000	0,0068
Mineral Premix	0,1000	0,1000	0,1000
Vitamin Premix	0,1000	0,1000	0,1000

Results

During this trial almost no significant effects of B on body weight could be detected. Due to an unknown reason, the product negatively influenced body weight on day 21. The impact on body weight was only temporary because at 42 days body weight was even numerically improved by B comparing to C.

Table 2. Body weight (mean ± standard deviation) (Prob>F: < 0,05 indicates significance)

	Body Weight (Kg)					
	7 days	14 days	21 days	28 days	35 days	42 days
C	0,175±0,006	0,394±0,016	0,826±0,034	1,487±0,057	2,200±0,066	2,964±0,077
B	0,169±0,012	0,383±0,031	0,812±0,043	1,466±0,062	2,205±0,089	3,046±0,246
Prob>F	0,1284	0,0623	0,0303*	0,0747	0,3494	0,197

Feed conversion rate was significantly decreased by B starting from day 21 of evaluation, indicating that animals fed with B had improved intestinal digestive and absorptive capacities.

Table 3. Feed conversion ratio (mean±standard deviation) (Prob>F:<0,05 indicates significance)

	Feed Conversion Ratio					
	7 days	14 days	21 days	28 days	35 days	42 days
C	0,872±0,039	1,359±0,080	1,461±0,085	1,499±0,055	1,585±0,041	1,690±0,028
B	0,889±0,049	1,341±0,122	1,42±0,063	1,471±0,04	1,557±0,04	1,628±0,084
Prob>F	0,5006	0,189	0,0465*	0,0298*	0,0498*	0,0045*

Conclusions

Findings from this trial reveal that the inclusion of a synergistic butyrate based product affects FCR significantly and body weight numerically. Animals that received a corn and soybean meal based diet supplemented with B, showed significant improvement in FCR starting from day 21 of the trial. Teirlinck et al. (2009) documented the protective effect of corn based diets on intestinal morphology and gut health comparing to wheat/rye based diets. One could expect it would be difficult to show any significant improvement by the addition of a gut health improving butyrate based product to this corn and soybean meal based diet. However this study has proven that a butyrate based product can significantly improve FCR. It may be concluded that, feeding a synergistic calcium butyrate based product on top of a gut health promoting corn based diet, still had beneficial effects on performance results of broilers.

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P-081 (ID 2)

IMPACT OF AN AFLATOXIN CONTAMINATED FEED AND A MYCOTOXIN ELIMINATOR (ELITOX®) ON CIRCULATING IMMUNE CELLS AND SERUM ALBUMIN IN BROILERS

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Abstract

The aim of the trial was to determine the impact of an early aflatoxicosis on serum albumin and circulating leucocytes and the protective effect of a mycotoxin eliminator. An *in vivo* trial was performed at UFPR, Brazil. Seventy two male day old Cobb® broilers were housed up to 21 days and divided into 3 treatments (T) (n=24): a negative control diet (C), a contaminated feed (500ppb of purified Aflatoxin B1 – AFB1) (AFLA), and contaminated feed supplemented with 0,2% of a mycotoxin eliminator (ME) (E-AFLA). At day 10 and 21, blood samples were collected (n=10/T) to quantify the amount of circulating activated cytotoxic T-cells, suppressor macrophages and B-cells through flow cytometry and to evaluate liver protein synthesis by quantifying serum albumin. Contamination with AFB1 led to a significant increase in activated cytotoxic T-cells at 10 days of age and a numerical increase in circulating suppressor macrophages comparing to C. The use of a ME reduced the amount of such cells down to the level of C. At d21, contamination with AFB1 resulted in numerically fewer B cells. The addition of ME again counteracted this effect. Serum albumin levels were significantly reduced in the AFLA group compared to the C group and brought back to control levels due to the inclusion of ME. The inclusion of a ME in broiler feed prevented early immunological impairment and liver damage induced by AFB1.

Keywords: Aflatoxin, Mycotoxin eliminator, Immune cells, Albumin, Liver

Introduction

Aflatoxin is a secondary metabolite produced by mold species, predominantly *Aspergillus flavus* and *Aspergillus parasiticus* (Do, 2007), being reported by many authors as carcinogenic, mutagenic, teratogenic and growth inhibiting (Ortatatli, 2005). Animals intoxicated with aflatoxins present anorexia, decrease in growth rate, weight gain, egg production and weight, as well as increased mortality rate (Leeson et al., 1995). Macroscopic and microscopic alterations in liver (Espada et al., 1992), kidneys and spleen (Glahn et al.,1991) and humoral and cellular immunity impairment may also be observed (Oguz et al., 2003).

Material and Methods

The trial was conducted at the Federal University of Paraná, Curitiba, Brazil. The experimental unit consisted of three 2m^2 isolators covered with wood shavings, into a negative pressure ventilation system. Seventy two male day old Cobb® broiler chickens vaccinated against Infectious Bronchitis at hatch were used. Trial duration was 21 days. Ad libitum access to water and feed was provided throughout the entire experiment, according to the CCAC guidelines (1993). On day 1, all animals were individually weighed, identified with a wing tag, and equally distributed according to body weight into groups providing 3 treatments(T) (n=24): a control group (C), a contaminated feed (500ppb of purified Aflatoxin B1 –AFB1) (AFLA), and a contaminated diet supplemented with 0,2% of mycotoxin eliminator (ME) (ELITOX®) (E-AFLA). On days 10 and 21, blood samples of 10 animals from each treatment were collected, to quantify different lymphoid cells (Imunova Biological Analysis Company) by flow cytometry and to evaluate the hepatic function (at the Veterinary Clinical Pathology Laboratory from the Federal University of Paraná). Serum was extracted by centrifugation to evaluate albumin content. Statistics were performed through ANOVA followed by the Tukey-Kramer Post Hoc Test, both considering $p \leq 0,05$.

Results

Albumin, an important protein produced in liver, was decreased by both E-AFLA and AFLA on day 10. However, on day 21, ME returned albumin levels close to the control, which indicates some protective effect of ME on this variable.

Table 1. Mean albumin levels. Same letters within the same column indicate similar means.

Treatment	Albumin	
	D10	D21
C	1.35±0.14a	1.54±0.2a
AFLA	1.13±0.12b	1.35±0.12b
E-AFLA	1.1±0.14b	1.46±0.15ab
p>F	0.02*	0.0109*

Flow cytometry determinations showed significant differences for several immune cells. Activated cytotoxic T-lymphocytes were significantly impaired due to AFB1 contamination. AFB1 induced an initial increase and a further decrease, comparing to C and E-AFLA. Activated cytotoxic T-lymphocytes are a subset of T-cells that already migrated to the secondary lymphoid organs, where they get in contact with antigens. Such process occurs naturally as birds grow and are exposed to antigens. An initial higher number of these cells in the AFLA treatment may indicate an increased earlier need for cell activation and a later suppressive effect caused by the toxin, which may compromise birds' response against challenges. This negative effect was counteracted by ME.

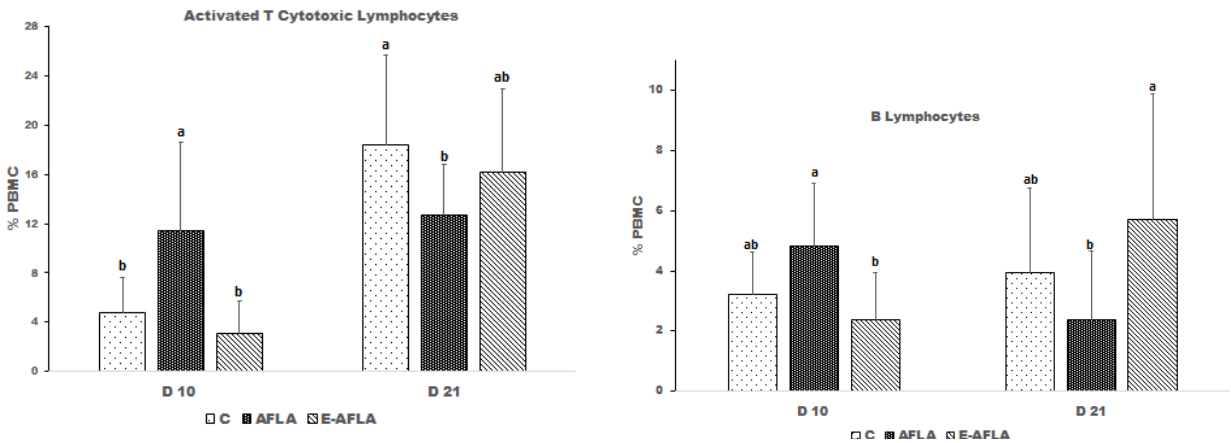

Fig. 1. Mean percentage of Activated Cytotoxic T-Lymphocytes related to Peripheral Blood Mononuclear Cells (PBMC) and mean percentage of B-lymphocytes. Same letters within the same points of evaluation indicate

The amount of B-lymphocytes was numerically increased when comparing AFLA to C and significantly increased when comparing the former to E-AFLA. However, after 21 days of exposure, such pattern shifted to a significant reduction of B-lymphocytes in AFLA, when compared to E-AFLA and a numerical reduction when compared to C. B-lymphocytes are cells responsible for antibody production. Hence, later reductions in this cell subset may reflect an impairment in humoral immune response.

Exposure to AFB1 led to a numerical increase of circulating suppressor phagocytic monocytes (termed here as suppressor macrophages) in untreated birds after 10 and 21 days of exposure, while both C and E-AFLA showed numerical similar number of cells.

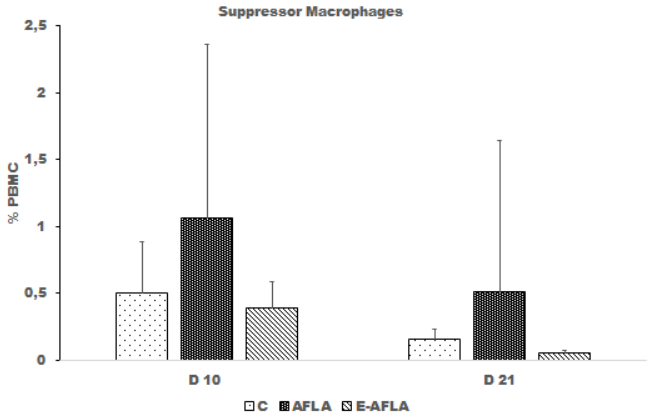

Fig. 2 Mean percentage of Suppressor Phagocytic Monocytes (Macrophages).

Conclusions

Findings of this trial could confirm Aflatoxin B1 at 500ppb significantly impaired liver function, as viewed through reduced albumin levels, and the immune system of animals. These negative effects could be counteracted by the use of a ME and thus prevent liver damage and early immunological impairment induced by AFB1.

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P-082 (ID 211)

EFFECT OF FEEDING POLYPHENOLS ON *CAMPYLOBACTER* COUNTS IN BROILERS

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Abstract

An experiment was conducted in order to evaluate the effect of a blend of polyphenols (PPh; 1% hidroxibenzoic acids) added to the feed on cecal *Campylobacter* counts in broilers. There were two treatments applied from 1 to 42 d of age, T1: Positive controls (*Campylobacter*, no additives) and T2: T1 + PPh at 2 kg/t from 0-21 d and 4 kg/t from 21-42d. A total of 84 one-day-old Ross 308 broilers (half male and half female) were divided into the experimental treatments. At 14 d of age, all broilers were orally gavaged with 100 µl of a solution containing 1 x 10^5 CFU/ml of ST-45 *C. jejuni* strain. On days 21, 35 and 42, ceca from 12 birds per treatment were collected and *Campylobacter* counts determined (ISO 10272). Data expressed as $\log_{10}$ CFU/g ceca content were first tested for normality and then analysed by the non-parametric test of Kruskall-Wallis (SPSS v.19.0). No significant differences in the *Campylobacter* counts were observed between treatments at 21d (7.83 *vs* 7.43 $\log_{10}$ CFU/g, for T1 *vs* T2; P = 0.85) or 42 d of age (6.77 *vs* 6.09 $\log_{10}$ CFU/g, for T1 *vs* T2; P = 0.14). However, *Campylobacter* counts tended to be reduced by PPh supplementation at 35 d of age (5.99 *vs* 7.12 $\log_{10}$ CFU/g; P = 0.07). It is concluded that supplementation of broiler diets with a blend of polyphenols might reduce *Campylobacter* infection in broilers.

Keywords: polyphenols, *Campylobacter jejuni*, broiler

Introduction

Campylobacteriosis is a foodborne disease associated with the infection of humans by thermophilic *Campylobacter spp*., mainly *C. jejuni* and *C. coli* (LASTOVICA AND SKIRROW, 2000). *Campylobacter* is the major cause of bacterial gastroenteritis in humans worldwide. In the EU, it is the most commonly reported gastrointestinal bacterial pathogen since 2005, with 214,779 human cases reported in 2013 (EFSA, 2015). *Campylobacter* preventive measures aim at reducing the probability of *Campylobacter* colonisation in birds (GHAREEB et al., 2013). For this purpose several approaches, as the use of bacteriophages (WAGENAAR et al., 2005), bacteriocins (LINE et al., 2008), organic acids (CHAVEERACH et al., 2004; VAN DEUN et al., 2008; SKÅNSENG et al., 2010) and their derivatives (HILMARSSON et al., 2006) or medium chain fatty acids (HERMANS et al., 2010) have been proposed over the past few years. Also phytochemicals could be considered as an alternative. These substances have low molecular weight and they are derived from the plant secondary metabolism and include glucosides, alkaloids, essential oils, saponins, mucilage, phenolic compounds, and flavonoids (MARTINS et al., 2000). Grape seeds and skins are good sources of phytochemicals such as gallic acid, catechin, and epicatechin and are suitable raw materials for the production of antioxidative dietary supplements. Flavonoids, as catechin and epicatechin, have been shown to be able to affect various biological functions: capillary permeability, cellular secretary processes involved in the inflammatory response and inhibition of enzymes, receptors and carriers (RHAMA and MADHAVAN, 2011). Thousands of phytochemicals, including phenols, have already been identified to be inhibitory towards microorganisms (COWAN, 1999). In fact, RHAMA and MADHAVAN (2011) proved that several flavonoids had even more *in vitro* antibacterial activity than tetracycline. Previous studies carried out by GANAN et al. (2009) also found that some phenolic compounds present in wine and grape residues significantly reduced *in vitro* the viability of *Campylobacter*. However, their ability to reduce *Campylobacter* has not been investigated *in vivo* and differences between *in vitro* and *in vivo* trials are not fully understood. Therefore, the objective of this study was to evaluate the effect of a blend of polyphenols (PPh; 1% hidroxibenzoic acids) added to the feed on cecal *Campylobacter* counts in broilers.

Material and Methods

A total of 84 Ross 308 broilers (50% male and 50% female) from 1 to 42 days of age were used and allocated to the experimental treatments. There were two treatments applied from 1 to 42 d of age, T1: Positive controls (*Campylobacter*, no additives) and T2: T1 + PPh at 2 kg/t from 0-21 d and 4 kg/t from 21-42d. The trial was carried out in an Animal Biosafety Level 2 Unit for broilers. Broilers were housed in cages in groups of three. The individual bird was the experi-

mental unit. Mash (non-pelleted) feeds were fed *ad libitum* with no added coccidiostat, growth promoter or veterinary antibiotics. Diets were cereal based diets similar to those used commercially. Prior to the infection of the animals, caecal samples of 6 birds randomly selected were collected to verify that chicks were all *Campylobacter*-free. At 14 d of age, all broilers were orally gavaged with 100 µl of a solution containing 1 x 10^5 CFU/ml of ST-45 *C. jejuni* strain. On days 21, 35 and 42, ceca were collected from 12 birds per treatment and *Campylobacter* counts determined (ISO 10272). Detection limit was 2 logs, and animals below limit of detection were assigned 2 logs for statistical analysis. Data expressed as $\log_{10}$ CFU/g ceca content were first tested for normality and then analysed by the nonparametric test of Kruskall-Wallis (SPSS v.19.0). Statistical significance is declared at $P \leq 0.05$, with $0.05 < P \leq 0.10$ considered as a near-significant trend.

Results

Although broilerd fed PPh always showed less *Campylobacter* counts, no significant differences in the *Campylobacter* counts were observed between treatments at 21d (7.83 *vs* 7.43 $\log_{10}$ CFU/g, for T1 *vs* T2; P = 0.85) or 42 d of age (6.77 *vs* 6.09 $\log_{10}$ CFU/g, for T1 *vs* T2; P = 0.14). However, *Campylobacter* counts tended to be reduced by PPh supplementation at 35 d of age (5.99 *vs* 7.12 $\log_{10}$ CFU/g; P = 0.07).

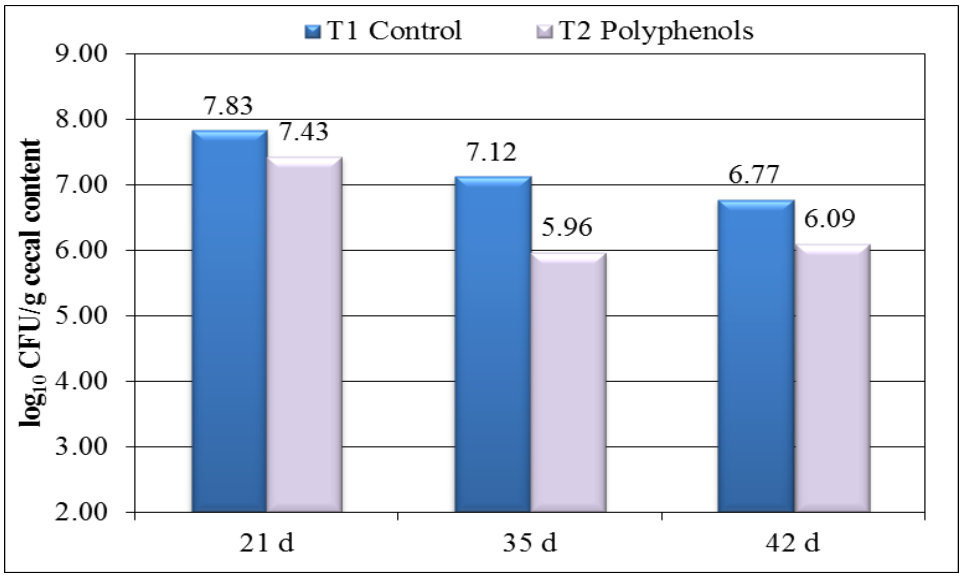

Figure 1. Effect of dietary treatment (T1: Negative Control and T2: Polyphenols) on *Campylobacter* counts (cfu/g of cecal content expressed as $\log_{10}$) at 21, 35 and 42 days.

Conclusions

It is concluded that supplementation of broiler diets with a blend of polyphenols might reduce *Campylobacter* infection in broilers.

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Feed enzymes

P-086 (ID 40)

AMINO ACIDS CONCENTRATION OF INTERNAL CONTENTS AND ELEMENTAL CONTENTS OF EGGSHELL OF QUAIL EGGS DUE TO ENZYME-SUPPLEMENTED HIGH OR LOW FIBRE DIETS

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Abstract

Three hundred and sixty 6 weeks old laying quails were utilized in an 8 week experiment to evaluate the effects of Maxigrain® enzyme supplementation of sugarcane scrapping meal-based diets on amino acids concentration of internal contents of quail eggs and the elemental contents of the eggshells. Six diets tagged $T10_0$, $T10_5$, $T10_{10}$, T15, $T15_5$ and $T15_{10}$ were formulated to be isonitrogenous (20%CP) and isocaloric (12.14 MJ/kg, ME). The enzyme was included at 0, 5 and 10mg/kg thus; treatments $T10_5$, $T10_{10}$, $T15_5$ and $T15_{10}$ contained 5 and 10mg/kg of the enzyme respectively. Treatments $T10_0$ functioned as the control for $T10_5$ and $T10_{10}$ while $T15_0$ was for $T15_5$ and $T15_{10}$, respectively. The birds were randomly allotted to the 6 dietary treatments at the rate of 60 birds per diet. Each treatment was replicated 4 times in a 3 x 2 factorial arrangement having 15 point-of-lay female birds per replicate. Three eggs of similar weights per replicate were randomly selected (n=72) for determination of amino acids concentration and their eggshells analyzed for mineral contents. Enzyme supplementation significantly (P<0.05) improved threonine (5.34 vs. 5.16 and 5.61mg/g), arginine (3.84 vs. 3.89 and 4.27 mg/g), methionine (2.66 vs. 2.72 and 2.86 mg/g), and phenylalanine (4.23 vs. 4.22 and 4.63 mg/g); all the acids evaluated were not influenced by the interactive effects of the enzyme and fibre. Enzyme or fibre did not influence (P>0.05) all the minerals analyzed; the interaction of the enzyme and fibre influenced all the minerals except for K which did not vary significantly (P>0.05). Birds fed the $T10_0$ produced better Ca values (11.92 and 12.75 %) compared to those fed the other diets. Same trend was recorded for Na (0.71 and 0.74 %) and Cl (0.69 and 0.73 %) concentration of the eggshells. Birds fed the $T10_0$ also produced better (P<0.05) result for P (8.75 %) than those fed the other diets. In view of the results gathered laying quails fed the high fibre-high enzyme supplemented diets will produce normal eggs.

***Keywords*:** Layer quails, sugarcane scrapping meal, enzyme, amino acids, minerals and eggshell

Introduction

Minerals are important for normal bone development, blood clot formation, muscle contraction and maintenance of good egg shell quality. They also take part in cellular membranes and are required for many metabolic functions. Some of them aid digestion as a component of hydrochloric acid (Olomu, 1979). The productivity, reproduction and health of flock greatly depend on the quality of feed they receive. Therefore, feeds should be properly formulated with appropriate requirement for particular group of animals (Adeola and Olukosi, 2008). The objective of this study is therefore; to evaluate the effect of replacing maize with sugarcane scrapping meal supplemented with exogenous enzyme on layer growth rate and layer parameters of laying quails.

Materials and Methods

Study area

The experiment was carried out at the Teaching and Research Farm of the Faculty of Agriculture, Nasarawa State University, Keffi, Shabu – Lafia Campus, Nasarawa State, Nigeria.

Sugarcane scrapping

The proximate analysis of sample of sugarcane scraping meal (Table 1) were carried using the procedure outlined by AOAC (1984) while the fibre fractions were determined by methods of Vansoest and Robertson (1985) and the values reported on a dry matter basis.

Table 1. Proximate and energy composition of sugarcane scrapping

Nutrient	CP	EE	CF	Ash	DM	NFE	[a]Energy (MJ/kg ME)
%	8.25	3.36	36.48	9.98	90.67	67.40	12.42

[a]Calculated from Pauzenga (1985)

Six experimental diets tagged $T10_0$, $T10_5$, $T10_{10}$, T15, $T15_5$ and $T15_{10}$ were compounded to be isonitrogenous (23% crude protein) and isocaloric (11.67 MJ/kg, ME) with two levels of crude fibre, 10 and 15%. The birds were randomly allocated to the treatments at rate of 60 birds per diet in an 8 week experiment. Each treatment was replicated 4 times in a 3x2 factorial having 15 birds per replicate. Treatments $T10_0$, $T10_5$, and $T10_{10}$ contained 10% crude fibre and had 0, 5 and 10mg/kg of the enzyme while treatments $T15_{0,}$ $T15_5$ and $T15_{10}$ contained 15% crude fibre level and had 0, 5 and 10mg/kg of the enzyme, respectively; such that treatments $T10_0$ and $T15_0$ served as the controls.

The birds were fed *ad-libitum* and allowed to have access to drinking water at all times. Other routine management practices were adopted as outlined by Musa *et al.* (2007). A total of 17 (2:15 male and female) sexually mature 9 weeks old quails per replicate were utilized for the experiment. Three eggs per replicate of similar weights were randomly selected (n=72) for determination of amino acids composition using the method of Speckman *et al*. (1958). The egg-shells so collected from each replicate were cleaned to be devoid of debris, oven-dried at 30°C for 18hrs to remove moisture, bulked, ground and thoroughly mixed to obtain a homogenous mixture. Samples of the mixture were analyzed for mineral according to standard methods outlined (AOAC, 1984).

Statistical analysis

Data obtained were subjected to Two Way Analysis of Variance (ANOVA) and where significant differences (P>0.05) are observed, means were separated using Duncan's Multiple Range Test as described by Steel and Torrie (1980).

Results and Discussion

Enzyme supplementation (Table 2) significantly (P<0.05) improved (5.34 vs. 5.16 and 5.61mg/g) threonine (3.84 vs. 3.89 and 4.27 mg/g), arginine (2.66 vs. 2.72 and 2.86 mg/g), methionine (2.66 vs. 2.72 and 2.86 mg/g) and phenylalanine (4.23 vs. 4.22 and 4.63 mg/g), but did not affect (P>0.05) all the minerals evaluated. This observation may be explained by the facts that availability of amino acids is affected by the presence of digestives endogenous enzyme, inhibitors, tannins and gossypol (Olomu, 1979); addition of exogenous enzyme in this study possibly increased the availability of these acids in the egg content. Dietary fibre (Table 2) reduced (P<0.05) the concentration of all amino acids except for cystine (0.42 mg/g) which remained uniform at both low and high fibre diets while mineral concentration was unaffected. The observations reported in this study agree with the earlier assertion of Olomu (1979).

All the amino acids evaluated were not influenced by the interactive effects of Maxigrain® enzyme and dietary fibre while all the minerals were reduced (P<0.05) except for K which did not vary significantly (P>0.05) (Table 3). Logically, the combination of high fibre diets with enzyme will reduce the negatives effects of fibre in the general performance of an animal (Adeola and Olukosi, 2008).

Conclusions and Recommendations

In view of the results gathered laying quails fed the high fibre-high enzyme supplemented diets will produce eggs with normal chemical composition.

Table 2. Effect of Maxigrain® enzyme or dietary fibre on amino acids concentration of internal contents of quail eggs and mineral composition of eggshell

Parameters	ENZYME TREATMENT MEANS					FIBRE TREATMENT MEANS			
	No Enzyme	100ppm Enzyme	200ppm Enzyme	SEM	LOS	Low fibre	High fibre	SEM	LOS
Amino Acids (mg/g)									
Lysine	4.90	4.87	5.11	0.13	NS	5.28 [a]	4.64 [b]	0.11	*
Histidine	2.22	2.21	2.25	0.03	NS	2.28 [a]	2.17 [b]	0.03	*
Arginine	5.34 [a]	5.16 [b]	5.61 [a]	0.08	*	5.55 [a]	5.19 [b]	0.07	*
Aspartic acid	8.21	8.02	8.49	0.13	NS	8.52 [a]	7.96 [b]	0.11	*
Threonine	3.84 [b]	3.89 [b]	4.27 [a]	0.12	*	4.33 [a]	3.66 [b]	0.10	*
Serine	3.55	3.64	3.74	0.08	NS	3.82 [a]	3.47 [b]	0.07	*
Glutamic acid	13.04	13.03	13.29	0.10	NS	13.31 [a]	12.92 [b]	0.08	*
Proline	3.29	3.17	3.52	0.10	NS	3.57 [a]	3.09 [b]	0.08	*
Glycine	6.30	6.23	6.69	0.18	NS	6.79 [a]	6.02 [b]	0.15	*
Alanine	5.21	5.30	5.46	0.11	NS	5.60 [a]	5.05 [b]	0.09	*
Cystine	0.41	0.42	0.43	0.01	NS	0.42	0.42	0.01	NS
Valine	5.07	5.05	5.45	0.15	NS	5.48 [a]	4.90 [b]	0.13	*
Methionine	2.66 [b]	2.72 [b]	2.86 [a]	0.05	*	2.85 [a]	2.64 [b]	0.04	*
Isoleucine	4.13	4.06	4.43	0.17	NS	4.47 [a]	3.94 [b]	0.14	*
Leucine	7.69	7.76	8.11	0.14	NS	8.26 [a]	7.45 [b]	0.11	*
Tyrosine	3.37	3.45	3.61	0.09	NS	3.69 [a]	3.27 [b]	0.07	*
Phenylalanine	4.23 [b]	4.22 [b]	4.63 [a]	0.12	*	4.69 [a]	4.03 [b]	0.10	*
Minerals (%)									
Calcium	12.33	4.94	5.14	0.25	NS	6.88	8.06	1.00	NS
Phosphorus	9.18	2.20	2.32	0.12	NS	4.26	4.87	1.01	NS
Potassium	1.64	0.72	0.91	0.34	NS	1.06	1.12	0.11	NS
Sodium	0.73	0.30	0.32	0.04	NS	0.44	0.46	0.06	NS
Chlorine	0.71	0.27	0.28	0.17	NS	0.40	0.44	0.06	NS

a,b- Means on the same row bearing different superscript differ significantly ($P < 0.05$), **NS**- No significant difference ($P > 0.05$), **LOS**- Level of significant difference.

Table 3. Effects of Maxigrain® enzyme and dietary fibre on amino acids concentration of internal contents of quail eggs and mineral composition of eggshell

Parameters	MAIN TREATMENT MEANS							
	$T10_0$	$T10_{100}$	$T10_{200}$	$T15_0$	$T15_{100}$	$T15_{200}$	SEM	LOS
Amino Acids (mg/g)								
Lysine	5.24	5.16	5.44	4.56	4.58	4.79	0.19	NS
Histidine	2.24	2.24	2.35	2.19	2.19	2.15	0.04	NS
Arginine	5.53	5.26	5.87	5.16	5.05	5.35	0.11	NS
Aspartic acid	8.39	8.27	8.88	8.02	7.77	8.10	0.19	NS
Threonine	4.22	4.11	4.67	3.45	3.67	3.87	0.17	NS
Serine	3.69	3.82	3.96	3.41	3.46	3.53	0.12	NS
Glutamic acid	13.20	13.13	13.62	12.88	12.94	12.96	0.14	NS
Proline	3.41	3.43	3.87	3.18	2.90	3.18	0.14	NS
Glycine	6.73	6.58	7.07	5.86	5.87	6.32	0.26	NS
Alanine	5.51	5.40	5.90	4.92	5.20	5.03	0.16	NS
Cystine	0.41	0.42	0.42	0.42	0.42	0.43	0.01	NS
Valine	5.41	5.18	5.87	4.74	4.93	5.03	0.22	NS
Methionine	2.78	2.82	2.97	2.55	2.61	2.76	0.07	NS
Isoleucine	4.51	4.12	4.78	3.75	3.99	4.08	0.25	NS
Leucine	8.19	8.04	8.56	7.20	7.48	7.68	0.20	NS
Tyrosine	3.53	3.61	3.93	3.22	3.30	3.30	0.12	NS
Phenylalanine	4.63	4.50	4.94	3.32	3.93	4.32	0.17	NS
Minerals (%)								
Calcium	6.92^a	4.27^b	4.45^b	6.75^a	5.60^b	5.83^b	0.26	*
Phosphorus	3.75^a	2.20^b	2.32^b	2.03^b	2.40^b	2.60^b	0.13	*
Potassium	1.80	0.66	0.72	1.49	0.78	1.11	0.05	NS
Sodium	0.71^a	0.30^b	0.30^b	0.74^a	0.30^b	0.35^b	0.03	*
Chlorine	0.69^a	0.27^b	0.25^b	0.73^a	0.27^b	0.32^b	0.02	*

a,b- Means on the same row bearing different superscript differ significantly (P<0.05), **NS**- No significant difference (P>0.05), **LOS**- Level of significant difference.

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P-087 (ID 39)

DRESSING PERCENTAGE, CARCASS CUTS AND PERCENT ORGAN WEIGHTS OF MALE QUAILS FED LOW OR HIGH FIBRE DIETS SUPPLEMENTED WITH MAXIGRAIN® ENZYME

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Abstract

Three hundred and sixty 3 weeks old male quails were used in an 8-week experiment to evaluate the effects of Maxigrain® enzyme supplementation of sugarcane scrapping meal-based (SCSM) diets on carcass characteristics. Six diets, $T10_0$, $T10_5$, $T10_{10}$, T15, $T15_5$ and $T15_{10}$ were formulated to be isonitrogenous (20%CP) and isocaloric (11.67 MJ/kg, ME); diets $T10_0$, $T10_5$ and $T10_{10}$ contained 10% crude fibre while $T15_0$, $T15_5$ and $T10_{10}$ contained 15% crude fibre. The enzyme was included at 0, 5 and 10mg/kg. The birds were randomly allocated to the treatments at the rate of 60 birds per diet; each treatment was replicated 4 times in a 3 x 2 factorial setting with 15 birds per replicate. Four birds per treatment were selected, fasted for 12hrs, slaughtered and their carcasses evaluated. There were no variations (P>0.05) in all the parameters measured except for percent thigh (20.31 vs. 20.41 and 22.20%) which increased (P<0.05) due to enzyme supplementation while dietary fibre decreased (P<0.05) dressed weight (93.30 and 83.70%) and dressing percentage (81.60 and 74.10%) but increased (P<0.05) weight of gizzard (5.02 vs. 6.49%). Similarly, the interaction of enzyme and dietary fibre did not affect (P>0.05) all the parameters measured. It was therefore, concluded that SCSM supplemented with Maxigrain® at 10mg/kg can be included in the diets of growing male quails without reducing the carcass quality.

***Keywords*:** male quails, sugarcane scrapping meal, enzyme, carcass cuts, dressing percentage and organ weights.

Introduction

Maize, most often, constitutes the highest proportion of ingredient in diet formulation of any poultry ration (Agbede *et al*., 2002). This high inclusion rate translates into high cost of feed because of the seasonality of maize production and competition for its use by man (Agbede *et al*., 2002). This indicates the need to replace maize in poultry diets to reduce feed cost and overall production cost.

The objective of this study is therefore, to investigate effect Maxigrain® enzyme supplementation on the carcass characteristics of male quails fed sugarcane scrapping meal-based diets.

Materials and Methods

Study area

The experiment was carried out at the Teaching and Research Farm of the Faculty of Agriculture, Nasarawa State University, Keffi, Shabu – Lafia Campus, Nasarawa State, Nigeria.

Sugarcane scrapping

Sugarcane scrapings were sourced from local sugarcane marketers within Lafia metropolis, sun-dried and milled to form the sugarcane scrapping meal.

The proximate analysis of sample of sugarcane scraping meal were carried using the procedure outlined by AOAC (2006) while the fibre fractions were determined by methods of Vansoest and Robertson (1985) and the values reported on a dry matter basis.

Description of diets and experimental design

Six experimental diets tagged $T10_0$, $T10_5$, $T10_{10}$, T15, $T15_5$ and $T15_{10}$ were compounded to be isonitrogenous (23% crude protein) and isocaloric (11.67 MJ/kg, ME) with two levels of crude fibre, 10 and 15%. The birds were randomly allocated to the treatments at rate of 60 birds per diet in an 8 week experiment. Each treatment was replicated 4 times in a 3x2 factorial having 15 birds per replicate. Treatments $T10_0$, $T10_5$, and $T10_{10}$ contained 10% crude fibre and had 0,

5 and 10mg/kg of the enzyme while treatments $T15_0$, $T15_5$ and $T15_{10}$ contained 15% crude fibre level and had 0, 5 and 10mg/kg of the enzyme, respectively; such that treatments $T10_0$ and $T15_0$ served as the controls. Table 2 shows the gross composition of the experimental diets for finisher quails.

Management of experimental birds

The birds were fed *ad-libitum* and allowed to have access to drinking water at all times. Other routine management practices were adopted as outlined by Musa *et al.* (2007).

Data collection

The birds were slaughtered by severing the carotid arteries and jugular veins and blood drained under gravity. Carcass data were collected from four randomly selected birds per treatment group. The relative weights were determined using the formula (Abdulmojeed *et al.,* 2010):

$$\text{Relative weight (cut part or organ)} = \frac{\text{Fresh weight of cut part or organ}}{\text{Final live body weight of bird}} \times 100$$

Statistical analysis

Data obtained were subjected to Two Ways Analysis of Variance (ANOVA). The separation of means was effected using Least Significant Difference (LSD) method and tested at probability level of 5% as described by Steel and Torrie (1980).

Results and Discussion

The calculated metabolizable energy of SCSM was about 11.67 MJ/kg, ME (Table 1). It contains low (8.25%) crude protein, high crude fibre (36.48%) and low (3.36%) either extract. The dry matter was about 90.67% while ash and nitrogen free extract were about 9.98 and 67.40% respectively. This composition makes SCSM a fibrous material which will require some level of processing or pre-digestion such as milling and or enzyme supplementation if it must be fed to monogastric animals.

Carcass characteristics of male quails

There were no variations (P>0.05) in the fasted live weight, dressing percentage, dressed weight, head, legs, visceral organs and wings except for thigh which was improved (P<0.05) by the supplementation of enzyme (Table 2). These observations were in contrast to that of Esuga *et al*. (2008) who observed a non-significant variation in percent thigh when they supplemented Maxigrain® enzyme in the diets of broiler chickens. There was significant reduction (P<0.05) in the percent dressed weight and dressing percentage due to dietary fibre. Similarly, all the carcass parameters measured (Table 3) did not vary (P>0.05) due to the interactive effects of enzyme supplementation and dietary fibre. The non-significant variation was in consonance with the earlier report (Alu *et al*., 2010).

Table 1. Proximate and energy composition of sugarcane scrapping

Nutrient	CP	EE	CF	Ash	DM	NFE	[a]Energy (MJ/kg ME)
%	8.25	3.36	36.48	9.98	90.67	67.40	12.17

Calculated from Pauzenga (1985).

Conclusion and recommendation

Based on the findings of this experiment, supplementing high level (15%) of sugarcane scrapping meal-based diets with Maxigrain® enzyme at 5mg/kg is safe and economical and is recommended for inclusion in the diets of finisher quails.

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Table 2. Effect of Maxigrain® enzyme supplementation or dietary fibre on carcass characteristics of male quails

Parameters	**ENZYME TMENT MEANS**					**FIBRE TREATMENT MEANS**			
	No Enzyme	**100ppm Enzyme**	**200ppm Enzyme**	**SEM**	**LOS**	**Low fibre**	**High fibre**	**SEM**	**LOS**
Cut parts									
Fasted weight (g/bird)	112.50	121.20	110.00	5.82	NS	115.00	114.20	4.75	NS
Dressed weight (g/bird)	85.00	91.00	89.50	2.60	NS	93.30 a	83.70b	2.12	*
Dressing percentage (%)	75.70	76.20	81.60	2.89	NS	81.60 a	74.10 b	2.36	*
Thigh (%)	20.31b	20.41b	22.20a	0.51	*	21.08	20.86	0.42	NS
Head (%)	7.13	6.71	6.94	0.20	NS	6.75	7.10	0.16	NS
Legs (%)	2.74	2.47	2.48	0.13	NS	2.52	2.61	0.10	NS
Wings (%)	10.15	9.15	9.73	0.40	NS	9.62	9.74	0.03	NS
Drumstick (%)	25.23	24.87	24.22	0.34	NS	25.34	25.67	0.04	NS
Breastplate (%)	38.55	38.90	37.23	0.44	NS	38.21	36.78	0.03	NS
Visceral organs (%)									
Heart (%)	1.13	1.06	1.13	0.06	NS	1.18	1.03	0.05	NS
Testes (%)	3.70	3.26	3.48	0.14	NS	3.30	3.65	0.12	NS
Gastrointestinal tract (%)	4.41	4.26	5.45	0.53	NS	4.65	4.76	0.43	NS
Liver (%)	2.06	1.84	2.04	0.16	NS	1.90	2.06	0.13	NS
Gizzard (%)	5.78	5.57	5.90	0.42	NS	5.02 b	6.49 a	0.35	*

a, b- Means on the same row bearing different superscript differ significantly (P < 0.05), **NS**- No significant difference (P > 0.05), **LOS**- Level of significance, **SEM-**Standard error of mean.

Table 3. Interactive effects of Maxigrain® enzyme supplementation and dietary fibre on carcass characteristics of male quails

Parameters	10			15				
	T_0	T_{100}	T_{200}	T_0	T_{100}	T_{200}	SEM	LOS
Cut parts								
Fasted weight (g/bird)	115.00	120.00	110.00	110.00	122.50	110.00	8.23	NS
Dressed weight (g/bird)	90.00	96.00	94.00	80.00	86.00	85.00	3.69	NS
Dressing percentage (%)	78.40	80.20	86.10	73.00	72.10	77.20	4.09	NS
Thigh (%)	22.30	20.42	20.52	22.09	20.40	20.10	0.73	NS
Head (%)	6.91	6.48	6.86	7.36	6.95	7.01	0.28	NS
Legs (%)	2.56	2.29	2.70	2.93	2.64	2.26	0.18	NS
Wings (%)	9.92	9.25	9.69	10.39	9.04	9.78	0.57	NS
Drumstick (%)	25.01	25.11	24.82	25.22	25.00	25.32	0.05	NS
Breastplate (%)	40.11	39.22	39.09	38.45	39.90	39.51	0.09	NS
Visceral organs (%)								
Heart (%)	1.10	1.24	1.20	1.16	0.89	1.06	0.08	NS
Testes (%)	3.49	3.18	3.25	3.91	3.34	3.72	0.20	NS
Gastrointestinal tract (%)	4.92	3.38	5.65	3.91	5.14	5.24	0.75	NS
Liver (%)	1.65	1.96	2.08	2.47	1.73	1.99	0.23	NS
Gizzard (%)	4.78	5.09	5.17	6.78	6.05	6.63	0.60	NS

NS- No significant difference (P > 0.05), **LOS**- Level of significance, **SEM-**Standard error of mean.

P-088 (ID 108)

COMPARATIVE EFFICACY OF THREE PHYTASES ON GROWTH PERFORMANCE AND APPARENT ILEAL DIGESTIBILITY OF PHOSPHORUS OF BROILER CHICKENS

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Summary

In a five-week study, the efficacy of three solid thermostable phytase products from *C. braakii* (PHY-1), *E. coli* (PHY-2) and *Buttiauxella spp* (PHY-3) was compared in broilers. Growth performance, apparent ileal P digestibility (AIDP) and bone mineralization were measured. The birds were allotted to 5 treatments fed in 2 regimes (starter and grower). There was a positive control (PC) diet adequate in all nutrient requirements, and a negative control (NC) diet formulated to be deficient in nutrients and energy at equivalent levels as proposed for a standard recommended dose (RD) of PHY-1 by supplier. NC diets (1.75 g nPP /kg starter and 1.57 g nPP/kg grower) were fed as such and supplemented with phytases PHY-1, PHY-2 and PHY-3 at RD meaning 1000, 500 and 500 U/kg, respectively.

Phytases improved the weight gain (P<0.001) and FCR (P=0.047) compared to NC. Phytases performed equally or even outperformed the PC, irrespective of the measured parameters.

Over the whole trial period, all the phytases at RD were equally potent in terms of growth performance, tibia ash percentage and apparent ileal digestibility of P.

Keywords: phytase, phosphorus, ileal digestibility, bone mineralisation, broilers

Introduction

Phytate as P source is poorly utilized by monogastric animals as they have insufficient intestinal phytase to effectively digest phytate. The poor digestive utilization of phytate P by monogastric animals and its consequences in terms of diet costs and environmental pollution have led to extensive research efforts directed toward improving phytate digestion. Research has demonstrated definitively that phytase is the only enzyme that was able to initiate the release of P from the phytate molecule, making it available for absorption and utilization (AURELI et al., 2014a). The industrial demand for phytase with greater potency in intestinal phytate hydrolysis and better heat stability continues to stimulate the search for new enzyme sources. Enzyme preparations with phytases derived from *A. ficuum*, *Peniophora. lycii* and *E. coli* are available commercially. More recently, new microbial 6-phytase produced by synthetic genes, mimicking a gene from *C. braakii* or isolated from *Buttiauxella*, were introduced into the market. In the present study, the efficacy of phytase products from either *C. braakii*, *E. coli* or *Buttiauxella spp* was compared in terms of performance, ileal P digestibility and bone mineralization in broiler chickens.

Material and Methods

The study was carried out at the Research Center for Animal Nutrition and Health (DSM Nutritional Products France) using a total of 960 day-old male Ross PM3 broiler chickens randomly assigned into 5 treatments (6 replicates of 20 birds per treatment). Each group was placed in one floor pen littered with wood shavings and allocated to one of the different treatments. The chickens were housed in an environmentally controlled room and had *ad libitum* access to feed in pellets and tap water. The composition of the basal diets used in the trial is presented in Table 1. Diets I were fed as positive control diet (PC) and were adequate to meet all nutrient requirements of growing broilers in accordance to their age. Diet II was formulated to be deficient in nutrients and energy equivalent to the matrix values determined for PHY-1 standard recommended dose (RD). Diet II was fed as such (NC) and with the addition of PHY-1 (*C. braakii*, Ronozyme® HiPhos (GT)) at 1000 U/kg, PHY-2 (*E. coli* phytase produced by *Schizosaccharomyces pombe*) at 500 U/kg and PHY-3 (*Buttiauxella*-phytase) at 500 U/kg respectively.

Table 1: Composition and nutrient contents of the experimental diets

	STARTER (Day 1-21)		GROWER (Day 22-36)	
Ingredients (%)	Diet I/PC	Diet II/NC	Diet I/PC	Diet II/NC
Soybean meal	39.40	38.80	37.20	36.80
Maize	55.00	56.40	55.64	56.20
Soya oil	2.50	1.50	4.00	3.50
DL-Methionine	0.20	0.20	0.10	0.10
$CaCO_3$	0.49	0.69	0.55	0.65
DCP	1.20	0.20	1.20	0.20
Sodium chloride	0.15	0.15	0.15	0.15
Vitamin & mineral premix	1.00	1.00	1.00	1.00
Coccidiostat (Lasalocid)	0.06	0.06	0.06	0.06
Sand	0.00	1.00	0.00	1.24
Titanium dioxide	-	-	0.10	0.10
Calculated content (g/kg)				
Crude protein	224	221	214	212
P total	5.87	4.01	5.77	3.79
Avalaible P	2.61	1.13	2.59	1.10
Calcium	7.48	5.77	7.79	5.66
Analysed content (g/kg)				
Crude protein	229	226	223	211
Metabolizable energy (MJ/kg)[1]	13.3	12.9	13.5	13.3
P total	5.46	3.89	5.44	3.87
Calcium	6.57	5.31	6.62	5.17

[1]Metabolizable Energy calculated with EC-equation based on analyzed crude nutrients according to the formula ME (MJ/kg) = ((15.51*crude protein+34.31*fat+16.69*starch+13.01*sugar)/1000)

On day 36, six chickens randomly chosen from each replicate were euthanized and the content of the terminal part of the ileum (17 cm and 2 cm proximal to the ileo-caecal junction), was sampled. The right tibiae were taken from the six chickens for bone strength determination and tibia ash analysis. The concentration of the marker in feed and digesta together with the concentration of P in the feed and digesta were used to calculate the apparent ileal digestibility coefficient (AID) of P according to the following formula:

$$\mathbf{AID\ (\%) = 100 - [(CM_f/CM_e) \times (CN_e/CN_f)] \times 100}$$

CM_f =concentration of marker in feed; CM_e =concentration of marker in ileal digesta;

CN_f = concentration of P in feed; CN_e =concentration of P in ileal digesta

Performance data, ileal digestibility data, and tibia analyses were subjected to a one-factorial analysis of variance (ANOVA) with the treatment as the experimental factor. Where significant treatment effects ($p < 0.05$) were indicated, the differences among treatment means were subsequently analysed and compared using the Newman-Keuls test.

Results

Over the whole period (day 1-36), the results on performance (Table 2) showed that inadequate dietary avP in the diet (NC) led to a significant decrease of WG whereas phytase supplementation was effective in improving significantly the WG and resulted in similar WG than that obtained with the adequate avP diet (PC). FCR was not significantly different among the supplemented phytase treatments. Dietary level of total P and phytase had significant effect on AIDP (Table 2). Indeed, addition of phytase to NC diet resulted in significant improvements of AIDP. Nevertheless, differences among the supplemented treatments were not significant, as already recorded by AURELI et al. (2014b). Whereas tibia-breaking strength was found to decrease with a reduction in dietary available P levels, the addition of phytase led to significant improvements of bone breaking strength and tibia ash percentages compared to NC diet. Although the dif-

ference among the supplemented treatments was not significant, the effects on parameters of bone mineralization were similar when compared to PC (Table 2), showing that bone mineralization criteria are sensitive indicators of P status in the bird (RAVINDRAN et al., 1995).

Table 2: Effect of phytase supplementation on growth performance (day 1-36), apparent ileal digestibility (AID) of P and bone mineralization

Product	Dose expected	Dose analysed[1]	Weigth gain	Feed intake	FCR	AIDP	Tibiae	
							strength	ash
	U/kg	U/kg	g/b	g/b		%	N	%DM
PC			2598[a]	3771[a]	1.452[b]	40.6[c]	298[ab]	56.5[a]
NC			1664[b]	2466[b]	1.482[a]	53.7[b]	212[c]	55.0[b]
NC+PHY-1	1000	1264	2590[a]	3823[a]	1.476[ab]	64.6[a]	290[ab]	57.0[a]
NC+PHY-2	500	485	2509[a]	3689[a]	1.470[ab]	65.8[a]	315[a]	56.8[a]
NC+PHY-3	500	529	2589[a]	3820[a]	1.476[ab]	66.4[a]	276[b]	56.8[a]
P values			*<0.001*	*<0.001*	*0.047*	*<0.001*	*<0.001*	*0.006*

[a,b,c] Means within a row, not sharing a common superscript, are significantly different (p<0.05)
[1] phytase activity after pelleting in the grower diet

Conclusions

The results obtained in this five-week efficacy study with broiler chicks indicated that phytases have a strong potency for improving P availability in maize/soybean meal based diets for broilers. Irrespective of the measured parameters, the phytase treatments performed equally to the PC. All the phytases at the recommended dose were equally potent in terms of growth performance, ileal digestibility of P and tibia ash percentage.

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P-089 (ID 109)

EFFECTS OF PROTEASE SUPPLEMENTATION ON THE STANDARDIZED ILEAL AMINO ACID DIGESTIBILITY IN TWO WHEAT BY PRODUCTS IN BROILER CHICKENS

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Summary

A study was conducted to evaluate the effect of a serine protease on the standardized ileal digestibility of amino acids (SIAAD) in two wheat by-products (wheat DDGS and wheat middlings) in broiler chickens. A total of 288 20-day old male broiler chickens were used in a 4-day digestibility study. A nitrogen-free diet was used to determine basal endogenous amino acids losses of broiler chickens. The test feed ingredients served as the sole source of amino acids in experimental diets. The efficacy of the protease for improving the SIAAD in broiler chickens was investigated at the dietary inclusion level of 15 000 PROT/kg. The addition of the protease numerically improved the SIAAD of Val (+3.7 %) and Thr (+ 4.8 %) of wheat DDGS. Furthermore, SIAAD of Gly (+5.5 %) and His (+8.0 %) were significantly improved in wheat middlings whereas numerical improvements by more than 2% for SIAAD of Ile-Phe (+2.8 %), Thr (+3.5 %), Ala and Tyr (+2.5 %) and Asp (+3.7 %) were also recorded. In this study, protease supplementation was effective in improving the standardized ileal digestibility of almost all the amino acids in wheat middlings.

Keywords: protease, ingredients, amino acid, standardized ileal digestibility, broilers

Introduction

Knowledge of amino acid (AA) digestibility of feed ingredients is necessary to feed broilers with properly balanced compound diets. For this reason, more attention has recently been given to the determination of AA digestibility of feedstuffs, recognizing that it may vary greatly depending upon the feed ingredient. There is evidence demonstrating that the ileal digesta sampling is a more exact method of estimating the AA digestibility in chickens than the measurement of total AA flow *via* the excreta (LEMME et al., 2004; GARCIA et al., 2007). However, values of endogenous AA secretion have been shown to depend on the method of determination and the age of the bird (ADEDOKUN et al., 2007). A correction for the endogenous fraction by using a nitrogen-free diet allows determining the standardized ileal amino acid digestibility (SIAAD) which represents a better basis for comparison of dietary treatments and their effects on amino acid digestibility. By using this method, the efficacy of a protease (Ronozyme® ProAct) for improving the SIAAD in broiler chickens fed experimental diets containing wheat by-products (wheat DDGS and wheat middlings) was evaluated.

Material and Methods

The study was performed at the Research Center for Animal Nutrition and Health (DSM Nutritional Products France, F-68305 Village-Neuf). Day-old male broiler chickens were housed in wire-floored battery cages, which were kept in an environmentally controlled room. The birds had *ad libitum* access to feed and tap water. They were given a standard broiler diet as mash from day old until day 20. On day 8, the chickens were divided by weight into groups of 8 birds, which were allocated to 6 treatments. 6 replicate groups were assigned to each treatment.

The 6 dietary treatments were as follows: Diets 1 and 4 were Nitrogen-free diet (NFD) to be used for estimating endogenous nitrogen and AA losses fed with or without protease supplementation. These values were used to standardize the apparent ileal digestibility (AID) and thus to determine SIAAD. Diets 2 and 3 were experimental diets containing corn starch and mineral sources in which wheat by-products such as wheat middlings (WM) and wheat DGGS (WDGGS) were the only source of dietary protein, respectively. Diets 5 to 6 were the same as diets 2 and 3, with the protease Ronozyme® ProAct added to supply 15000 PROT per kg feed. To enable the ileal digestibility determinations, titanium dioxide (TiO_2) was added to the feed as indigestible marker at a concentration of 1000 mg/kg.

The birds were provided the experimental diets in mash form from day 20 to day 24. On day 24 all the chickens per group were sacrificed and the content of the terminal part (17 cm proximal to a point 2 cm before the ileo-caecal junction) of the ileum was collected.

The contents of AA as well as the concentration of TiO_2 as indigestible marker were determined in the digesta samples and in the feed. Ileal endogenous AA flow in birds fed the NFD was calculated as mg of amino acid (AA) flow per 1 kg of DM intake basis using the following formula reported by MOUGHAN et al. (1992):

IEAA (g/kg DM) = [AA in ileal digesta (mg/kg)*(diet TiO_2, mg/kg / ileal TiO_2, mg/kg)].

The concentration of the marker in feed and digesta together with the content of AA in the feed and digesta were used to calculate the standardized ileal amino acid digestibility (SIAAD %) using the formula given by LEMME et al. (2004):

SIAAD (%) = AID (%) + ((IEAA flow, g/kg)/ (amino acid content of diet, g/kg))*100

where AID (%) apparent ileal digestibility= 100 - [(diet TiO_2, mg/kg /ileal digesta TiO_2, mg/kg) x (AAs in diet, g/kg/ AAs in ileal digesta, g/kg)] x 100

Data were subjected to one-factorial analysis of variance per tested sample of wheat (factor: protease supplementation), using the software 'Stat Box Pro Agri', version 7.1.9 (Grimmer soft, 1985-2011).

Results

Quantification of endogenous intestinal AA losses indicated low losses (<3% of total AA) for Met, His and Cys whereas losses between 6.5% and 13.6% were recorded for Pro, Leu, Ser, Asp, Glu and Thr (Table 1). Although various methods are available to determine the endogenous losses, all indicate that the loss of Glu, Asp and Thr are high in comparison with the other essential AA (LEMME et al., 2004) and are due to mucin secretion.

Whereas several publications are available on AID of feed ingredients for poultry (GARCIA et al., 2007; ADEDOKUN et al., 2007), no data were reported on AID of feed ingredients supplemented with protease. SIAAD values of Lys (+ 2.0 %), Val (+ 3.7 %) and Thr (+ 4.8 %) from wheat DDGS were numerically increased with protease supplementation. Furthermore, SIAAD values of His and Gly from wheat middlings were significantly increased by 8.0 % and 5.5 %, respectively. Numerical improvements by more than 2% for SIAAD values of Ile & Phe (+2.8 %), Thr (+3.5 %), Ala & Tyr (+2.5 %) and Asp (+3.7 %) were also recorded. Improvement of SIAAD in the presence of protease is not the same for all AA and varies from one ingredient to another one. COWIESON and ROOS (2014) have shown that the response to the addition of the protease on AA digestibility may range from >5% for Thr and Cys to < 3% for Trp and Glu. The effects of added protease are dependent on feed composition and on intrinsic digestibility of AA. Indeed, the current results showed that SIAAD values of WM were much higher than those recorded for wheat DDGS which maybe influenced during the industrial processing (e.g. drying procedure).

Moreover, factors such as type and quality of the ingredients and the presence of anti-nutritional substances such as tannins, phytates, trypsin inhibitors in plant species also modulate the AID in poultry feed and thus the effectiveness of exogenous protease. Finally, the physiological state of animals (growth or maintenance), feed consumption or the nutritional feed quality could also influence the digestibility values. Characterization of raw materials is important to guide diet composition and to maximize the effects of protease supplementation.

Conclusion

In this study, protease supplementation was effective in improving the standardized ileal digestibility of almost all the amino acids in wheat middlings.

Table 1: Ileal endogenous amino acid flow and standardized ileal digestibility coefficients (%) of amino acids (SIAAD) for broilers fed experimental diets

	IEAA (mg/kg DM)				SIAAD (%)							
Diet	1		4		2	5			3	6		
Enzyme[1]	-		+		-	+			-	+		
	Mean	SD	Mean	SD	WM		*p*=	SEM	WDDGS		*p*=	SEM
EAAs[2]												
Arg	248	90.7	259	100.7	81.5	82.2	*0.684*	0.27	72.1	72.9	*0.516*	0.24
His	83	24.8	86	31.0	74.9	80.9	*0.007*	0.35	88.0	87.3	*0.197*	0.16
Ile	159	54.6	165	72.9	77.5	79.9	*0.293*	0.32	67.8	65.8	*0.239*	0.28
Leu	338	124.1	359	135.7	81.3	82.4	*0.449*	0.26	78.1	78.8	*0.490*	0.21
Lys	269	105.3	298	104.4	80.2	78.1	*0.292*	0.31	73.0	74.5	*0.409*	0.28
Met	68	16.6	67	30.9	83.4	83.4	*0.945*	0.24	74.2	75.2	*0.490*	0.25
Phe	203	74.7	214	68.9	81.4	83.5	*0.142*	0.26	83.3	83.4	*0.990*	0.19
Thr	651	175.1	701	166.3	68.9	71.3	*0.272*	0.31	65.4	68.6	*0.115*	0.31
Val	170	66.2	188	69.4	78.2	78.9	*0.743*	0.30	70.0	72.6	*0.175*	0.30
NEAAs[3]												
Ala	270	76.8	287	90.9	74.8	76.4	*0.358*	0.28	67.6	65.5	*0.178*	0.27
Asp	473	127.2	515	176.1	73.5	76.0	*0.177*	0.29	47.7	42.7	*0.053*	0.36
Cys	138	23.7	152	13.8	75.2	74.8	*0.809*	0.28	67.9	68.7	*0.707*	0.31
Glu	570	178.3	620	221.5	88.7	89.6	*0.314*	0.21	85.0	83.7	*0.129*	0.20
Gly	266	73.3	286	62.6	85.6	90.3	*0.058*	0.35	60.4	52.7	*0.068*	0.45
Pro	311	86.0	351	62.0	86.2	86.9	*0.410*	0.20	84.4	84.5	*0.869*	0.19
Ser	395	104.0	439	105.7	76.8	75.8	*0.752*	0.38	72.7	72.7	*0.990*	0.23
Tyr	168	56.8	184	53.4	79.2	80.7	*0.278*	0.31	79.6	79.8	*0.898*	0.26

[1] - No added enzyme, + Added enzyme (Ronozyme® ProAct at 15000 PROT/kg); [2]EAAs: essential amino acids; [3]NEAAs: non-essential amino acids

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EFFECTS OF INCLUSION OF A MONO COMPONENT SERINE PROTEASE ON APPARENT ILEAL DIGESTIBILITY OF CRUDE PROTEIN AND AMINO ACIDS OF SOYBEAN MEALS OF DIFFERENT ORIGINS IN BROILERS

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Abstract

We studied the effects of including in the diet a mono component serine protease (PRO; RONOZYME® ProAct, DSM Nutritional Products, Basel, Switzerland) on apparent ileal digestibility (AID) of DM, CP and AA of soybean meals (SBM) of USA (n=3), Brazil (n=3) and Argentina (n=3) origin, in 22 d-old broilers. The 6 experimental treatments formed a 3 x 2 factorial with 3 SBM origins (USA, BRA and ARG) and 2 levels of PRO (0 and 200 mg/kg to give 0 and 15,000 PROT units/kg feed). Three different batches of each SBM were nested within each of the 3 SBM origins. Each of the 6 treatments were replicated 24 times (8 times per batch). The experimental unit was a cage with 8 broilers. Birds were fed a commercial corn-soybean meal diet from 1 to 18 d of age followed by the experimental diets that consisted of a mixture of 42.6% SBM and 57.4% of a nitrogen free diet for 4 d. The supplementation with PRO improved the AID of all the AA (ranging from 0.5 to 1.6 percentage units) but the differences were not significant. The AID of DM, CP and Lys was 1.5%, 1.1% and 1.0% higher in bird fed PRO than in birds fed the un-supplemented diets ($P > 0.10$). In general, the AID of DM, CP and AA was higher for the USA meals than for the South America meals but the differences were not significant. In addition, the AMEn of the diets increased by 1.1% as an average, with PRO inclusion ($P < 0.001$). In summary, the inclusion of RONOZYME® ProAct increased numerically the AID of all the AA of the SBM and the DM of the diets but the differences were not significant.

Keywords: amino acid digestibility, broiler, protease, soybean meal origin

Introduction

Soybean meal (SBM) is the most common source of protein and amino acids (AA) in poultry diets worldwide. This is due to its high CP content, and the excellent AA profile that complements that of the cereals, and the high AA digestibility. SBM contributes up to 70% of dietary CP in a typical corn-soybean meal broiler diet. The United States (US), Brazil (BRA), and Argentina (ARG) are the main suppliers of meals in the global SBM market. (RAVINDRAN et al., 2014). The AA profile and quality of the CP of commercial SBM depend on factors such as processing conditions during oil extraction as well as soil, latitude and environmental variables during the growing and harvesting of the beans. Therefore, the nutritive value of SBM might vary depending on the crushing plant and the country of origin of the beans (GARCÍA-REBOLLAR et al., 2014). Research on the use of exogenous enzymes in broiler diets has been ongoing for decades. However, the commercial use of enzymes, especially of proteases, is more recent. The interpretation of results from studies conducted with proteases is sometimes difficult because of the confounding effects of the presence of more than one enzymatic activity in a single treatment (FREITAS et al., 2011). The objective of this study was to study the influence of a mono component serine protease on the AA digestibility of soybean from different origins in broilers of 22d of age.

Material and Methods

In total 1,152 one-d-old (Ross 308) broiler chicks (divided into 2 blocks) were collected from a commercial hatchery (Avícola Moraleja, S. A., Madrid, Spain). After arrival to the experimental farm, chicks were weighed and distributed in groups of 8 in battery cages in an environmentally controlled room. All cages had wire flooring and were equipped with 2 nipple drinkers and an open trough feeder. Birds were fed a commercial corn-soybean meal diet from 1 to 18 d of age followed by the experimental diets that consisted of a mixture of 42.6% SBM and 57.4% of a nitrogen free diet, for 4 d. At d 22, birds were weighed and euthanized by CO_2 asphyxiation and fresh digesta was collected from the terminal ileum, defined as the segment of the gastrointestinal tract from 15 to 2 cm anterior to the ileocaecal junction. Ileal digesta was collected by gently flushing the contents with distilled water. The contents from the 8 birds within the cage

were pooled and frozen (-20 ºC) until the AID of the DM, CP and AA could be determined. The 6 experimental treatments formed a 3 x 2 factorial with 3 SBM origins (USA, BRA and ARG) and 2 levels of PRO (0 and 200 mg/kg to give 0 and 15,000 PROT units/kg feed). Three batches of SBM were nested within each of the 3 SBM origins. Each of the 6 treatments was replicated 24 times (8 times per batch) and the experimental unit was a cage with 8 broilers.

Results

The interactions between origin and supplementation with protease was not significant and therefore only main effects are presented (Table 1). The supplementation with PRO improved the AID of all the AA (ranging from 0.5 to 1.6 percentage units) but the differences were not significant. The AID of DM (73.5 vs. 72.4%), CP (77.8 vs. 77.0%) and Lys (81.0 vs. 80.1%) was 1.5%, 1.1% and 1.0% higher in birds fed PRO than in birds fed the un-supplemented diets (P > 0.10). In general, the AID of DM (73.9 vs. 72.5 and 72.6%) and CP (78.2 vs. 76.8 and 77.4%) was higher for the USA meals than for BRA and ARG meals but the differences were not significant. Also for most AA the AID was higher for USA SBM than BRA and ARG SBM but the differences were not significant. The AMEn of the diets increased by 1.1% as an average with PRO inclusion (P < 0.001) (data not shown).

Conclusions

The inclusion of RONOZYME® ProAct in the diet increased the AID of all the AA in the SBM and the DM of the diets, regardless of SBM origin, but the differences were not significant. The apparent ileal digestibility of DM, CP and almost all AA were numerally higher for the USA than for the ARG and BRA SBM.

Table 1. Apparent ileal digestibility of the amino acids of soybean meal of different origins with and without protease

	DM	Thr	Cys	Val	Met	Iso	Lys	Total	CP
Origin									
USA	73.9	73.5	65.2	79.3	79.2	81.2	81.4	80.1	78.2
BRA	72.5	72.5	64.4	79.0	79.5	80.9	79.8	79.4	76.8
ARG	72.6	72.1	65.1	78.8	77.9	80.4	80.4	79.1	77.4
SEM[1]	0.548	0.861	0.773	0.568	0.566	0.572	0.793	0.577	0.487
Protease									
Without	72.4	72.9	64.2	78.4	78.0	80.4	80.1	79.1	77.0
With	73.5	72.6	65.5	79.6	79.6	81.3	81.0	79.9	77.8
SEM[2]	0.470	0.688	0.756	0.622	0.825	0.582	0.616	0.571	0.571
P-value									
Origin	0.200	0.551	0.658	0.869	0.178	0.599	0.423	0.460	0.371
Protease	0.101	0.729	0.231	0.194	0.174	0.297	0.274	0.327	0.325

[1] Standard error of the mean (16 replicates of 8 chicks each one).
[2] Standard error of the mean (72 replicates of 8 chicks each one).
The interaction was not significant

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P-091 (ID 284)

INFLUENCE OF PROTEASE ENZYME SUPPLEMENTATION ON APPARENT ILEAL DIGESTIBILITY OF AMINO ACIDS OF CORN, WHEAT AND BARLEY GRAINS

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Abstract

We studied the effects of a commercial multi-enzyme complex [with a minimum of 8,000 U/g of endo-1,4-β-glucanase, 18,000 U/g of endo-1,3(4)-β-glucanase and 26,000 U/g of endo-1,4-β-xylanase (XYL) (Roxazyme G2 G, DSM Nutritional Products)] and a mono component serine protease (PRO; Ronozyme ProAct, DSM Nutritional Products) on the apparent ileal digestibility (AID) of DM, CP and AA of 3 varieties of the 3 cereals in 22-d old broilers. The 12 experimental treatments formed a 3 x (2 x 2) factorial, with 3 main cereals (corn, wheat and barley), 2 levels of XYL (0 vs. 100 mg/kg), and 2 levels of PRO (0 vs. 200 mg/kg) with cereal variety nested within the corresponding cereal. Each treatment was replicated 24 times. Birds were fed the experimental diets from 18 to 22 d (92:8 mixture of the cereal and a N free diet). The AID of DM, Met and Leu, across variety and enzyme supplementation, was higher for corn than for barley with that of wheat being intermediate ($P < 0.05$). The inclusion of PRO increased ($P < 0.05$) Gly AID and tended to increase ($P < 0.10$) the AID of DM and of most AA, including Cys, Glu, Met, Thr and Val. The inclusion of XYL improved ($P < 0.05$) the AID of DM and of CP, and all AA, except Met and Lys. The combination of both enzymes did not improve AA digestibility further. Trends observed, when combining different enzyme activities in short term digestibility trials, need further study and may not reflect the response seen combining different enzyme combinations on animal performance studies or when fed as part of a complete diet from hatching to slaughter.

Keywords: amino acid digestibility, broiler, cereal, protease, xylanase

Introduction

Cereals are the most widely used ingredients as energy sources in poultry feeds. In addition to supplying starch, cereals also provide part of the CP and amino acids (AA) requirement of the bird. Starch utilization by poultry depends on the cereal used, because starches differ in their nature and structure. Corn and wheat are the two most commonly used cereals in poultry diets. In some countries, barley is also a commercial alternative. Corn has less protein and dietary fiber and more starch, fat and AMEn than wheat or barley (FEDNA, 2010). In addition, the chemical composition and nutritive value of corn is quite uniform compared to that of wheat and barley, but in all grains the energy and AA contents vary depending on factors such cultivar, agronomic practices, weather conditions and length of storage (FRIKHA et al., 2011; PÉREZ BONILLA et al., 2011). Wheat contains relatively high levels of non-starch polysaccharides (NSP) in cell walls and these cannot be digested in the small intestine of non-ruminants because of the lack of adequate presence of enzymes in their digestive tract. The accumulation of NSP in the alimentary tract can result in water retention and an increase in the viscosity of the digestive contents. High digesta viscosity has a negative effect on nutrient digestion and absorption including that of the AA's. Consequently, exogenous enzymes (carbohydrases) are required to improve nutrient utilization (REBOLÉ et al., 2010). The objective of this work was to determine the effect of exogenous carbohydrases used with or without proteases on energy and amino acids (AA) digestibility of cereal grains.

Material and Methods

A commercial multi-enzyme complex with β-glucanase and β-xylanase activity (XYL; ROXAZYME® G2 G, DSM Nutritional Products) and a mono component serine protease (PRO; RONOZYME® ProAct, DSM Nutritional Products) were used to study the apparent ileal digestibility (AID) of DM, CP and AA in three different cereals. The experiment was carried out in 4 time blocks due to facility limitation. In each block, 576 one-d-old male broiler chicks (Ross 308) were used. On arrival at the experimental farm, chicks were weighed and distributed in groups of 8 into 72 battery cages in

an environmentally controlled room. All cages had wire flooring and were equipped with 2 nipple drinkers and an open trough feeder. Birds were fed a commercial corn-soybean meal based diet from 1 to 18 d followed by the experimental diets (92:8 mixture of the cereal and a nitrogen free diet) to 22 d. On d 22, birds were weighed and euthanized by CO_2 asphyxiation and fresh digesta was collected from the terminal ileum, defined as the segment of the gastrointestinal tract from 15 to 2 cm anterior to the ileocaecal junction. Ileal digesta was collected by gently flushing the contents with distilled water. The contents, from the 8 birds within the cage were pooled and frozen (-20 ºC) until the AID of the DM, CP and AA could be determined. The 12 experimental treatments were formed by a 3 x (2 x 2) factorial arrangement of 3 main cereals (corn, wheat and barley), 2 levels of XYL (0 vs. 100 mg/kg), and 2 levels of PRO (0 vs. 200 mg/kg) with cereal variety (n= 3) of each of the 3 main cereals nested within the corresponding cereal. In total, each main treatment was replicated 24 times.

Results

The AID of DM (81.8 vs. 71.3 and 72.1%, respectively), Met (85.6 vs. 78.4 and 82.2, respectively) and Leu (87.3 vs. 78.2 and 82.3%, respectively), across variety and enzyme supplementation, were higher for corn than for barley with that of wheat being intermediate ($P < 0.05$) (Table 1). The AID of Ala (85.9 vs. 74.6 and 72.0; $P < 0.01$, respectively) was higher for corn than for wheat and barley. However, the AID of Pro was higher for wheat than for corn and barley (90.9 vs. 84.5 and 84.3; $P < 0.05$, respectively) and the Glu was higher for wheat than for barley, with that of corn being intermediate (92.5 vs. 85.4 and 86.6; $P < 0.05$, respectively). The inclusion of xylanases improved the digestibility of DM (75.4 vs. 74.5; $P < 0.01$), CP (77.8 vs. 75.7; $P < 0.01$) and of all the AA ($P < 0.01$) except for Met and Lys that were not affected. The inclusion of PRO increased ($P < 0.05$) AID of Gly and tended to increase ($P < 0.10$) that of DM and of most AA, including Cys, Glu, Met, Thr and Val The combination of both enzymes did not improve AA digestibility further.

Conclusions

The AID of most of the AA was higher for corn and wheat than for barley. The inclusion of a multi-enzyme complex with β-glucanase and β-xylanase activity increased the AID of DM, CP and of all the AA, except Met and Lys. The inclusion of the mono component serine protease increased the AID of Gly and tended to increase the digestibility of most of the other AA. The combination of both enzymes did not improve AA digestibility further. Trends observed when combining different enzyme activities in short term digestibility trials need further study and may not reflect the response seen combining different enzyme combinations on animal performance studies or digestibility studies when fed as part of a complete diet from hatch to slaughter.

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Table 1. Apparent ileal amino acid digestibility (%) of the amino acid of the main cereal of the diet with and without enzymes

		DM	Thr	Glu	Pro	Gly	Ala	Cys	Val	Met	Leu	Lys	CP
Cereal													
Corn		81.3[a]	66.6	86.6[ab]	84.5[b]	72.8	85.9[a]	76.4	76.2	85.6[a]	87.3[a]	76.5	76.4
Wheat		72.1[ab]	69.4	92.5[a]	90.9[a]	75.2	74.6[b]	81.8	76.1	82.2[ab]	82.3[ab]	73.1	79.7
Barley		71.3[b]	66.7	85.4[b]	84.3[b]	70.3	72.0[b]	76.6	74.5	78.4[b]	78.2[b]	73.7	74.1
SEM[1] (n=32)		2.252	2.772	1.424	1.534	2.568	1.499	2.325	2.270	1.262	1.395	2.138	2.260
Enzyme													
Xylanase	Protease												
Without	Without	71.8[b]	63.3[b]	86.2[b]	84.2[b]	68.9[b]	74.2[c]	74.6[c]	71.8[b]	79.6[b]	80.1[c]	71.3[b]	73.5[c]
	With	76.4[a]	69.1[a]	88.7[a]	87.2[a]	74.3[a]	79.0[ab]	79.7[ab]	77.0[a]	83.5[a]	83.5[ab]	75.9[a]	78.0[ab]
With	Without	77.1[a]	70.2[a]	89.5[a]	88.2[a]	75.1[a]	79.8[a]	81.0[a]	78.2[a]	83.4[a]	84.5[a]	76.4[a]	79.0[a]
	With	74.5[ab]	67.5[a]	88.3[a]	86.7[a]	72.8[a]	77.0[b]	78.0[b]	75.6[a]	81.6[ab]	82.2[b]	74.2[ab]	76.5[b]
SEM[1] (n=72)		0.709	0.781	0.404	0.407	0.727	0.687	0.587	0.731	0.623	0.549	1.021	0.669
Xylanase													
Without		74.1	66.2	87.5	85.7	71.6	76.6	77.1	74.4	81.6	81.8	73.6	75.7
With		75.8	68.9	88.9	87.5	74.0	78.4	79.5	76.9	82.5	83.4	75.3	77.8
Protease													
Without		74.5	66.8	87.8	86.2	72.0	77.0	77.8	74.9	81.5	82.3	73.9	76.3
With		75.4	68.3	88.5	87.0	73.5	78.0	78.8	76.3	82.6	82.9	75.0	77.2
SEM[1] (n=144)		0.502	0.552	0.285	0.288	0.514	0.486	0.415	0.517	0.441	0.388	0.722	0.473
P-value													
Cereal		0.029	0.673	0.027	0.038	0.458	0.001	0.254	0.837	0.019	0.011	0.538	0.288
Xylanase x protease		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	<0.001
Xylanase		0.002	<0.001	<0.001	<0.001	0.001	0.007	<0.001	<0.001	0.123	0.004	0.109	0.003
Protease		0.096	0.069	0.082	0.055	0.041	0.142	0.090	0.065	0.069	0.233	0.268	0.134

[1] The experimental unit was a cage with 8 chicks.

P-093 (ID 202)

THE EFFECT OF SUPPLEMENTING A PURIFIED BACTERIAL ENDO-XYLANASE IN THE DIET OF OLD LAYING HENS ON PRODUCTION AND EGG SHELL QUALITY

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Abstract

A trial was set up to investigate the effect of a bacterial endo-xylanase (B) in the diet of laying hens, 54 weeks old, on performance and egg shell quality. Two dietary treatments were studied: a standard wheat-corn based diet supplemented without (NC) or with B (100 mg/kg feed). Five replicates of 18 laying hens were allocated to each of these two treatments during five consecutive periods of 28 days. Supplementing the feed with B significantly improved the laying percentage (87.9 *vs* 83.7%) and egg mass (58.3 *vs* 55.3 g) resulting in a significant better feed conversion ratio (1.974 *vs* 2.085). The incidence of broken and dirty eggs was moderate and comparable between treatment groups, whereas the incidence of soft-shelled eggs was significantly lower (0.04 *vs* 0.47%) for the B supplemented group. Egg quality was not influenced by treatment, only for the Haugh units (HU) a significantly higher value for the laying hens fed the diets supplemented with B (90.14 *vs* 87.60) was noticed. To conclude, supplementing a bacterial endo-1,4-β-xylanase resulted in a more efficient egg production and increased HU when compared to the NC diet. Based on these results, we hypothesize that this bacterial xylanase not only reduces the anti-nutritional effects of the non-starch polysaccharides but also creates prebiotic arabinoxylan-oligosaccharides which are fermented in the ceca to produce high levels of butyrate.

Keywords: xylanase, bacterial, laying hens, performance, egg quality

Introduction

The objective of the current trial is to provide efficacy data of non-starch polysaccharide (NSP) enzymes for use in laying hens. For this purpose a bacterial endo-xylanase (B) was supplemented in a standard wheat corn based diet. The bioefficacy of the NSP enzyme was determined in a zootechnical performance trial.

Material and Methods

Laying hens (Lohmann brown), 54 weeks of age at the start of the experiment, were used in this trial. In each pen unit 18 laying hens were housed divided over 2 enriched cages (9 hens/cage) and reared under conventional conditions for lighting, heating and ventilation. Drinking water and feed (in finely ground meal form) was provided *ad libitum*. Two dietary treatments were studied: a standard wheat-corn based diet supplemented without (NC) or with a bacterial endo-xylanase (B) (Nutrase Xyla, 100 mg/kg feed). The trial was designed as a 2-factorial Complete Block Design (2 treatments x 5 replicates) with 18 medium weight laying hens per pen with a total number of 180 birds. Duration of the trial was 140 days as there were 5 periods of each 28 days.

Average pen body weight was recorded at the start and at the end of the experiment. Feed intake was recorded and feed conversion and daily feed intake per bird were calculated for each period per 18 laying hens. Daily number of eggs per pen unit of 18 laying hens were counted and every 2 weeks eggs were weighed. Furthermore, every day the incidence of cracked, soft-shelled and dirty eggs was recorded. Daily mortality and cullings were recorded for each pen. Corrections for mortality calculating zootechnical performances were done using the number of 'animal days' (number of animals x days alive). Egg quality was measured in terms of egg shell deformation (under a 500 g load, in micrometer), yolk colour, egg shell thickness and Haugh Units at the start, during period 3 and at the end of the trial. The thickness of the eggshell, after the removal of the egg content and the egg membranes, was measured (µm) with a micrometer at 3 different areas. The egg content was broken onto a glass-top table with a mirror, the thick albumen height was measured at the distance of approximately 1 cm from the yolk. The height of thick albumen in mm (H) and the egg weight in g (W) were used to calculate the Haugh Units (HU) from following formula (Monira et al., 2003): HU = 100 log (H - (1.7 *W^0.37) + 7.6)). The yolk colour was determined with the 15-point scale of Roche.

All parameters were subjected to a factorial analysis of variance ("2 x 5 x 5 / 2 dietary treatments x 5 blocks x 5 periods") and the corresponding LSD-multiple range test (with P ≤ 0.05 as the threshold value) (STATISTICA 64.0).

Results

There was a significant effect of period on laying rate, egg weight and daily feed intake. Laying rate significantly decreased (P = 0.030) whereas egg weight increased with the age of the laying hens. In general, egg mass decreased with ongoing age of the laying hens but not at the 5% significance level. Supplementing the feed with B significantly affected the laying percentage (P = 0.020), daily egg mass (P = 0.016) and feed conversion (P = 0.016). Hens fed the diet supplemented with B had a significantly higher laying percentage compared to the other group of laying hens. Due to the differences in laying percentage, daily egg mass was significantly higher resulting in a significantly better feed conversion for the B supplemented broilers compared to their counterparts. The percentage of broken and dirty eggs was moderate and comparable between treatments but the incidence of soft-shelled eggs was significantly lower for the B supplemented group (P = 0.028 (Table 1).

Table 1: Effect of dietary treatment on laying rate (%), egg mass (g), feed conversion, incidence of soft-shelled eggs (%) and haugh units (n = 5)

	Laying rate (%)	Egg mass (g)	Feed conversion	Soft-shelled eggs (%)	Haugh Units
Control diet	83.7[b]	55.3[b]	2.085[b]	0.47[b]	87.60[b]
B-supplemented diet	87.9[a]	58.3[a]	1.974[a]	0.04[a]	90.14[a]
P-value	0.020	0.016	0.016	0.028	0.010

[a-b] means with the same letter are not significantly different from each other at P ≤ .05.

It is obvious from the results that egg quality changed with the age of the hen: with increasing age of the hens egg shell deformation was higher, egg shells became thinner and Haugh unit values decreased. A significantly higher percentage broken and dirty eggs were obtained during the last periods of the experiment this is in accordance with the thinner egg shell and higher egg shell deformation at this stage of the trial.

There was no treatment effect on egg quality only for the Haugh units a significant higher value was reached if laying hens were fed the B supplemented diet (Table 1). No interaction between period and treatment was present indicating that these effects were similar for each period.

At the start and the end of the trial, body weight of the hens was not significantly different between treatments. During the experiment laying hens lost some weight, but this was comparable between treatments. No significant effect of dietary treatments on body weight or body weight loss was observed.

Mortality percentage during the trial was rather high and main causes were cannibalism and age of the laying hens. Due to the low number of birds per treatment no statistical conclusions can be drawn.

Conclusions

Feeding laying hens the diet supplemented with B significantly influenced their performance results. By supplementing the bacterial endo-xylanase a significantly higher laying percentage, daily egg mass and lower feed conversion was obtained. The incidence of broken and dirty eggs was comparable between groups whereas the incidence of soft-shelled eggs was significantly higher if laying hens were given the control diet. No dietary treatment effect could be detected for egg shell quality, but internal egg quality was significantly higher for the B supplemented laying hens. Based on these results, we hypothesize that this bacterial xylanase not only reduces the anti-nutritional effects of the non-starch polysaccharides but also creates prebiotic arabinoxylan-oligosaccharides which are fermented in the ceca to produce high levels of butyrate inducing an increased bioavailability of minerals (COURTIN et al., 2008).

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P-094 (ID 249)

REPLACEMENT VALUE OF RAW FULL-FAT SOYBEAN FOR COMMERCIAL SOYBEAN MEAL SUPPLEMENTED WITH A NEW-GENERATION PROTEASE

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Abstract

The aim of this study was to assess the potential of replacing commercial soybean meal with raw soybean meal (RSBM) in broiler diets supplemented with a protease product. Birds were housed in a cage rearing system in climate controlled rooms. The concentrations of antinutritive factors (ANF), particularly trypsin inhibitors, in the RSBM (13098.0 TIU/g) and diets (ranging from 1730.5 to 9913.2 TIU/g) were measured while bird response to the diets was assessed at 10, 24 and 35 days of age. Feed intake (P<0.01) and body weight gain (P<0.001) to 35 days were reduced by rising levels of RSBM in the diet. Feed conversion efficiency was also reduced (P<0.001) as a result of inclusion of RSBM. Body weight gain to 35 days was marginally (P<0.06) improved as a result of enzyme supplementation of the diets. There were no significant interactions between RSBM levels and enzyme supplement on the gross responses. Inclusion of RSBM in the diet tended to reduce carcass dressing percentage and parts yield but this effect was not significant. Breast meat yield and thigh weight were increased (not significantly) by enzyme supplementation. The weight of drumsticks and wings was also increased (P<0.05) as a result of enzyme supplementation. The relative weight of visceral organs was unaffected except for the weight of the small intestine at 10 d, which was increased (P<0.05) by rising levels of RSBM but reduced (P<0.01) by enzyme supplementation. Inclusion of RSBM in the diets increased the weight of the pancreas in entire study periods. It can be concluded that the enzyme supplement may enable the utilization of low levels of RSBM in diets for broiler chickens.

Keywords: Commercial SBM; proteases enzymes; raw full-fat soybean meal; trypsin inhibitors.

Introduction

The price of soybean meal (SBM) has consistently been on the increase over the years and this trend is likely to continue into the future (SHI et al., 2012; PETTERSSON & PONTOPPIDAN, 2013). POPESCU and CRISTE (2003) have suggested that full-fat SBM could be used as an alternative to both SBM and supplemental oils, which are included in various diets for poultry feeding. However, raw full-fat soybean meal (RSBM) contains antinutritional factors (ANF) such as protease inhibitors, lectins and phytate (CHEN et al., 2013). Exogenous feed enzymes have been routinely used in commercial poultry diets for several decades to improve dietary quality, through a reduction in or modification of the structure of ANF. The development of new enzyme products offers the incentive to the exploration of ingredients such as RSBM in poultry feeding. The objective of the current research was to assess the benefits of a protease enzyme product in diets containing graded levels of RSBM for broiler chickens.

Materials and methods

A 3 x 3 factorial study was designed to assess the potential of replacing commercial soybean meal with raw soybean meal (RSBM) at 0, 10 or 20 %. The diets were supplemented with a protease product (DSM Nutritional Products, Australia Pty. Ltd) at 0.1, 0.2 or 0.3 g/kg and were formulated to be iso-energetic and iso-nitrogenous. The recommended level of microbial phytase (0.1 g/kg DSM Nutritional Products, Australia Pty. Ltd) was uniformly added on each diet. Each of the nine treatments was replicated 6 times, with 9 broiler chicks per replicate. Birds were housed in cages, in climate controlled housing and fed on starter (1-10 d), grower (11-24 d) and finisher (25-35 d) diets.

Results and discussion

The analysed trypsin inhibitor (TI) concentrations ranged from 1730.5 to 9913.2 TIU/g diets). Some of these TI values are beyond the threshold levels for birds as set by FRIEDMAN & BRANDON (2001), who reported that diets containing more than 0.77 mg TI/g diets can affect the performance of non-ruminant animals. Increasing RSBM contents of the diets reduced (P<0.01) the feed intake of birds; however, their BWG and FCR over 1-35 days were unaffected due to

protease supplementation. This result agrees with those of other scholars (JACELA *et al*., 2013; BARLETTA, 2011), who reported that microbial proteases can break down both stored proteins and proteinaceous anti-nutrients and improve the nutritional values of feeds. The weight of drumsticks and wings of birds was improved as a result of protease enzyme supplementation of diets containing RSBM. However, the dressing percentage weights of other meat parts such as breast, thighs and neck were not affected. This result partly disagrees with those of RADA et al. (2014), who reported that the meat part of broilers is positively affected by addition of microbial enzymes.

Table: Feed intake (g/b), live weight gain (g/b) and feed conversion ratio (FCR) of broiler chickens between hatch and 10, 24 or 35 days of age

RSBM[1] (%)	Enzyme level	Feed intake (days)			Live weight gain (days)			FCR (days)		
		1-10	1-24	1-35	1-10	1-24	1-35	1-10	1-24	1-35
0	L	253.6	1894.7	3897.9[a]	238.1[ab]	1411.2	2453.3	1.08[b]	1.36	1.60
	M	261.6	1753.8	3669.6[ab]	242.8[a]	1414.3	2474.6	1.07[b]	1.29	1.48
	H	267.0	1817.8	3776.0[a]	245.1[a]	1442.0	2562.4	1.07[b]	1.25	1.47
10	L	264.4	1839.1	3581.4[b]	231.7[b]	1383.1	2348.7	1.09[b]	1.33	1.53
	M	267.6	1747.2	3418.6[b]	241.8[a]	1349.4	2350.7	1.10[b]	1.30	1.46
	H	263.3	1734.2	3502.4[b]	242.8[a]	1407.5	2475.2	1.07[b]	1.24	1.42
20	L	259.9	1735.7	3458.2[b]	219.7[c]	1356.4	2348.4	1.16[a]	1.32	1.48
	M	245.7	1820.6	3586.0[b]	220.1[c]	1361.3	2437.1	1.14[ab]	1.31	1.47
	H	271.1	1786.1	3529.4[b]	235.9[ab]	1418.7	2484.6	1.14[ab]	1.26	1.45
Polled SEM		2.0	15.4	34.7	1.6	8.6	21.4	0.01	0.01	0.02
Sources of variation										
RSBM[1] level		NS	NS	**	***	NS	NS	***	NS	NS
Enzyme level		NS	NS	NS	**	*	0.06	NS	**	NS
RSBM[1] x enzyme		NS	NS	NS	NS	NS	NS	NS	NS	NS

[a,b,c] Means bearing uncommon superscript within a column are significantly different; [1]RSBM = Raw soybean meal; NS= not significant; * P<0.05;**P<0.01;***P<0.001; L= low level of enzymes (0.1 g/kg); M= mid-level of enzymes (0.2 g/kg); H= high level of enzymes (0.3 g/kg).

Regardless RSBM contents of diets, protease supplementation reduced the weight of the small intestines, but unaffected other visceral organs such as gizzard + proventriculus (G+P), liver, heart, bursa and spleen.

Conclusion

It can be concluded that the inclusion of RSBM is detrimental to the physiological functions and growth of broiler chickens. However, some of these effects can be ameliorated by supplementation with the test protease enzyme.

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P-096 (ID 285)

AMYLASE IMPROVES RESISTANT STARCH DIGESTIBILITY OF DIETS

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Abstract

Enzymes are added to broiler diets in order to improve the utilization of nutrients. Amylase as an isolated enzyme is not commonly available on the market. The objective of this study was to investigate the effect of increasing levels of amylase on the starch digestibility of diets. For this, 315 male Cobb 500 chicks were used in a completely randomized design. Increasing levels of amylase (0, 66.7, 133.3, 200, 233.3 ppm) with 9 replicates of seven broilers were used. For ileal digestibility, 1% of acid insoluble ash was added to the diet. Data were analyzed by analysis of variance (P<0.05) and when significant it was presented as a linear and quadratic regression (P <0.05).Treatments, with or without amylase were compared and contrasted. No enzyme effect in available starch digestibility was observed. However, a linear improvement was found in the resistant starch digestibility associated with increasing levels of amylase (P <0.01). In addition, a quadratic effect on digestibility of the total starch (P = 0.03) was seen. It was concluded that amylase improves RS digestibility of corn-soy diets.

Keywords: enzymes, starch.

Introduction

Feed enzymes are used in animal nutrition in order to improve the digestibility of the diet. Recent studies have shown that the addition of a mixture of amylase, protease, and xylanase brings benefits for birds fed diets based on corn and soybean meal (Zanella et al., 1999; Café et al., 2002). However, information on the influence of amylase as an single exogenous enzyme added to broiler diets is scarce. Starch is a major fraction of the diet and is the main energy source of broilers (Weurding et al., 2003). Thus the objective was to investigate the effect of increasing levels of amylase on the starch digestibility of diets.

Materials and Methods

For this, 315 male Cobb 500 chicks were used in a completely randomized design. Increasing levels of amylase (0, 66.7, 133.3, 200, 233.3ppm) with 9 replicates of seven broilers were used. The birds were housed in cages with water and feed *ad libitum*. At 25 days of age the birds were euthanized and the ileal content was collected. The material was frozen in liquid nitrogen and stored at -18 ° C. Subsequently, the samples were lyophilized (Liofilizador ModulyoD, Thermo Electron Corporation, Waltham, MA, USA). The diets and ileal content samples were analyzed for available starch (AS), resistant starch (RS) and total starch (TS). For ileal digestibility, 1% of acid insoluble ash was added to the diet. Data were analyzed by analysis of variance (P<0.05) and when significant it was presented as a linear and quadratic regression (P <0.05).Treatments, with or without amylase were compared and contrasted.

Results

No enzyme effect was observed on available starch digestibility, Table 1. However, a linear improvement was found in the resistant starch digestibility associated with increasing levels of amylase (P <0.01; $y = 0.041x + 72.46$; $R^2 = 0.594$), Figure 1. In contrast the inclusion of the enzyme improved the resistant starch digestibility. In addition, a quadratic effect was seen on digestibility of total starch (P = 0.03; $y = -0.001x^2 + 0.024x + 93.33$; $R^2 = 0.161$), Figure 2.

Table 1. Available starch (AS), resistant starch (RS) and total starch (TS) apparent ileal digestibility coefficients of diets with different levels of amylase.

Amylase (ppm)		AS	RS	TS
0		94.81	70.77	93.13
66.7		95.70	78.01	95.11
133.3		94.73	77.61	93.97
200		95.59	78.66	94.12
266.7		93.48	84.31	91.97
P-value	Linear	0.316	<0.001	0.210
	Quadratic	0.208	0.660	0.031
	Contrast(S)	0.948	<0.001	0.486

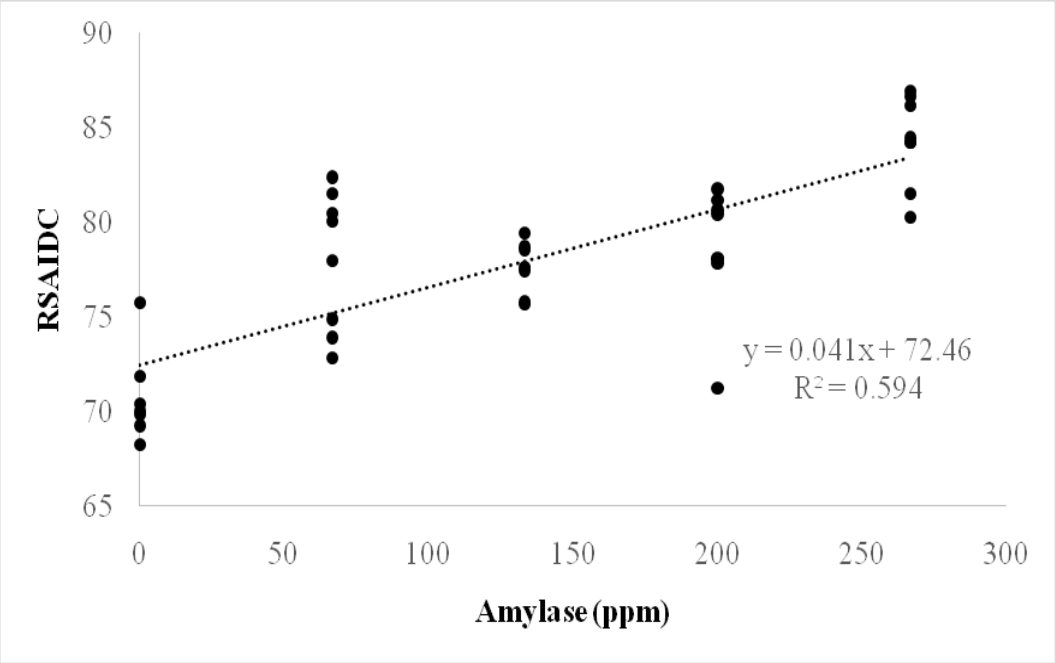

Figure 1. Resistant starch apparent ileal digestibility coefficient of diets with increasing levels of amylase (ppm).

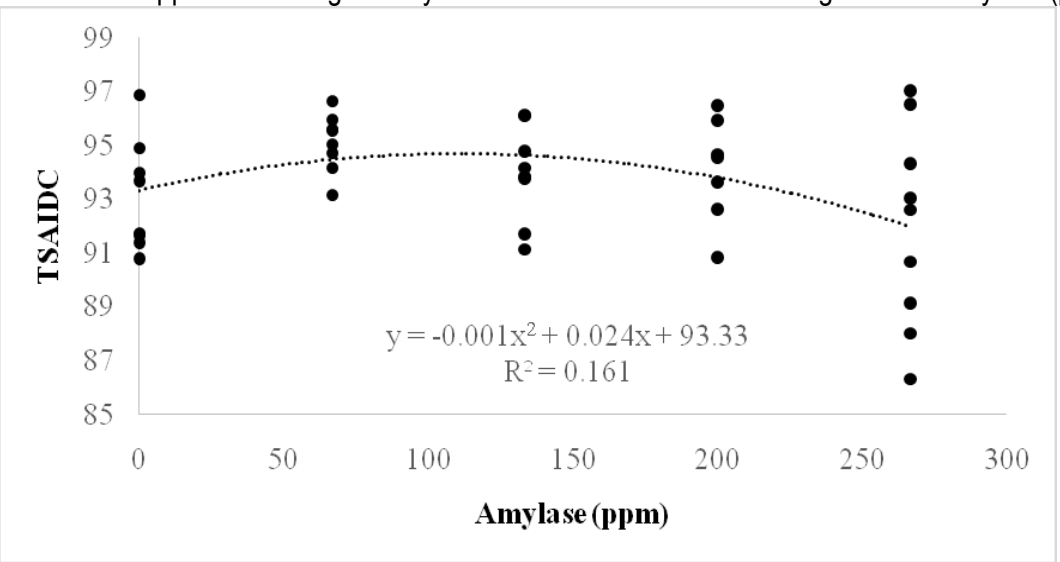

Figure 2. Total starch apparent ileal digestibility coefficient with increasing levels of amylase (ppm).

Resistant starch is divided into 3 fractions. but in starch analysis is stated that only the RS3 is included. which is retro-graded. because fractions RS1 and RS2 are solubilized by grinding and gelatinization during the analysis process (ASP, 1996; WOLF et al., 1999).

Conclusions

It was concluded that amylase improves resistant starch digestibility of corn-soy diets.

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P-098 (ID 73)

MICROBIAL PHYTASE IMPROVES PERFORMANCE AND BONE TRAITS IN BROILERS FED DIETS BASED ON SOYBEAN MEAL AND CONTAINING LUPIN MEAL

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Abstract

A completely randomized design study with a 3 x 2 factorial arrangement was conducted. A total of 480 male broiler chickens were used to determine the influence of phytase addition, plant protein source (soybean meal, soybean meal + 200 g/kg of blue lupin meal (*Lupinus angustifolius L.*) or soybean meal + 200 g/kg yellow lupin meal (*Lupinus luteus L.*)) on performance of broiler chickens, ileal Ca, P and protein digestibility, tibia bone characteristics and AMEn. All diets were deficient in Ca, available P as well as lysine and total sulphur amino acids. Birds fed different protein sources were characterized by similar BWG and FI during trial but FCR was higher when birds were fed containing yellow lupin meal ($P < 0.05$). Phytase improved BWG and FCR regardless of protein source ($P < 0.05$). Diets containing 200 g/kg blue or yellow lupin meal were found to be lower in AMEn than soybean meal diets ($P < 0.05$). Birds fed diets supplemented with phytase were characterized by higher utilization of Ca and P than birds whose feed was not supplemented with phytase ($P < 0.05$). Tibia bone ash as well as Ca content was similar across protein sources ($P > 0.05$), while tibia P content was lower when SBM or blue lupin meal was used ($P < 0.05$). Birds fed diets containing blue lupin meal were characterized by the highest tibia P content ($P < 0.05$). Birds fed phytase-supplemented diets were characterized by higher tibia Ca and P content irrespective of protein source.

Keywords: lupines, legumes, exogenous enzyme, tibia, performance

Introduction

Whole lupin seeds contain meaningful concentrations of phytate P possibly limiting the use of lupin in poultry feeding. Phytate in plant feedstuffs can interact electrostatically with dietary (and possibly endogenous) proteins and amino acids which negatively influences protein utilization and increases endogenous protein loss (Kong and Adeola, 2011, Gehring et al., 2013).

The research performed earlier by Steiner et al. (2007), showed that about two thirds of total P in legume seeds, are bound to phytate and lupin seeds may contain up to 4.4 g phytate-P/kg DM. The presence of phytate P in common maize/wheat SBM diets is no longer a problem because of widespread phytase use. However, the effectiveness of phytase in SBM-lupin diets has attracted less attention. As reported by Biehl and Baker (1997), supplemental phytase failed to elicit a gain or feed efficiency response in broiler chickens fed a maize-peanut meal diet, in contrast to SBM-based diets. (Steiner et al., 2007) found that the location of phytate in cereal grains and oilseeds varies among sources and in lupins strong binding of phytate to protein occurs. The objective of the research was to test the hypothesis that phytase efficacy could be affected by a diet protein source (representing phytate-protein matrix). In addition, ileal digestibility was performed to determine whether phytase improved protein, Ca and P utilization.

Material and Methods

The study consisted of a completely randomized experimental design with a 3 x 2 factorial arrangement of treatments, three sources of protein (SBM, yellow or blue lupin meal) and with or without phytase (RONOZYME HiPhos, DSM Nutritional Products, Kaiseraugst, Switzerland, contained 5000 U of phytase activity/g). The experiment was conducted with 480 broilers (Ross 308). Chicks (initial individual weight: 40±2 g) were divided into six dietary treatments with 10 replications with 8 birds in each. On day 36, 10 chickens from each group were sacrificed by CO_2 gas, according to the recommendations for euthanasia of experimental animals and the ileum was removed. Ten digesta were flushed from the terminal ileum (15 cm, adjacent to the ileo-caecal junction) and pooled by cage (2 birds/sample) to provide sufficient material for chemical analysis (n=5). The left tibiae of experimental birds were taken from 5 randomly chosen birds per treatment.

All diets were deficient in protein (-13 g/kg), Ca (- 3.9 g/kg), available P (- 2.3 g/kg) as well as lysine (-3.4 g/kg) and total sulphur amino acids (-2.4 g/kg). Other nutrients met or exceeded Aviagen recommendations for 1-10d old broiler chickens (Aviagen 2009). One phase feeding model was used to keep phytate and vegetable protein in the diet at constant level throughout the whole trial.

Results

From day 1 to day 35 there were no interactions between any of the main treatment terms. Birds fed different protein sources were characterized by similar BWG and FI during the whole trial. Feed conversion ratio was not affected by dietary treatments during the first two weeks of the experiment. However, considering the grower period (d14-35) of the trial or the whole experiment, birds fed diets containing YLM were characterized by the highest FCR ($P < 0.05$). There were no differences in FCR between birds fed SBM and blue lupin meal.

The use of phytase improved ($P < 0.05$) BWG and FCR regardless of the lupin type implemented during the grower period and the whole experiment. Diets containing 20% lupin meal were found to be lower in AMEn than SBM diets ($P < 0.05$). There was no effect of enzyme on AMEn and no significant treatment interaction. The ileal digestibility of protein of SBM and BLM diets was found to be lower than those based on YLM ($P<0.05$).

Table 5. Bird performance fed diets with soybean meal, blue lupin or yellow lupin, supplemented without (-) or with (+) phytase – main effects only.

Protein source	Phytase addition	1-35d			At 35d				
		BWG	FI	FCR	AMEn	CP	P	Ash	P
	-	2039	3249	1.60	11.7	0.770	0.519	39.4	11.5
	+	2122	3267	1.54	11.8	0.783	0.697	49.0	18.0
SBM		2106	3235	1.54^{b}	12.2^{a}	0.755^{b}	0.615	43.8^{b}	14.5^{b}
SBM+BLM		2102	3218	1.53^{b}	11.5^{b}	0.768^{b}	0.617	43.1^{b}	15.0^{a}
SBM+YLM		2035	3321	1.63^{a}	11.4^{b}	0.806^{a}	0.591	45.2^{a}	14.6^{b}
Phytase		<0.001	n.s.	<0.001	n.s.	n.s.	<0.001	<0.001	<0.001
Protein source		n.s.	n.s.	<0.001	<0.05	<0.05	n.s.	n.s.	n.s.

+, phytase added; –, no enzyme added. n.s., Not significant ($P > 0.05$); ^{abc} – $P < 0.05$, SBM – soybean meal, BLM – blue lupine meal, YLM – yellow lupine meal.

Birds fed YLM diets retained less N than those fed SBM or BLM ($P < 0.05$). Phosphorus as well as Ca digestibility was affected by phytase addition ($P < 0.05$) but not by plant protein source. Birds fed diets supplemented with phytase were characterized by higher utilization of Ca and P than birds whose feed was not supplemented with phytase ($P < 0.05$). There was no interaction between protein source and phytase addition for apparent ileal digestibility. Tibia bone ash as well as Ca content was similar across protein sources ($P > 0.05$), while tibia P content was different ($P < 0.05$). Birds fed diets containing blue lupin were characterized by the highest tibia P content ($P < 0.05$). Tibia ash, Ca and P content was affected by phytase supplementation. Birds fed phytase-supplemented diets were characterized by higher tibia Ca and P content irrespective of protein source. There was no interaction between protein source and phytase addition for all tibia traits.

Conclusions

It can be concluded that the use of YLM as a protein source in poultry diets may be limited due to antinutrients concentrations, when compared to other plant protein sources such as SBM. The benefits of phytase addition seem independent of the protein meal employed. It can be suggested that the use of phytase was an effective tool to improve phosphorous availability from maize-SBM and maize-SBM-YLM or maize-SBM-BLM diets. The extent to which the effect of phytase addition on nutrient digestion is associated with employed plant protein source, requires further research.

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P-099 (ID 326)

EFFECTS OF AN NSP-ENZYME SUPPLEMENTATION ON PRECAECAL NUTRIENT DIGESTIBILITY AND ARABINOXYLAN DEGRADATION IN TURKEYS.

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Abstract

The influence of NSP enzyme supplementation on arabinoxylan degradation in the ileal chyme of turkeys was investigated in order to gain more insight in their effects on nutrient digestibility. 72 1-day old turkeys were randomly allocated to 3 treatments: T1-control; T2–enzyme blend (ENZY CARBOPLUS; Lohmann Animal Nutrition GmbH), with 100 mg/kg feed; T3–with 300 mg/kg feed. Birds were kept in pens from day 1 to 28. On day 28 birds were transferred to balance cages and diets were supplemented with TiO_2. Fecal samples were collected for ME calculation over 5 days. At study end, birds were sacrificed and digesta collected for determination of prececal protein digestibility and enzymatic hydrolysis of arabinoxylan. Enzyme supplementation significantly improved weight gain (80.3 *vs.* 73.6 g/day, T3 *vs.* T1; P=0.009) and FCR (1.44 *vs.* 1.58 g/day, T3 *vs.* T1; P=0.001). This is most likely the consequence of improved ME which was numerically increased in T2 turkeys compared to T1 while ME and prececal protein digestibility of T3 was significantly increased compared to T1. The analyses of free sugars in the ileal chyme showed a significant increase of arabinose and xylose and a significant decrease of glucose in turkeys fed the enzyme blend. The significant decrease of glucose in the ileal chyme of birds fed enzymes might be a result of increased resorption before the terminal ileum as a result of higher starch degradation in the enzyme groups.

Keywords: turkeys, NSP enzymes, arabinoxylan, free sugars

Introduction

Cereal-based turkey diets are characterized by high amounts of NSP such as water-soluble arabinoxylan (AX) and β-glucans. However, the digestibility of diets containing high levels of NSP is limited and the apparent metabolizable energy (AME) and the amount of soluble NSP are negatively correlated (Choct & Annison, 1992). Both can be improved by the addition of enzymes which hydrolyze AX to low molecular compounds and monomer sugars such as xylose and arabinose. The current study investigates the prececal (pc) protein digestibility and enzymatic hydrolysis of AX in the ileal chyme in response to xylanase and β-glucanase in turkeys.

Material and methods

72 one-day old turkeys were randomly allocated to 3 treatments. The control group received a diet without enzymes (T1). T2 was fed a diet with 100 mg/kg diet of a xylanase/ β-glucanase blend (Enzy Carboplus; Lohmann Animal Nutrition GmbH, xylanase activity 7000 LXU/g and β-glucanase activity 600 LXU/g). T 3 received 300 mg of the enzyme blend. The turkeys were kept in 12 pens of 6 animals each and were fed a diet P1 (day 1-14) and a diet P2 (day 14-36). The diet composition is presented in Table 1.

At day 28, all animals were transferred to individual balance cages. Excreta were collected for ME calculation over 5 days. During the balance period feed was restricted.

At the end of the study, animals were sacrificed and digesta were collected from the proximal two-thirds of the intestine between Meckel's diverticulum and 2 cm anterior to the ileo-ceco-colonic junction. Samples from 3 animals were pooled for analyses. Samples were freeze dried and free sugars were analyzed after solution in water and after derivatization to alditol acetates by gas chromatography. Individual body weight was measured at 0, 14, 28 and 36 days. Feed intake, average daily gain (ADG) and FCR were calculated for all phases.

Experimental data were analysed statistically by means of analysis of variance (ANOVA) using the program STATISTIKA FOR WINDOWS™. In case of significant F values (P<0.05), mean values of the treatment groups were tested for significant differences by means of Tukey-HSD-test.

Table 1: Ingredient composition (%); AME and protein content of diets

	P1	P2
Wheat	44.6	44.9
Rye	5.0	10.0
Soybean meal (48% CP)	40.0	36.0
Potato protein	2.0	0
Palm oil	3.0	3.2
Premix*	1.0	1.0
Monocalcium phosphate	2.5	2.5
Limestone	1.0	1.0
NaCl	0.3	0.3
Choline chloride	0.1	0.1
Lysine HCl	0.3	0.32
DL-Methionine	0.2	0.18
TiO_2	0	0.5
AME (MJ)	11.7	12.0
Protein (g)	269	241

*per kg premix: Ca 330 g, Vit. A 1200000 IU, Vit. D_3 400000 IU, Vit. E 4000 mg, Vit. K_3 240 mg, Vit. B_1 210 mg, Vit. B_2 720 mg, Vit. B_6 400 mg, Vit. B_{12} 2.3 mg, niacin 6,6 g, Ca-P- pantothenate 1.4 g, folic acid 150 mg, biotin 17,5 mg, iron 5.0 g, copper 1g, zinc 6.0 g, manganese 8.0 g, cobalt 25 mg, iodine 100 mg, selenium 35 mg

Results and discussion

Turkeys receiving the enzyme blend showed significantly improved ADG and FCR during the performance period from day 1-28 days on trial compared to the control (Tab. 2).

Table 2. Zootechnical performance of turkeys (1 to 28 days on trial)

Parameter	**T1** Control	**T2** (100 mg/kg)	**T3** (300 mg/kg)	**SEM**	**p-value**
BW, 1 day (g)	62.3	63.3	63.3	0.48	0.710
BW, 28 days (g)	840^{a}	1170^{b}	1090^{b}	5.0	<0.001
ADFI (g/d)	49.3^{a}	60.8^{b}	57.2^{ab}	1.8	<0.011
ADG (g/d)	27.9^{a}	39.4^{b}	36.8^{b}	1.6	<0.001
FCR	1.77^{b}	1.54^{a}	1.55^{a}	0.04	0.006

During the balance period, birds fed the enzymes also had significantly higher body weights (P<0.001), improved ADG (P=0.006) and improved FCR (P=0.002) compared to control birds.

This is most likely the consequence of improved ME which was numerically increased in T2 and significantly increased in T3 turkeys compared to the control as well as the improved prececal protein digestibility which was numerically increased in T2 and significantly increased in T3 compared to control (Tab. 3).

The analyses of free sugars in the ileal chyme showed a significant increase of arabinose and xylose in treatment groups. A significant decrease in the concentration of glucose and mannose was observed in turkeys fed the enzyme blend (Tab. 3).

Table 3: Free sugar concentrations, nutrient digestibility and energy utilization

Parameter	**T1** Control	**T2** (100 mg/kg)	**T3** (300 mg/kg)	**SEM**	**p-value**
AX diet (% DM)	4.69	4.66	4.69		
Free sugars in the prececal digesta					
Arabinose (% DM) [1]	0.13 [a]	0.17 [ab]	0.21 [b]	0.01	0.003
Xylose (% DM) [1]	0.06 [a]	0.10 [b]	0.13 [c]	0.01	<0.001
Mannose (% DM) [1]	2.01 [b]	0.68 [a]	1.14 [ab]	0.21	0.026
Glucose (% DM) [1]	16.5 [b]	4.8 [a]	8.5 [ab]	1.44	<0.001
Galactose (% DM) [1]	0.19	0.19	0.14	0.03	0.655
Prececal protein digestibility (%) [1]	81.2 [a]	83.2 [ab]	83.7 [b]	0.43	0.029
ME (MJ/kg feed) [2]	11.7 [a]	11.8 [ab]	12.0 [b]	0.05	<0.004

[1] 8 replicates/treatment of 3 pooled bird samples/replicate, [2] 24 replicates/treatment
[a,b] Values in the same row with no common superscript are significantly different ($P \leq 0.05$).

Data reveal that turkeys receiving the enzyme blend had lower amounts of glucose in the digesta than the control. We assume that the low glucose content of digesta is caused by a higher starch digestibility and an improved absorption of glucose as it was found in broiler chickens fed xylanase (Choct et al., 1999).

Conclusion

Dietary inclusion of the enzyme blend significantly improved ADG and FCR of turkeys. This is the result of an improved ME content and precaecal digestibility of protein, improved NSP degradation and therefore increased availability of free sugars.

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P-101 (ID 150)

THE EFFECT OF DIETARY FEED STRUCTURE, PHYTASE AND XYLANASE APPLICATION ON BROILER PERFORMANCE

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Abstract

900 broilers were distributed randomly to one of 10 treatments (10 pens x 9 birds each). The birds were fed pelleted wheat-soybean-based diets, in 5 treatments with part of the wheat as whole grain. Starter, grower and finisher diets were adequate (PC, 4.5, 4.4 and 4.1 g/kg P_{av} and 10, 8.8 and 8.2 g/kg Ca; respectively) or reduced by 1.5g/kg P_{av} and 1.6g/kg Ca (CC_{500}). All CC_{500} diets contained 500 FTU/kg phytase or were supplemented with a further 1000 FTU/kg phytase, and were fed with or without 16,000 BXU/kg xylanase. Performance was measured at d10, d24, and d42, and data analysed as a 5 x 2 factorial ANOVA (5 treatments, with or without whole kernel inclusion). Differences at $P<0.05$ were declared as significant. No interactions were detected ($P>0.05$). Birds fed CC_{500} diets achieved the same ADG and FCR as birds fed PC diets ($P<0.05$). Compared to CC_{500}, the additional phytase improved ADG and FCR from the beginning of the trial and outperformed birds fed the PC diets. Addition of xylanase improved ADG and FCR in the last feeding period (d24-42) and this was significant over the whole trial period (d1-42). When added on top of the high phytase application, xylanase further improved FCR (from 1.52 to 1.49 $P<0.05$). The inclusion of whole wheat grains resulted in similar improvements in FCR from d25 onwards ($P<0.05$) as the xylanase. The data shows that addition of xylanase and/or whole wheat grains into diets containing a high dosage of phytase results in further improvements in broiler performance.

Keywords: phytase, xylanase, whole wheat grain, broiler

Introduction

Addition of whole wheat grains to broiler diets is common practice but different strategies in respect of the inclusion rate and application are applied commercially (fed along with or included in the pellet). Benefits of whole wheat grain feeding include improved growth and feed efficiency due to increased gastric retention time and endogenous enzyme secretion, resulting in better nutrient digestibility (Svihus et al., 2004). Because phytases and xylanases are typically added to wheat-based broiler diets and experiments following the effect of whole wheat grains often do not contain these enzymes, the objective of this trial was to examine the interaction between whole or ground wheat, xylanase and phytase dose on bird performance.

Materials and Methods

900 male broilers (Ross 308) were distributed to 10 treatments (10 pens, 9 birds each). The birds received starter (d1 to 10), grower (d11-24) and finisher diets (d25-42) based on wheat, soybean meal, corn and soya oil (for wheat, Na, Ca and P_{av} contents, see Table 1). Adequate diets (PC) were not supplemented by enzymes whereas all mineral reduced diets (CC) contained phytase (Quantum Blue, 6-phytase produced by *T. reesei*) at 500 (CC_{500}) or 1500 (CC_{1500}) FTU/kg feed, without or with xylanase (Econase XT, endo-1,4-beta-xylanase produced by *T. reesei*, 16,000 BXU/kg feed; CC_{500} + Xyl or CC_{1500} + Xyl). All feeds were pelleted, with the wheat included either milled or partly added as whole grains (50, 150 and 250 g/kg in starter, grower and finisher feeds, respectively).

Table 1. Wheat, P, Ca and Na contents (g/kg) in the positive (PC) and commercial diets (CC)

	PC (starter, grower, finisher)	CC (starter, grower, finisher)
Wheat	400, 464, 567	400, 481, 585
P available	4.8, 4.4, 4.1	3.3, 2.9, 2.6
Ca	10, 8.8, 8.2	8.4, 7.2, 6.6
Na	1.7, 1.7, 1.6	1.4, 1.4, 1.3

Birds were individually weighed at the start and end of each feeding phase and feed consumption measured accordingly. Nutritional contents of the diets were analysed following AOAC (2000) methods and enzyme contents analysed by the supplier using internal ELISA methods. Data was analysed as a 5 x 2 factorial ANOVA (5 treatments, with or without whole grains) with the pen as experimental unit, and differences at $P<0.05$ declared as significant.

Results and Conclusion

The analytical composition of experimental feeds was in good agreement with the calculated values, with P and Ca contents being slightly above the estimated values, but the targeted differences between PC and CC diets were as expected. Analysed phytase activities were <50 (PC), 793-990 FTU/kg (CC_{500} treatments), 2240-2930 FTU/kg (CC_{1500} treatments) and analysed xylanase activities were 14200-20700 BXU/kg in diets with and <2000 BXU/kg in those without added xylanase.

Performance results of birds achieved during the entire trial period are shown in Table 2. No interactions were analysed between wheat form and enzyme application.

The addition of whole wheat grains improved FCR ($P<0.05$) over the whole trial period without any effect on BW.

Birds fed diets including a standard phytase content (500 FTU/kg feed, CC_{500}), accompanied with the dietary P, Ca and Na reduction as recommended for the phytase tested, achieved breeder target BW and FCR in all test periods and gave the same performance as PC birds ($P>0.05$). Feeding an increased phytase level (1500 FTU/kg feed) further improved FCR (CC_{1500} compared to CC_{500} and PC treatments, $P<0.05$).

These results confirmed recently published data showing that high dosages of the present phytase tested in diets reduced in P, Ca and Na as recommended for a standard phytase dose improves broiler performance above the level achieved by nutritionally well-balanced diets (Walk et al., 2013).

Xylanase, when added to diets which already contained a standard phytase level, further improved BW and FCR (CC_{500} +Xyl compared to CC_{500}, $P<0.05$)

On top of the high phytase application, the xylanase effect on broilers BW was not significant but FCR was further improved (CC_{1500} +Xyl compared to CC_{1500}, $P<0.05$). This additional effect mainly occurred in the final feeding phase (data not shown). The most beneficial FCR was in birds fed the higher phytase dose plus xylanase (CC_{1500}+Xyl, $P<0.05$ compared to all other treatments).

Whether the additional effect of xylanase is due to an increased nutrient digestibility or has influenced phytate degradation, as shown for the tested enzymes by Zeller et al., 2015, needs further investigation. Additionally, xylanase also might increase digesta retention time through changes in the caecal microbiome inducing a hormonal effect on gastric retention time (Singh et al., 2012).

It can be concluded that dietary inclusion of whole wheat grain, a high phytase level and xylanase can improve FCR of broilers.

Table 2. Performance of broilers (day 1 to 42, n = 10)

Treatment	BW at day 42 (kg)	ADFI (g)	FCR (kg/kg)
Wheat milled:			
PC	3.314 ± 0.039	125.2 ± 5.7	1.569 ± 0.056
CC_{500}	3.362 ± 0.096	122.2 ± 3.5	1.546 ± 0.029
CC_{500} +Xyl	3.428 ± 0.092	126.2 ± 9.2	1.516 ± 0.016
CC_{1500}	3.388 ± 0.084	124.0 ± 2.7	1.532 ± 0.035
CC_{1500} +Xyl	3.422 ± 0.093	123.3 ± 4.3	1.495 ± 0.021
Whole wheat:			
PC	3.361 ± 0.107	124.1 ± 5.1	1.543 ± 0.033
CC_{500}	3.345 ± 0.127	123.5 ± 9.8	1.537 ± 0.030
CC_{500} +Xyl	3.350 ± 0.109	123.7 ± 5.3	1.511 ± 0.031
CC_{1500}	3.414 ± 0.061	121.2 ± 2.7	1.506 ± 0.025
CC_{1500} +Xyl	3.450 ± 0.089	120.5 ± 4.7	1.482 ± 0.022
Wheat form			
Wheat milled	3.383 ± 0.106	124.2 ± 5.5	1.532^{b} ± 0.042
Whole wheat	3.404 ± 0.106	122.6 ± 5.9	1.516^{a} ± 0.035
Enzyme			
PC	3.338^{b} ± 0.121	124.7 ± 5.3	1.556^{c} ± 0.047
CC_{500}	3.354^{b} ± 0.110	122.9 ± 7.2	1.541^{c} ± 0.029
CC_{500} +Xyl	3.439^{a} ± 0.099	125.0 ± 7.4	1.514^{b} ± 0.024
CC_{1500}	3.401^{ab} ± 0.073	122.6 ± 3.0	1.519^{b} ± 0.032
CC_{1500} +Xyl	3.436^{a} ± 0.090	121.9 ± 4.6	1.489^{a} ± 0.022
SEM	0.011	0.576	0.004
$P_{(wheat\ form)}$	0.287	0.180	**0.013**
$P_{(enzyme)}$	**0.004**	0.374	**0.001**
$P_{(interaction)}$	0.900	0.771	0.737

[ab] Values with different superscripts within column and parameter differ significantly (P<0.05)

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P-102 (ID 327)

EFFICACY OF PHYTASE SUPPLEMENTATION IN UNCOATED AND COATED FORM IN MALE BROILER CHICKENS

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Abstract

Heat stability of enzymes is important in pelleted feed. Coating is one option to increase the survival rate but might affect the enzyme release in the intestine. The aim of this study was to evaluate the effect of uncoated and coated phytase on performance and bone mineralization in broilers. 100 day-old chicks were housed in balance cages and allotted to 5 treatments. Starter and grower diets were formulated according to recommendations, except that P was reduced to allow a response to phytase addition. Treatments were control, 250 FTU/kg uncoated, 500 FTU/kg uncoated, 250 FTU/kg coated and 500 FTU/kg coated *E.coli* derived 6-phytase (Enzy Phostar, Lohmann Animal Nutrition). Due to inadequate P supply control birds reached 80% of the breed specific body weight (BW). Phytase addition led to significantly improved BW gain (13.4%) and FCR (10.4%) compared to control birds. Animal performance correlated with the dose level irrespective of the phytase form. Based on ileal P-digestibility the coated phytase was numerically (250 FTU/kg) or significantly (500 FTU/kg) better compared to the uncoated form. Both, ileal digestibility and tibia bone deposition of P and Ca also were dose-dependent (partly significant). In conclusion, for ileal digestibility and tibia mineralization the coated phytase at dose levels of 250 and 500 FTU/kg was similar or superior to the uncoated form. It was shown that the coating technology did not affect the biological efficacy of this new phytase.

Keywords: phytase, coating, tibia mineralization, broiler

Introduction

Enzymes like all proteins are heat sensitive. However, as the biggest proportion of feed is pelleted heat stability of enzymes is very important. Coating is one option in order to increase enzyme survival rate during the pelleting process. Nevertheless, coating might affect the release of the enzyme in the gastro-intestinal tract and therefore compromise its efficacy. The objective of the present study was to demonstrate the efficacy of a new phytase as uncoated or coated formulation when added to low phosphorus diets for broiler chickens.

Material and methods

100 one-day-old male broiler chickens (COBB) were housed in balance cages with 2 birds per cage and allotted to 5 treatments with 10 cages per treatment. The feeding period included starter (1-14 d) and grower diets (15-35 d) formulated according to the recommendations of GfE (1999), except that phosphorus was reduced in order to allow for a response to additional phytase supply as coated or uncoated product (*E. coli*-derived 6-phytase, Enzy Phostar, Lohmann Animal Nutrition) at dose levels of 250 or 500 FTU per kg feed. Diet composition is shown in table 1. Investigated parameters were body weight gain, feed intake, FCR as well as ileal digestibility (P, Ca), and left tibia bone mineralization at the end of the trial period.

Statistical analyses were performed with SPSS (IBM SPSS Version 21) and based on Oneway Anova. All treatment least squares means were compared with each other and the Tukey adjustment was used to control for experiment wise error.

Table 1. Ingredients and nutritional characteristics of the basal starter and grower diet

Ingredients		**Starter (1-14 d)**	**Grower (15-35 d)**
Wheat	%	46.33	49.46
Maize	%	10.00	10.00
Soybean meal	%	34.50	30.15
Soybean oil	%	5.60	6.80
Limestone	%	1.56	1.36
Monocalcium phosphate	%	0.50	0.13
DL-Methionine	%	0.23	0.24
L-Lysine HCl	%	0.08	0.16
Premix	%	1.20	1.20
Nutritional characteristics			
ME_N*	MJ/kg	12.20	12.60
Crude protein	g/kg	220.00	205.00
Lysine	g/kg	12.40	11.80
Methionine / cystine	g/kg	9.40	9.10
Calcium	g/kg	8.00	6.50
Total phosphorus	g/kg	5.00	4.00
Digestible phosphorus	g/kg	2.50	2.20

* Estimated according to equation of WPSA 1984

Results and discussion

Due to the inadequate phosphorus supply, productive performance (table 2) of birds fed diets without phytase addition reached approximately 80% of the intended weight gain given by the breeding company. Phytase supplementation led to significantly increased body weight gain (+13.4%) and significantly reduced (10.4%) FCR compared to birds receiving no phytase. When feeding the new phytase at the dose level of 500 FTU the body weight gain was slightly (3.3%) higher than that recorded for the lower dose level (250 FTU). Feed intake showed no consistent preparation or dose dependent effects. FCR of the control was 1.69. Phytase supplementation significantly improved FCR in all groups by an average of 10.4% due to the enhanced body weight. Regarding body weight gain and FCR there were no preparation dependent effects detected.

As expected, the present study demonstrated that dietary inclusion of phytase significantly improved apparent ileal P-digestibility (table 2) in comparison to broilers fed a low phosphorus diet without phytase addition. Feeding phytase (uncoated, coated) at 500 FTU resulted in significantly better apparent ileal P-digestibility when compared to the lower dose level (250 FTU). Dose dependent effects on ileal digestibility were also found for crude ash and Ca. However, phytase effects in comparison to the control diet were lower than those recorded for digestibility of phosphorus. At each phytase dosage, coating did not influence ileal digestibilities of ash and Ca when compared to the uncoated formulation, while for P-digestibility it was found that the coated phytase gave numerically (250 FTU) or significantly (500 FTU) higher figures in comparison to the uncoated preparation.

Significant enzyme effects on tibia mineralization (table 3) were seen for crude ash, P and Ca contents in the fat-free dry matter in comparison to broilers fed the control diet (A). Deposition of crude ash, P and Ca showed no statistically firm effect of coating except for P-deposition at the 500 FTU dosage. In general, tibia parameters also exhibited a numerical, dose dependent effect.

For demonstrating the efficacy of the new phytase in uncoated or coated form, ileal digestibility and tibia mineralization were more sensitive than productive performance. Therefore, based on apparent ileal P-digestibility it could be shown that the coated form of the phytase was numerically (250 FTU) or significantly (500 FTU) better in comparison to the uncoated preparation. These findings were not expected and are in contrast to the report by Owusu-Asiedu et al. (2007) who could not find any significant difference in the efficacy of a coated and uncoated *E. coli* phytase.

Table 2. Effect of phytase on performance and digestibility during the total feeding period

Treatment		A	B	C	D	E
Phytase	FTU kg $^{-1}$	0	250 n-ct	500 n-ct	250 ct	500 ct
BW gain	g	1546.4 ± 68.5^{a}	1736.1 ± 79.6^{b}	1783.7 ± 58.5^{b}	1713.8 ± 63.0^{b}	1781.8 ± 34.8^{b}
FCR		1.691 ± 0.081^{a}	1.536 ± 0.063^{b}	1.485 ± 0.060^{b}	1.534 ± 0.044^{b}	1.504 ± 0.048^{b}
Ileal digestibility						
Crude ash	%	46.89 ± 5.56^{a}	50.80 ± 4.86^{ab}	54.07 ± 3.73^{b}	50.10 ± 2.93^{ab}	53.64 ± 4.95^{b}
Phosphorus	%	42.95 ± 6.15^{a}	52.41 ± 3.30^{b}	60.29 ± 2.95^{c}	55.65 ± 3.98^{bc}	65.93 ± 3.29^{d}
Calcium	%	52.75 ± 6.03^{a}	58.38 ± 4.98^{abc}	62.02 ± 2.83^{bc}	57.04 ± 4.12^{ab}	63.84 ± 4.25^{c}

^{ab} Values with different superscripts within lines differ significantly (P<0.05) n-ct = uncoated, ct = coated

Table 3. Effect of phytase on tibia bone mineralization of broilers at 35 days

Treatment		A	B	C	D	E
Phytase	FTU kg $^{-1}$	0	250 n-ct	500n-ct	250 ct	500 ct
Tibia bone weight (as is)	g	10.1 ± 1,9	10.9 ± 1.6	11.6 ± 1.1	11.1 ± 1.2	10.7 ± 1.0
Tibia fat-free dry matter	%	36.88 ± 4.10	37.98 ± 2.54	37.10 ± 1.33	37.45 ± 2.99	37.07 ± 2.08
Tibia bone mineralization						
Crude ash	g kg^{-1} DM	262.0 ± 21.3^{a}	308.2 ± 23.8^{b}	313.1 ± 13.6^{b}	311.9 ± 32.1^{b}	320,3 ± 22.1^{b}
Phosphorus	g kg^{-1} DM	34.6 ± 2.9^{a}	40.5 ± 2.9^{b}	43.3 ± 2.9^{b}	42.4 ± 4.9^{b}	49.6 ± 4.5^{c}
Calcium	g kg^{-1} DM	92.8 ± 9.8^{a}	106.4 ± 7.1^{bc}	110.5 ± 6.6^{bc}	108.7 ± 6.6^{bc}	113.1 ± 5.5^{c}

^{ab} Values with different superscripts within lines differ significantly (P<0.05) n-ct = uncoated, ct = coated

Conclusions

Based on apparent ileal digestibility and tibia bone mineralization it can be concluded that the coating had no negative influence on the biological efficacy of the tested phytase. The new phytase in coated form at the dose levels of 250 and 500 FTU per kg of diet was superior (partly significant) to the uncoated form. Moreover, the benefits of uncoated or coated new phytase correlated with the dose level.

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P-104 (ID 203)

ADDITIONAL EFFECT OF ENDOXYLANASES ON TOP OF SUPERDOSED PHYTASE ON BROILER PERFORMANCE

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Abstract

To study the additional effect of xylanase on top of superdosed phytase (SD), a trial was organised at KU Leuven using 450 day-old Ross 308, floor pen housed, male broilers. Three dietary treatments were included: a standard wheat-corn based diet supplemented with 1500 U/kg feed of 6-phytase (= SD), SD with additional bacterial (*Bacillus subtilis*) endo-1,4-β-xylanase (100 mg/kg feed) (= BX), and SD with additional fungal (*Trichoderma citrinoviride)* endo-1,4-β-xylanase (100 mg/kg feed) (=FX). Ten replicates of 15 broilers were allocated to each of these 3 treatments during 42 days.

Supplementing SD with BX or FX significantly improved body weight (BW) (2925 *vs* 3085 and 3022 g) and corrected FC ($FC_{corr-2\,kg}$) (1.31 *vs* 1.21 and 1.26). With a FC of 1.65, BX was numerically better than FX (FC = 1.68) and SD (FC = 1.67). Comparing the results of both endo-1,4-β-xylanase products, the bacterial showed to be more efficient on $FC_{cor-2\,kg}$ ($p < 0.05$), BW ($p < 0.1$) and FC (numerically).

To conclude, endo-1,4-β-xylanases proved to be beneficial on top of superdosed phytase, and clear differences were seen between fungal and bacterial endo-1,4-β-xylanase.

Keywords: xylanase, bacterial, broilers, performance

Introduction

Since superdosing of phytase is gaining interest in the broiler industry and speculations are made about the mode of action of the "extra-phosphorous" effect, some doubts can arise whether there will still be a sufficient additional effect of NSP enzymes. Phytases and xylanases act on complete different substrates and the mode of action, improving broiler performance, is different as well. Maybe the effect of combining both classes of enzymes is not strictly additive, enough arguments exist to believe in cost effectiveness of using both enzymes. The bio-efficacy of 2 different xylanases, on top of superdosed phytase, was determined in a zootechnical performance trial.

Material & methods

The experiment was organised and carried out by the Catholic University of Leuven and consisted of a floor pen trial, using 30 pens (3 treatment * 10 replicates) of 15 male broiler chickens per pen. The experimental house is divided into pens of equal size, arranged along a central aisle. The pens had a surface of 0.8 m² each, with built up wood shavings as bedding. The temperature of the building was monitored and environmental conditions during the trial were optimum to the age of the animals. The lighting scheme was 24 hours light per day from day 1 to day 14 and a repeating cycle of 1 hour light and 3 hours dark from day 15 to day 42. The diets were provided ad libitum in one feeding trough per pen. Water was provided ad libitum with two drinking nipples per pen. Standard floor pen management practices were used throughout the experiment.

Male Ross 308 chickens were obtained from Belgabroed, Belgium. The breeder flock history and vaccination record at the hatchery were recorded. The broilers were vaccinated at the farm on day 14 against Gumboro and Newcastle Disease via drinking water. Broiler weights by pen by sex were recorded on days 1, 14 and 42. Feed intake was measured immediately after weighing on day 14 and day 42.

Three dietary treatments were included: a standard wheat-corn based diet supplemented with 1500 U/kg feed of 6-phytase (= SD), SD with additional bacterial (*Bacillus subtilis*) endo-1,4-β-xylanase (100 mg/kg feed) (= BX), and SD with additional fungal (*Trichoderma citrinoviride)* endo-1,4-β-xylanase (100 mg/kg feed) (= FX). Ten replicates of 15 broilers were allocated to each of these 3 treatments during 42 days.

All experimental diets (composition in table 1) were manufactured by Research Diet Services in the Netherlands. All feeds were fed in pellet form. Starter feed was fed from day 1 to 14. On day 14, non-consumed starter was weighed by

pen and discarded. Grower and finisher feeds were issued and fed until day 28 and 42 respectively. On day 42, non-consumed feed was weighed by pen and discarded.

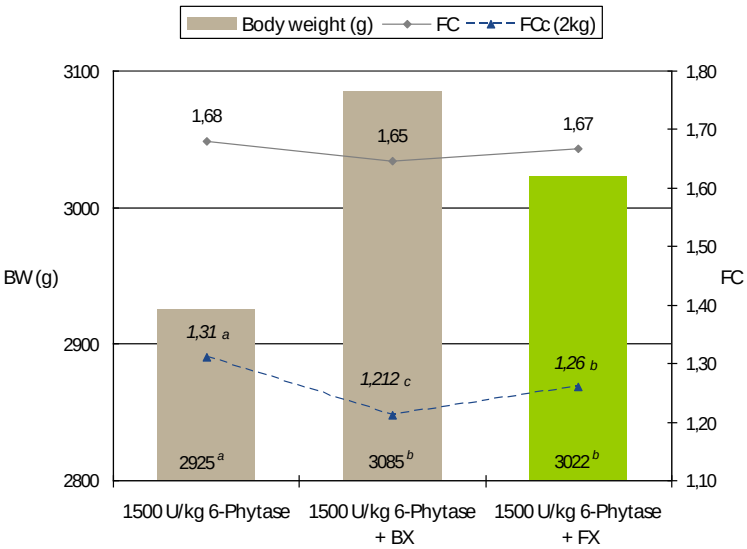
TABLE 1: DIET COMPOSITION			
	Starter	**Grower**	**Finisher**
Ingredients (%)			
Wheat	40,57	44,58	52,84
Corn	20,00	20,00	20,00
Soybean Meal 47	33,22	29,37	21,89
Fats/oils	2,23	3,09	2,75
Monocalciumphosphate	0,89	0,46	0,22
Limestone	1,23	0,87	0,64
Nutrients (%)			
Crude protein	22,00	20,50	18,00
Crude fat	4,19	5,01	4,64
Crude fibre	2,66	2,61	2,53
Calcium	0,72	0,51	0,36
Phosphorous P	0,60	0,49	0,41
Sodium	0,16	0,15	0,14
Potassium	0,99	0,91	0,79
6-phytase (U/kg)	1500	1500	1500

Statistical analysis of average daily gain, feed intake and feed conversion was done with a linear model (SAS software). Model choice was based on the R^2-value, on the root MSE and the distribution of the residuals.

Results

In this trial significant treatment effects were observed for final body weight (P = 0.001) and corrected feed conversion (P = 0.001), while feed intake was not influence by the treatments (P = 0.48).

The results in Figure 1 show that both NSP enzymes were able to increase significantly the final body weight of the animals. Adding xylanases increased final body weight by 5.5 % and 3.3 % for resp. BX and FX. Improving daily gain without changing feed intake means that feed utilisation became more efficient after xylanase supplementation. Corrected feed conversion improved by adding xylanase in a superdosed phytase diet. $FC_{cor-2\,kg}$ improved by 7.5 and 3.8 % for resp. the BX and FX supplemented group.



Although both xylanase sources improve the performance of the broilers, it's important to mention that they cannot be considered as equally effective. BX supplemented broilers were 63 g heavier (P < 0.1) at the end of the trial and their $FC_{cor-2\,kg}$ was 0.05 lower (P < 0.05) compared to the FX animals.

These data confirm the hypothesis that the mode of action of xylanase - which is a xylanase-specific combination of reducing viscosity, liberating entrapped nutrients and creating prebiotic substances - is not the same as the mode of action of phytase, even when superdosed.

Conclusions

Based on this trial, there shouldn't be any doubts about the technical or economical benefits of adding xylanase in combination with superdosed phytase. A closer look however to the source and type of xylanase is important. Efficacy may vary considerably between different types of xylanases.

P-105 (ID 307)

EFFECT OF A CARBOHYDRASES COMPLEX IN FEEDS WITH DIFFERENT METABOLIZABLE ENERGY AND AVAILABLE AMINO ACIDS LEVELS FOR BROILERS REARED UNDER A HOT CLIMATE CONDITION

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Abstract

The objective of this study was to evaluate the effect of a carbohydrase complex (CC) added to corn-soybean-based broiler feed formulated with different apparent metabolic energy (AME) and digestible amino acids (DAA) levels. Four diets were prepared as following: T1, Positive Control (PC) with regular commercial nutritional levels; T2, Negative Control (NC) 1, with less 80 kcal of AME kg^{-1} and less 2% of DAA; T3, NC 2, -80 kcal kg^{-1} and -4% of DAA; and T4, NC 3, -80 kcal kg^{-1} and -6% of DAA. CC (Rovabio® ADVANCE L) was added or not in each of those four diets, totalling 8 treatments, with 12 repetitions and 40 male birds in each. Air temperature inside the facility was kept above the breeder recommendations (29 °C) and humidity was in constant rise during the experimental (0-36 d). DAA reduction in feed linearly reduced (P<0.01) all performance parameters. No interaction between feed nutritional levels and the addition of the CC was observed. For the overall period, the addition of CC improved (P<0.01) BWG (+3.3%) and FCR (-2.7%), regardless of nutritional reduction levels. The efficacy of energy and protein deposition (calculated as g of BWG for each cal of AME or mg of digestible Lysine ingested) were significantly improved by the CC addition (+2.7% and +2.8%, respectively). This study also allowed to estimate the ideal DAA reduction thanks to CC addition between -2% and -4%, when AME is reduced by 80 kcal.

Keywords: NSP-enzymes, broilers, amino acids, nutritional matrix, nutrient deposition

Introduction

The benefits of adding carbohydrase complex (CC) to the diets of non-ruminant animals, particularly poultry, are well established (Campbell and Bedford 1992). Enzymes have been shown to improve performance and nutrient digestibility when added to poultry diets containing cereals (Bedford and Schulze 1998). Therefore, data illustrating practical reformulation were scarce. Improved knowledge of CC effect on practical feed formulation utilization could result in development of CC directed towards far more specific targets. Improvement of diet AME content have already been evaluated previously. Data on amino acid deposition were less important. The aim of the present study was (1) to investigate the effect of a reduction of DAA on performance and (2) to measure the effect of enzyme supplementation on diets.

Material and Methods

Experiment were carried out in a commercial farm. The study was conducted as a 4 × 2 factorial blocked arrangement of treatments with 4 levels of apparent metabolizable energy adjusted for zero nitrogen retention (AMEn), and digestible essential amino acids (DAA), with or without CC supplementation. Diet nutrient constraints for positive control (PC) were fixed to meet the minimum requirements relative to energy content according to the Rhodimet Nutrition Guide (Adisseo 2013). Feed were based on corn soybean meal. Digestible amino acids and metabolizable energy for negative control (NC) were obtained by reduction of vegetable oil and synthetic amino acid/soybean meal reduction, respectively. The feeding program consisted of 3 phases: starter diet from 0 to 14 d, grower diet supplied from 15 to 28 d and finisher diet from 29 to 36 d. Reformulation resulted in 3 diets: negative control (NC) 1, with less 80 kcal of AME kg^{-1} and less 2% of DAA; T3, NC 2, -80 kcal kg^{-1} and -4% of DAA; and T4, NC 3, - 80 kcal kg^{-1} and -6% of DAA. Enzyme was included at 200ml/ton.

For this experiment, 3840 one-d old Ross PM3 male broilers (body weight 40±1.4g) were used and allocated into 96 pens, with 40 chickens per pen. Pens were used in factorial and completely blocked design with 12 pens per treatment. High temperature was maintained during the experiment at 29°C from day 4 to 36. Humidity was gradually increased from 45 to 78% from day 0 to 36. Feed and water were provided *ad libitum* throughout the experiment. Animal feed intake (FI) and body weight gain (BWG) were recorded 4h after the removal of feed at 21 and 35 d of age corresponding to the end of the starting, the growing and the finishing periods, respectively. Mortality was recorded daily. Carcass

analysis were performed at the end of the experiment. Carcass, breast and legs were weighted and mass relative to the live body weight were estimated.

Results

All the animal remained healthy during the experiment. Performance of the positive control were in agreement with standard performances observed in the facility. Results are presented Table 1. During the overall period, DAA reduction resulted in a linear reduction BWG and increase of FCR of animal fed control diet (R^2 = 0.65 and 0.73, respectively; P < 0.001). Enzyme effect significantly increased the performances of broilers. Average increase of BWG and Feed efficiency were +3.2 and 2.6%, respectively. Effect were more pronounced for FCR during the starter period (-5.0%) and for BWG during the growing period (+3.9%).

Table 1. Combined effect of the reduction of digestible amino acids (DAA) content and the addition of a carbohydrase complex (CC) in feeds for broilers reared under hot climate condition in different periods (n=96).

DAA Reduction	0		2%		4%		6%		Statistic[1]		
CC adition	wo	with	wo	with	wo	with	wo	with	CC	DAA Red	Inter
Starter period											
BWG	369^{ab}	372^{ab}	369^{ab}	383^{a}	359^{b}	368^{ab}	353^{b}	366^{ab}	***[2]	**	NS
FI	431^{bc}	414^{c}	442^{b}	447^{ab}	456^{ab}	445^{b}	472^{a}	453^{ab}	NS	*	NS
FCR	1.168^{cd}	1.112^{d}	1.199c	1.168^{cd}	1.272^{ab}	1.211^{bc}	1.337^{a}	1.236^{bc}	*	***	NS
Grower period											
BWG	856^{ab}	895^{a}	825^{bc}	857^{ab}	794^{bc}	813^{bc}	780^{c}	816^{bc}	*	**	NS
FI	1357	1376	1360	1378	1362	1382	1375	1400	NS	NS	NS
FCR	1.585^{cd}	1.542^{d}	1.649^{bc}	1.615^{c}	1.717^{ab}	1.694^{b}	1.768^{a}	1.717^{ab}	*	***	NS
Finisher period											
BWG	586	608	586	609	602	607	588	603	*	NS	NS
FI	1159	1150	1169	1193	1203	1197	1223	1238	NS	NS	NS
FCR	1.981^{ab}	1.903^{b}	1.998^{ab}	1.966^{ab}	2.007^{ab}	1.977^{ab}	2.086^{a}	2.057^{a}	*	**	NS
Overall period											
BWG	1811^{b}	1875^{a}	1780^{c}	1849^{a}	1755^{cd}	1788^{b}	1721^{d}	1785$^{b\ c}$	**	**	NS
FI	2947	2940	2971	3018	3021	3024	3070	3091	NS	NS	NS
FCR	1.627^{d}	1.568^{e}	1.669^{cd}	1.632^{d}	1.721^{bc}	1.691^{c}	1.784^{a}	1.732^{b}	**	***	NS

[1] Data were subject to variance analysis with fixed effect CC (n=2) and two covariables DAA and AME content and interaction AA content*CC (n=8). 2 NS = P>0.05; * = P<0.05; ** = P<0.01; *** = P<0.001.

Table 2. Equations performance parameters (BWG and FCR during overall period) based on reduction of digestible amino acids (DAA) content and the addition of carbohydrases in feeds for broilers reared under hot climate condition in different periods (n=96).

Parameter	CC adition	Equation[1]	R^2
BWG	without	BWG = 1884 - 30 * red DAA	0.58
	with	BWG = 1949 - 33 * red DAA	0.41
FCR	without	FCR = 1.532 + 0.051 * red DAA	0.77
	with	FCR = 1.488 + 0.052 * red DAA	0.85

[1] Data (n= 96) were analysed by regression using DAA red as predictive variable and CC as fixed effect.

No interaction were observed among AA reduction and enzyme inclusion for all parameters. Linear equation were established between DAA reformulation and performance with and without CC. The equation were presented in Table 2. From this equation, BWG difference between supplemented and non-supplemented diets was estimated to 2.09% increase DAA whereas corresponding value for FCR was 0.85%. As a result, enzyme effect could be consider with a potential of 2.09 + 0.85 = 2.94 % increase DAA. All effects were observed on FCR adj. For this parameter, difference between supplemented and non-supplemented diets was estimated to 2.09%. Carcass measurement performed at the end of the trial did not suggested difference among treatment and not alteration

Improvement was observed regardless of the level of DAA of the diet. This results were in agreement with studies suggesting improvement of DAA using enzyme (Nian et al. 2011; Kalmendal et Tauson 2012). Reformulation of poultry diet taking into account metabolizable and DAA improvement might increase the CC value.

Conclusions

Present study suggest important impact of DAA on animal BWG and FCR. Additive CC allowed an increase of animal performance. These improvement was estimated to 3% improvement of DAA.

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P-106 (ID 305)

EFFECT OF THE ADDITION OF A CARBOHYDRASE COMPLEX ON PERFORMANCE AND CARCASS YIELD OF BROILER CHICKENS FED WHEAT-SOYBEAN-BASED DIETS

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Abstract

The objective of this study was to evaluate the effect of the addition of a carbohydrase complex (CC) on the performance and nutrients' deposition of broiler chickens fed wheat-soybean-based diets. Six control diets were formulated with different digestible amino acids (dAA) content (-7.5%, -5.0%, -2.5%, 0.0%, +2.5%, and +5.0%) based on commercial recommendations, but with the same ideal protein profile by controlling essential dAA content (Met, Met+Cys, Lys, Thr, Val, Iso, Arg and Trp) in formulation of feeds. Six treatment diets were prepared by adding CC (Rovabio® ADVANCE L, 200 mL ton^{-1}) to the six control diets reduced in energy (85 kcal kg^{-1}) in order to isolate the energy effect. Each of the 12 treatments included eight repetitions of 15 male birds. Performance parameters (BWG, FI and FCR), efficacy of energy and protein deposition (calculated as g of BWG for each cal of AME or mg of dig. Lys ingested) and carcass and cuts yield were calculated for the period between 0-42 d. The reduction of DAA linearly degraded ($P<0.05$) all performance, nutrient deposition and carcass parameters. Carcass and cuts yield were not affected by CC addition or DAA reduction ($P>0.05$). No interaction was observed between DAA content and the addition of the CC. The addition of the CC significantly improved ($P<0.001$) BWG (+3.2%), FCR (-2.7%), and deposition of energy (+5.7%) and protein (+2.8%). In conclusion, the addition of CC can improve the performance and deposition of nutrients in order of 3%, regardless the level of tested DAA in diets.

Keywords: NSP-enzymes, broilers, amino acids, nutritional matrix, nutrient deposition.

Introduction

The quality of feedstuffs for feed production can be highly variable, especially considering their nutritional composition (Maisonnier et al. 2001). Plant feed ingredients have different types of anti-nutritional factors and indigestible components. Monogastric animals lack enzymes to digest cellulose, arabinoxylan, beta-glucans, pectins, among other non-starch polysaccharides that make up the cell wall of some feeds (Ribeiro et al., 2011). To improve the availability of nutrients and minimize the anti-nutritional factors, the use of diets containing carbohydrase is becoming common (Avila et al. 2012). When added to poultry diets, carbohydrases usually result in numerous beneficial effects, such as increased utilization of nutrients, improved the utilization of apparent metabolizable energy (AME), increased growth rate, improved feed to gain ratio, decreased viscosity of intestinal *digesta* (Costa et al. 2008). The objective of this study was to evaluate the effect of the addition of a carbohydrase complex on the performance and nutrients' deposition of broiler chickens fed wheat-soybean meal-based diets.

Material and Methods

This study was carried out in the Centre of Expertise and Research in Nutrition (CERN), Adisseo France SAS, in Malicorne, France (46°18'14" N, 2°44'07" E), between October and December 2014. The 1,440 male Ross PM3 broilers were divided in 12 treatments, with 8 repetitions of 15 birds each. Repetition consisted floor pens (2.5 m^2, disposed in bloc) between 1 and 42 days of age, in 3 phases: initial (d1-14), grower (d15-28) and finisher (d29-42). Six control wheat-soybean meal-based diets were formulated with different digestible amino acids (dAA) content (-7.5%, -5.0%, -2.5%, 0.0%, +2.5%, and +5.0%) relative to the recommendations of Rhodimet Nutrition Guide (Adisseo 2013). Essential dAA (Met, Met+Cys, Lys, Thr, Val, Iso, Arg and Trp) were controlled by the use of their industrial sources to ensure adequate inclusion of soybean meal (Table 2). Six treatment diets were prepared by adding of a carbohydrase complex (Rovabio® ADVANCE L, 200 mL ton^{-1}) to the diets reduced in energy (85 kcal kg^{-1}) (Table 1). Broilers and feed were weighted at d0, d14, d28 and d42 in order to calculate the performance parameters (BWG, FI and FCR) for each single phase and the overall period. Energy and protein efficiency were estimated dividing BWG by either metabolizable energy or digestible lysine intake (expressed as g of BWG AME or mg of dig. Lys ingested) for the overall period (d1-42). At d42, five birds per pen were killed and submitted to a controlled slaughtering where carcass and cuts were weighted, in order to determine their yield.

Table 1. Experimental design and information of treatments.

Treatments	Additio of Carbohydrase Complex[1]	Energy (AME) Reduction	Digestible Amino Acids Reduction[2]
T1	No	0	-7.5%
T2	No	0	-5.0%
T3	No	0	-2.5%
T4	No	0	0.0%
T5	No	0	+2.5%
T6	No	0	+5.0%
T7	With	85 kcal (2.8%)	-7.5%
T8	With	85 kcal (2.8%)	-5.0%
T9	With	85 kcal (2.8%)	-2.5%
T10	With	85 kcal (2.8%)	0.0%
T11	With	85 kcal (2.8%)	+2.5%
T12	With	85 kcal (2.8%)	+5.0%

[1] Rovabio ADVANCE L, 200 mL ton^{-1}, Adisseo France SAS, Antony, France. [2] Reductions were based in the recommendation of Rhodimet Nutrition Guide (Adisseo 2013), as expressed in the level 0.0% of reduction.

Table 2. Composition of experimental diets.

Composition (%)	Starter (d 0-14)		Grower (d 15-28)		Finisher (d 29-42)	
	T1-T6	T7-T12	T1-T6	T7-T12	T1-T6	T7-T12
Wheat	58.4	60.3	65.5	67.5	70.0	72.0
Soybean meal	30.5	30.0	23.5	23.0	19.0	18.5
Soybean oil	6.1	4.7	6.5	5.0	7.0	5.5
Nucleus[1]	5.0	5.0	4.5	4.5	4.0	4.0

[1] Nucleus composition: Premix (vitamins, trace minerals and other additives), macro minerals (sodium sulphate, salt, dicalcium phosphate and limestone), and industrial amino acids (calcium salt of methionine hydroxy analogue, L-Lysine, L-Threonine, L-Tryptophan, L-Valine, L-Arginine, and L-Isoleucine).

Results

No effects from bloc or interaction between dAA reduction and the addition of the carbohydrase complex were observed for any evaluated parameter, and no differences of variances were observed among treatments. Treatments with the addition of carbohydrase complex, compared to those without enzyme addition, obtained better ($P < 0.05$) BWG and FCR in the initial and finisher phases as well as during the overall experimental period (+3.2% in BWG and +2.7% in FCR). The addition of the complex promoted significant improvement ($P<0.01$) of efficacy of energy (+5.7%) and protein (+2.8%) deposition. However, no difference ($P>0.05$) was observed for carcass and cuts yield with the addition (Tables 3 and 4).

Table 3. Performance results during the three production periods of broilers fed diets with or without the addition of a carbohydrase complex (CC).

Production Periods	Initial (d0-14)			Grower (d15-28)			Finisher (d29-42)		
CC Addition	BWG[1]	FI[2]	FCR[3]	BWG	FI	FCR	BWG	FI	FCR
Without	412	541	1.314	1010	1665	1.650	1495	2905	1.947
With	422	543	1.286	1028	1675	1.631	1559	2911	1.871
P-Values[4]	<0.01	0.55	<0.01	0.08	0.51	0.14	<0.01	0.88	<0.01

[1] Body weight gain (in g) for the respective period. [2] Feed intake (in g) for the respective period. [3] Feed conversion ratio (in g g-1) for the respective period. [4] All variables were tested for normality and homoscedasticity, allowing parametric statistics application. Treatments were compared by T-Test ($P<0.05$).

Table 4. Performance during the overall experimental period (d1-42), efficacy of energy and protein deposition, and carcass yield of broilers fed diets with or without the addition of a carbohydrase complex (CC).

Period	Performance			Efficacy of Deposition		Carcass Yield		
CC Addition	BWG	FI	FCR	Energy[1]	Protein[2]	Carc.[3]	Brest[4]	Legs[5]
Without	2917	5111	1.754	0.185	56.69	72.13	30.05	13.51
With	3009	5129	1.706	0.196	58.27	72.69	30.72	13.46
P-Values[4]	<0.01	0.70	<0.01	<0.01	<0.01	0.62	0.12	0.86

[1] Calculated as g of BWG for each calorie of AME ingested in the same period. [2] Calculated as g of BWG for each g of Dig. Lysine ingested in the same period. [3] Calculated as carcass weight compared to body weight (presented in %). [4] Calculated as entire breast weight (with muscle and bones) compared to carcass weight (in %). [5] Calculated as one entire leg weight (with muscle and bones) compared to carcass weight (in %).

On the other hand, the reduction of dAA linearly degraded ($P<0.05$) all performance, nutrient deposition, and carcass parameters.

Conclusions

Based in the analysis of the results obtained in this study, the addition of the evaluated carbohydrase complex can improve the performance, carcass yield and deposition of energy and protein in the order of 3% of broilers, regardless the level of dAA used in their diets.

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P-109 (ID 169)

EFFECT OF HOSTAZYM® X ON DIGESTIBILITY OF CEREALS AND PROTEINACEOUS BY-PRODUCTS IN BROILERS

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Abstract

A trial was conducted to evaluate the effect of Hostazym® X on digestibility of wheat, corn, barley, soybean meal, rapeseed meal and DDGS (of wheat origin) in broilers. In total 56 cages, of 6 birds each, were fed a general starter diet until 13 days of age. From 13-22 days, birds were fed the different test feeds with or without Hostazym® X (1500 EPU/kg). Feeds contained either 65 % of wheat, corn or barley (supplemented with a 35 % soybean meal based protein concentrate), or 30 % of either DDGS or soybean meal (supplemented with a 70 % corn/wheat based carbohydrate concentrate). Furthermore, also 2 test diets containing 10 or 20 % rapeseed meal were incorporated in the trial. Digestibility was measured between 18 and 22 days, based on the method of total faecal collection. Firstly, results showed that Hostazym® X inclusion led to an increase in metabolisable energy of 45, 104, 94, 89, 120, 33 and 111 kCal/kg of feed. Secondly, the Hostazym® X treatment yielded an increment-in overall fat digestibility by 1.3, 5.0, 3.7, 1.2, 8.8, 2.0 and 3.0 g fat/kg of feed for wheat, corn, barley, soybean meal, DDGS, 10 and 20 % rapeseed meal based feeds respectively. Thirdly, faecal droppings were reduced by 4 – 14.5 % when using Hostazym® X, and water intake was reduced at a rate of 0.5 up to 6.1 %. From this trial it could be concluded that the inclusion of Hostazym® X in different types of feed, gave a clear improvement in digestibility while reducing faecal output and water consumption.

Keywords: xylanase, Hostazym® X, digestibility, wet litter

Introduction

Enzymes are used to degrade non-starch polysaccharides, mainly to reduce viscosity in the intestine and to liberate more nutrients which are entrapped by insoluble fibrous structures. This improves the digestibility of the nutrients present in the feed, while reducing excretion, optimizing performance and improving gut health status. Hostazym® X is a well-established enzymatic complex product used in many types of diets, and is known to exert a consistent and beneficial effect on the digestibility of nutrients in poultry diets. However, new data on this effect needs to be generated continuously, in order to confirm the existing matrix values.

Trial description

The digestibility study was composed out of 56 cages of 6 female broilers (ROSS 308) per cage. Broilers were fed a starter feed till 13 days of age. From 13 till 22 days of age, animals were fed with the treatment feeds. Day 13-18 was considered as the adaptation period, while the digestibility assay was conducted from day 18 till day 22. Different feeds were produced with or without Hostazym® X at 1500 EPU/kg of feed. The first composed diets contained a 65 % inclusion level of wheat, corn or barley (each 2 different batches), combined with a 35 % protein based (soybean meal) complementary feed. Secondly, feeds were composed containing either 30 % soybean meal or 30% wheat-based DDGS (of each 2 different batches), which were added to a complementary feed containing corn/wheat as cereal sources in a 50/50 ratio. Finally, feeds containing 10 or 20 % rapeseed meal (at the expense of soybean meal) were produced, in which corn/wheat was dosed in a 50/50 ratio. Eight cages per feedstuff were used, of which 4 cages received the control feed and 4 cages received the control feed + Hostazym® X at 1500 EPU/kg. During the period of digestibility (d18-22) daily feed intake, total faecal droppings and water intake were measured. Digestibility was determined by analyzing the feed (dry matter, gross energy, starch and fat) and quantifying the total amount of faecal material produced (dry). After homogenisation of the faecal material and subsampling, 250 g was freeze-dried to determine the dry matter content. The dry matter was then analyzed for energy, starch and fat. The digestibility was calculated as follows: digestibility (%) = (total nutrient intake by feed – total nutrient output by faeces)/(total nutrient intake by feed) x 100 %

Results

First of all, the inclusion of Hostazym® X increased the metabolisable energy (ME) of all feeds, ranging from 33 kCal/kg (rapeseed meal 10 %) up to 120 kCal/kg (DDGS 30 %) (Fig. 1). Secondly, the inclusion of Hostazym® X increased the fat digestibility by 1.2 % (soybean meal 30 %) up to 7.7 % (DDGS 30 %) (Fig. 2).

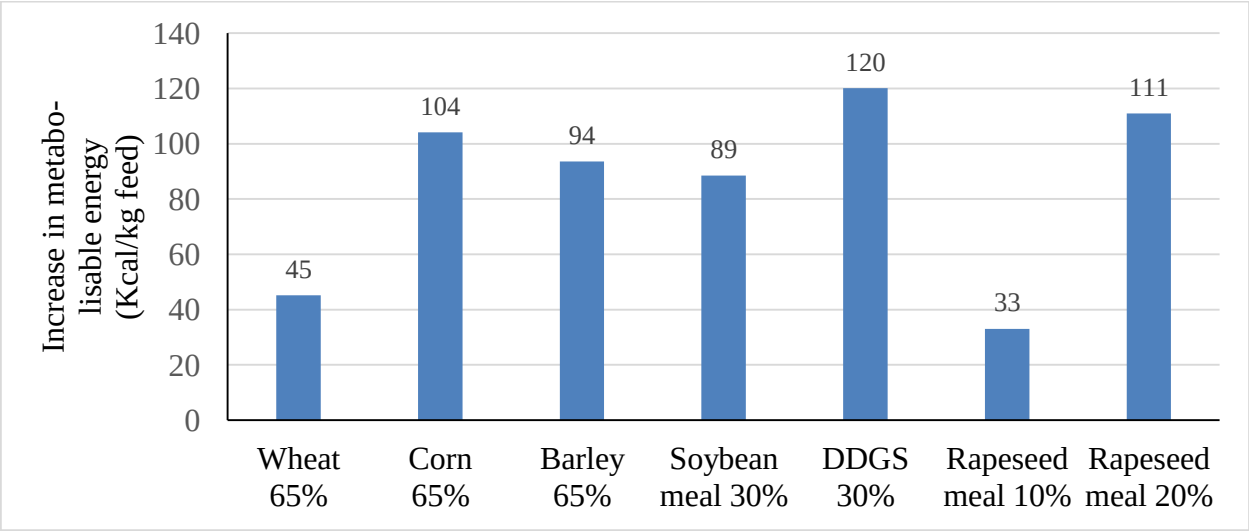

Fig. 1: increase in ME (kCal/kg feed) when including Hostazym® X at 1500 EPU/kg feed

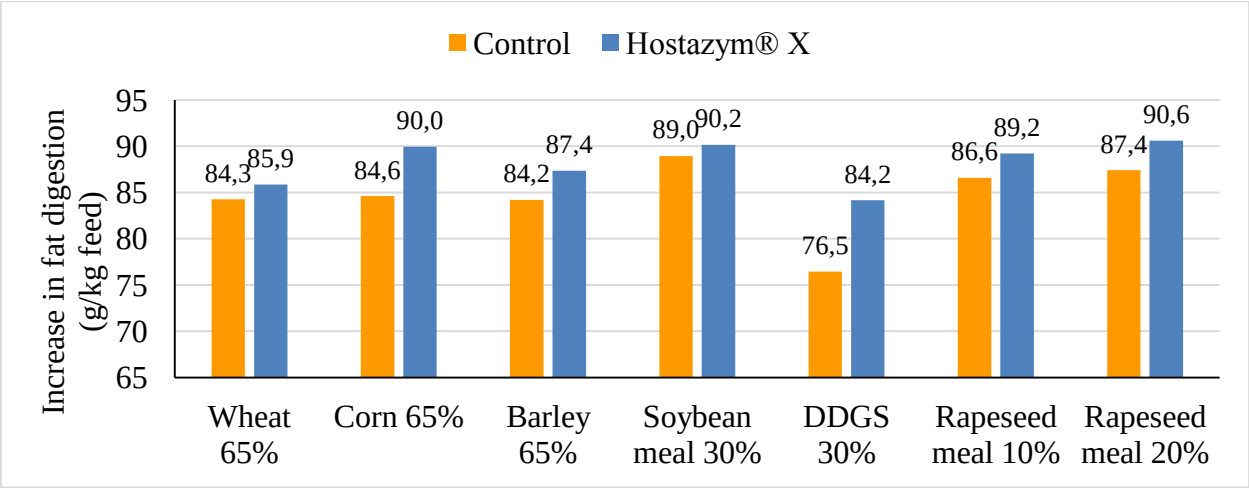

Fig. 2: fat digestibility (%) with or without Hostazym® X at 1500 EPU/kg feed

The inclusion of Hostazym® X led to a reduction in faecal droppings, indicating a better digestion of the feed (Fig. 3). Additionally, the inclusion of Hostazym® X resulted in a reduced daily water consumption, which might be linked to a reduced viscosity in the gut when using Hostazym® X (Fig. 4). As a consequence of the effects shown in Fig. 3 and 4, a reduction in wet litter in field conditions can be expected when using Hostazym® X.

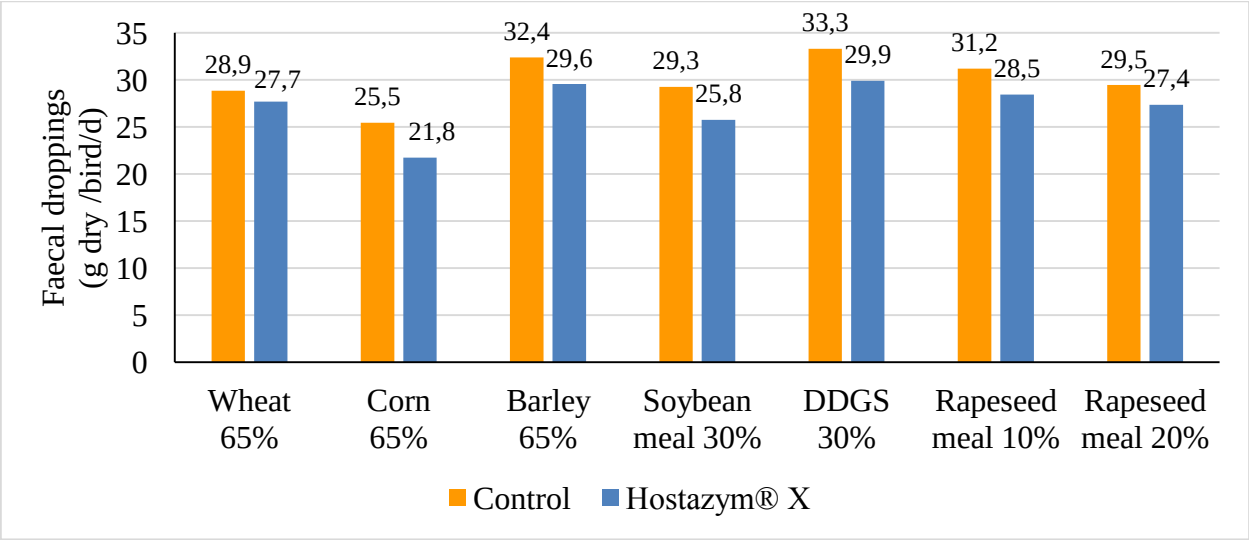

Fig. 3: daily faecal droppings (g/bird/d) with or without Hostazym® X at 1500 EPU/kg feed

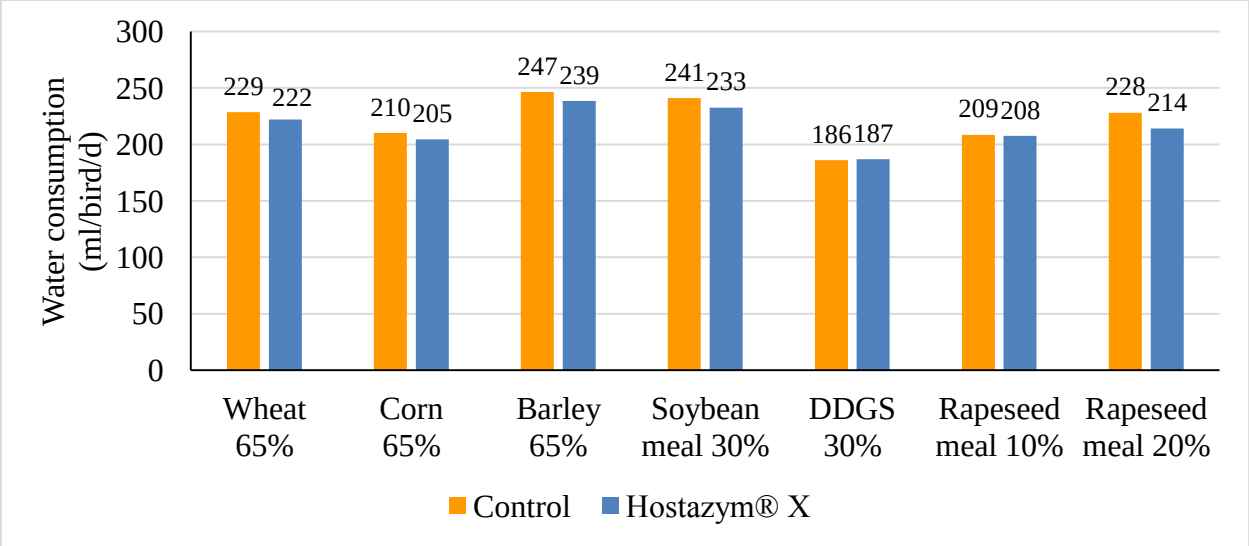

Fig. 4: daily water consumption with or without Hostazym® X at 1500 EPU/kg feed

Conclusions

The inclusion of Hostazym® X at 1500 EPU/kg led to an increase in ME which was highest in the DDGS-based diet, followed by the diets containing 20 % rapeseed meal, 65 % corn or 65 % barley. It also resulted in an increase in fat digestibility (highest in the corn diets followed by the 30 % DDGS diet), as well as reduction in daily faecal droppings, thereby showing an increased digestibility of the feed dry matter and also a educed water consumption. Both of these key findings effectuate a lower risk of wet litter.

P-110 (ID 70)

OPTIPHOS® IMPROVES PERFORMANCE AND SHOWS A HIGH AP VALUE IN BROILERS

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Abstract

A 42 d broiler pen trial was set-up at Valley View Research Farm (USA), consisting of 5 treatments having 8 replicates of 22 male birds each (Cobb x Cobb). Feeds were corn/soy based with low phosphorous level (starter 0.8 % Ca and 2.0 g/kg aP (available P); grower 0.7 % Ca and 1.4 g/kg aP; finisher (0.7 % Ca and 1.0 g/kg aP). To this feeds either no addition (negative control), or increasing levels of inorganic P as DCP (di-calcium phosphate) were added to yield an extra 1.0, 1.5 or 2.5 g/kg feed of aP (positive controls). To the remaining feed a dose of OptiPhos® at the rate of 550 OTU/kg feed was applied. Body weight, daily growth, feed intake, and mortality-corrected feed conversion (FCR) were measured for every phase. Also at 42 days of age, two tibias were collected per pen for ash analysis. Results showed that higher aP levels through OptiPhos® addition or through DCP addition, increased the weight gain while reducing FCR. The inclusion of OptiPhos® at 550 OTU brought the body weight performance to the same level/height as for the negative control + 1.5 g aP level. However 550 OTU OptiPhos® reduced FCR to come close to the FCR observed for the negative control + 2.5 g aP treatment. From the response curve between tibia ash and added P as DCP, an equivalence could be established between the OptiPhos® phytase unit (OTU) and aP in the ratio/ of 1.67 g aP for 550 OTU OptiPhos®.

Keywords: phytase, poultry, available P, matrix values, OptiPhos®

Introduction

Phytases are used in commercial broiler nutrition to reduce the need of and dependency on inorganic P sources by liberating P from phytate, present in vegetal feed materials. The correct estimation of the matrix value of the different phytases ~~are~~ is therefore essential to allow a nutritionist to formulate feeds with optimal P levels. This correct estimation of the matrix values requires multiple trials which have to be set-up in a scientifically correct manner.

Material and methods

A broiler trial was conducted at the trial site of Valley View Research Farm (USA) in the period of November/December 2013 (46 days). Male broilers (Cobb x Cobb) were split over 5 treatments, with 8 replicates of each 22 birds per treatment. Feeds were corn/soy based and were pelleted, except for the starter feed which was crumbled. There were 5 treatments including one negative control NC (starter: 0.8 % Ca and 2.0 g/kg aP; grower: 0.7 % Ca and 1.4 g/kg aP and finisher: 0.7 % Ca and 1.0 g/kg aP), and also three positive controls using increasing levels of extra inorganic P as DCP (dicalciumphosphate) added in the NC feed to yield an extra 1.0, 1.5 or 2.5 g/kg feed of aP. In the last treatment group, OptiPhos® was added at a rate of 550 OTU/kg in the NC feed. Body weight, daily growth, feed intake, and mortality-corrected feed conversion (FCR) were measured, while at day 42 two tibias were collected per pen for bone ash analysis.

Results

Higher aP levels by adding OptiPhos® or by adding DCP increased the weight gain while reducing FCR (Table 1). The inclusion of OptiPhos® at 550 OTU/kg feed brought the body weight performance to the same level as the one observed for the NC + 1.5 g aP treatment. However 550 OTU OptiPhos® reduced FCR to the extent that it came close to the FCR observed for the NC + 2.5 g aP treatment.

From the response curve between tibia ash and added P as DCP, an equivalence between the OptiPhos® phytase unit (OTU) and aP in the ratio of 1.67 aP for 550 OTU OptiPhos® could be established. This is very close to the commercially advised matrix value of 1.68 aP for 500 OTU OptiPhos®.

Table 1: effect on technical performance

	End weight (kg)	FCR	Tibia ash (%)
NC*	2462[a]	1.84[a]	37.7[a]
NC + 1.0 g aP	3115[b]	1.76[bcd]	42.7[b]
NC + 1.5 g aP	3187[b]	1.75[c]	43.1[b]
NC + 2.5 g aP	3268[b]	1.71[b]	44.4[b]
NC + OptiPhos® 550 OTU	3189[b]	1.72[bcd]	43.1[b]

*NC= negative control
a-d: values in a column followed by a different letter are significant different (P< 0.05)

Conclusions

OptiPhos® added at 550 OTU/kg feed gave similar to better technical results than the addition of 1.5 g aP as DCP. Equivalence calculations between OptiPhos® and P as DCP, revealed an aP value of 1.67 g aP for 550 OTU.

P-111 (ID 72)

OPTIPHOS® OUTPERFORMS QUANTUM® BLUE ON PH ACTIVITY RANGE AND SPEED OF PHYTIC ACID HYDROLYSIS

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Abstract

OptiPhos® and Quantum® Blue were purified from commercial sources to obtain the pure phytase proteins of both enzyme products. Research was performed on the pure phytase protein *in vitro*, to determine the pH profile and Michaelis-Menten kinetics. The pH activity assay was conducted by measuring the phytase activity in Glycine-HCl buffers at pH 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5 during a period of 10 min. Michaelis Menten kinetics were determined in a glycine-HCl buffer at pH 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5, after which the Vmax (maximum speed) and Kcat (turnover number) were both calculated. OptiPhos® showed a much higher activity at multiple pH levels than Quantum® Blue did, which indicates that OptiPhos® is performing better, especially under more acidic conditions. The Kcat value of OptiPhos® was also higher at all measured pH levels, in particular at lower pH. On average, the Kcat value of OptiPhos® was 893 vs. 645 per second for Quantum® Blue (i.e. +28 %). The Vmax value of OptiPhos® was also higher at most pH levels, in particular at lower pH. On an average basis covering all pH levels, the Vmax value of OptiPhos® was 1045 vs. 886 µmol P/min. per mg enzyme for Quantum® Blue (i.e. +15 %). It could be concluded that OptiPhos® has a higher activity at a broader pH range than Quantum® Blue, while OptiPhos® also works faster in breaking down phytic acid at *in vivo*-relevant pH levels.

Keywords: phytase, pH, kinetics, speed, OptiPhos®

Introduction

Phytase releases P from phytic acid present in vegetal feed materials, so decreasing the need for dietary supplementation of inorganic P sources and thereby reducing feed costs and P excretion. However, phytic acid can only be degraded by a phytase when it is in a solubilised state. As the solubility of phytic acid in feeds decreases with increasing pH due to formation of e.g. Ca salts, the phytase should especially work in a pH range below 4.5. With regard to the gastro-intestinal tract of poultry, this corresponds to the area of the proventriculus and the gizzard. Furthermore, considering that the transit time in this part of the gastro-intestinal tract is quite fast, a phytase should definitely be working at a high speed. The specific aim of this set-up was to evaluate purified OptiPhos® phytase on these intrinsic parameters, and to empirically compare it with Quantum® Blue.

Material and methods

The pure/naked phytase molecules of OptiPhos® and Quantum® Blue, were purified/extracted from the commercially available OptiPhos® and Quantum® Blue products. In brief, the phytases were solubilized overnight at 18 °C in 200 ml Tris-HCL buffer followed by a filtration on glass fibre pre-filter of 1 µm, on a membrane filter of 0.45µm and finally on a 0.22 µm GP Express PLUS Membrane. The protein solutions were concentrated by diafiltration. Proteins were precipitated by ammonia sulphate, and the phytase fraction was separated by chromatography.

The pH activity assay was conducted by measuring the phytase activity using phytate as a substrate in Glycine-HCl buffers at pH 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5 for a period of 10 min (according to ISO 30024:2009).

Michaelis-Menten kinetics were determined for the phytases in a Glycine-HCl buffer at pH levels of 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5, after which Vmax and Kcat were calculated. Vmax represents the maximum rate of speed for P release from phytate, at maximum (see saturating) concentrations of the (phytate) substrate. Kcat is the turnover number, which is the maximum number of substrate (phytate) molecules converted per enzyme molecule per second.

Results

OptiPhos® showed a much higher activity at different pH levels than Quantum® Blue (Fig. 1), which indicates that OptiPhos® is also performing better under more acidic conditions.

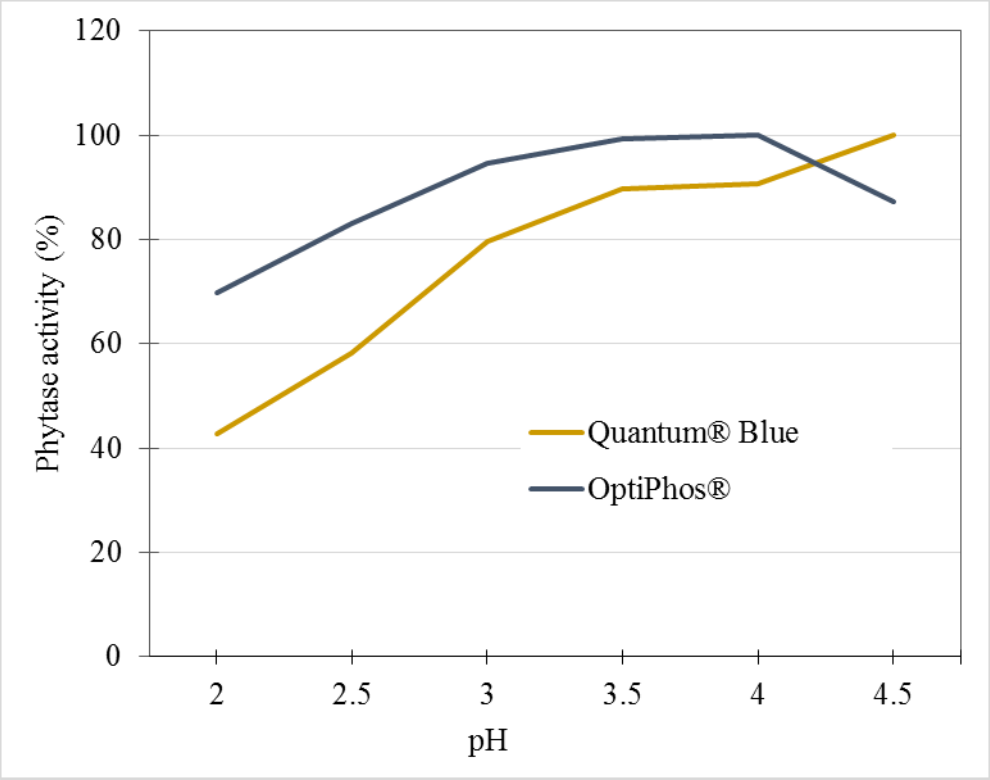

Fig. 1: *relative phytase activity at different pH levels after incubation during 10 minutes*

The Kcat value of OptiPhos® is also higher at all pH levels, in particular at lower pH. On an average basis, the Kcat value of OptiPhos® was 893 per second, compared to 645 per second for Quantum® Blue (+28 %, Fig. 2). The Vmax value of OptiPhos® was higher at most pH levels, in particular at lower pH. In this view the mean Vmax value of OptiPhos® was 1045 vs. 886 µmol P/min. per mg enzyme for Quantum® Blue (+15 %, Fig. 3).

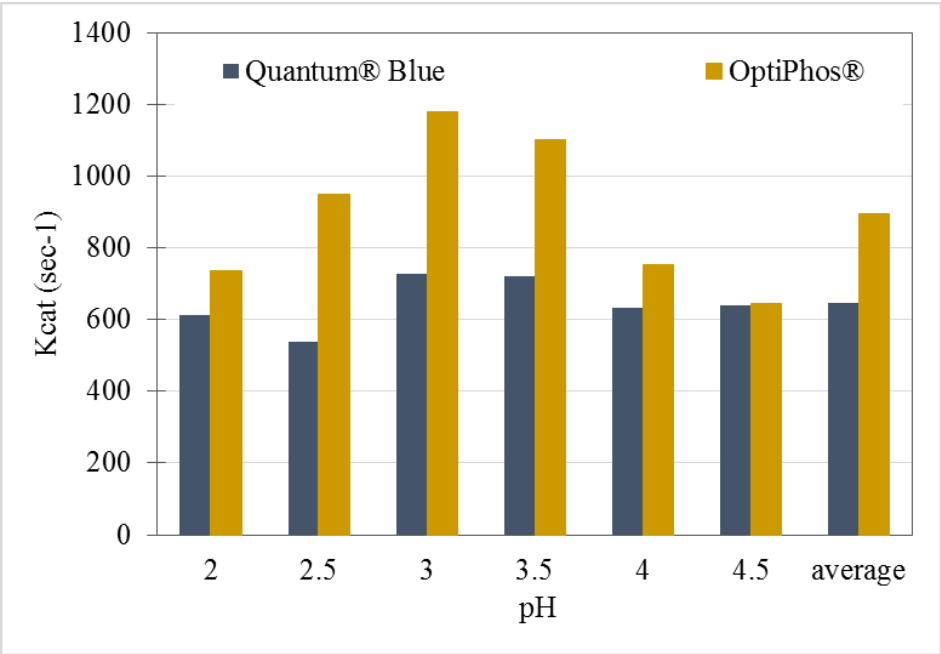

Fig. 2: *the Kcat value of OptiPhos® and Quantum® Blue at different pH levels*

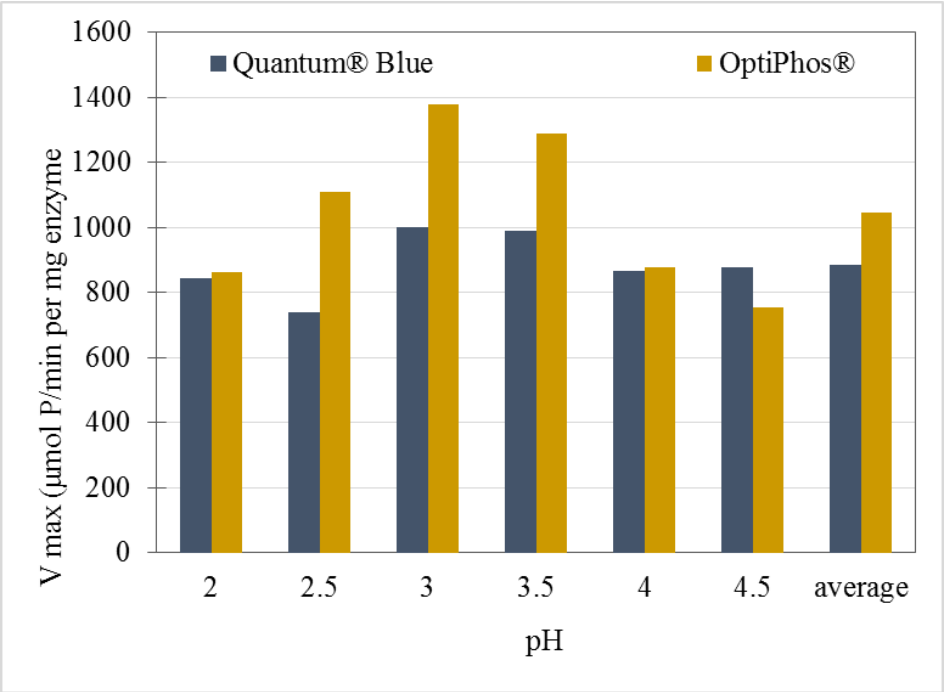

Fig. 3: *the Vmax value of OptiPhos® and Quantum® Blue at different pH levels*

Conclusions

OptiPhos® has a higher phytase activity at a broader pH working range compared with Quantum® Blue, and it also acts much faster in breaking down phytic acid at *in vivo*-relevant pH levels.

P-112 (ID 71)

OPTIPHOS® OUTPERFORMS RONOZYME® HIPHOS ON PH ACTIVITY RANGE, RESISTANCE TO PEPSIN AND SPEED OF PHYTIC ACID HYDROLYSIS

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Abstract

OptiPhos® and Ronozyme® HiPhos were purified from commercial available sources, to obtain the pure phytase protein. On the pure phytase protein *in vitro* determination of the pH profile, pepsin resistance and Michaelis-Menten kinetics was done. The pH activity assay was conducted by measuring the phytase activity in Glycine-HCl buffers at pH 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5 during 10 min. Pepsin resistance was measured by incubating pepsin and phytase at different pepsin/phytase ratios at 37°C. This was done/carried out for different time periods in 15 mM HCl (pH 2), after which the remaining phytase activity was measured in a 0.2 M Glycine-HCl buffer (pH 3.5) at 39°C. Michaelis Menten kinetics was determined in glycine-HCl buffer at pH 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5 from which Vmax (maximum speed) and Kcat (turnover number) were calculated. Results showed that OptiPhos® had a much higher activity at a number of different pH levels than Ronozyme® HiPhos, indicating that OptiPhos® is a truly superior phytase at (mild) acidic conditions. OptiPhos® was by far more resistant to pepsin, both when incubated with relative low doses of pepsin for a long time (2 h), as well as when incubated with a higher pepsin/phytase ratio during a shorter period of time (40 min.). The Kcat value of OptiPhos® was also higher at all measured pH levels averaging at 893 per second, compared to 668 per second for Ronozyme HiPhos® (i.e. +35%). The Vmax value of OptiPhos® was higher at most pH levels averaging at 1045 vs. 806 µmol P/min per mg enzyme for Ronozyme® HiPhos (i.e. +23 %). It can be concluded that OptiPhos® has a more suitable pH profile, is more resistant to pepsin and will work faster than Ronozyme® HiPhos at *in vivo*-relevant pH levels.

Keywords: phytase, pH, pepsin resistance, kinetics, OptiPhos®

Introduction

Phytase releases P from phytic acid present in vegetal feed materials, so decreasing the need for dietary supplementation of inorganic P sources and thereby reducing feed costs and P excretion. However, phytic acid can only be degraded by a phytase when it is in a solubilised state. As the solubility of phytic acid in feeds decreases with increasing pH due to formation of e.g. Ca salts, the phytase should especially work in a pH range below 4.5. With regard to the gastro-intestinal tract of poultry, this corresponds to the area of the proventriculus and the gizzard. As phytases are in fact proteins and thus degradable by proteases, a good phytase should also be resistant to pepsin. Furthermore, considering that the transit time in this part of the gastro-intestinal tract is quite fast, a phytase should definitely be working at a high speed. The specific aim of this experimental set-up was to evaluate purified OptiPhos® phytase on these intrinsic parameters, and to empirically compare it with Ronozyme® HiPhos.

Material and methods

The pure/naked phytase molecules of OptiPhos® and Ronozyme® HiPhos were purified/extracted from the commercially available OptiPhos® and Ronozyme® HiPhos products. The phytases were solubilized overnight at 18 °C in 200 ml Tris-HCL buffer followed by a filtration on glass fibre pre-filter of 1 µm, on a membrane filter of 0.45µm and finally on a 0.22 µm GP Express PLUS Membrane. The protein solutions were concentrated by diafiltration. Proteins were precipitated by ammonia sulphate, and the phytase fraction was separated by chromatography. The purified enzymes were tested *in vitro* on their pH profile, pepsin resistance and Michaelis-Menten kinetics. The pH activity assay was conducted by measuring the phytase activity using phytate as a substrate in Glycine-HCl buffers at pH 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5 for a period of 10 min (according to ISO 30024:2009). Pepsin resistance was measured by dissolving pepsin into 15 mM of HCl (final pH 2). In a final volume of 100 ml, 10 mg of purified phytase was incubated with pepsin at different pepsin/phytase (w/w) ratios at 37°C. This was carried out for different periods of time, after which the remaining phytase activity was measured in a 0.2 M Glycine-HCl buffer (pH 3.5) at 39°C. Michaelis-Menten kinetics was determined for the phytases in a Glycine-HCl buffer at pH levels of 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5, after which Vmax and Kcat were calculated. Vmax represents the maximum rate of speed for P release from phytate, at maximum (see saturating)

concentrations of the (phytate) substrate. Kcat is the turnover number, which is the maximum number of substrate (phytate) molecules converted per enzyme molecule per second.

Results

OptiPhos® showed a much better activity at different pH ranges compared with Ronozyme® HiPhos, which indicates that OptiPhos® is also performing better at acidic conditions (Fig. 1).

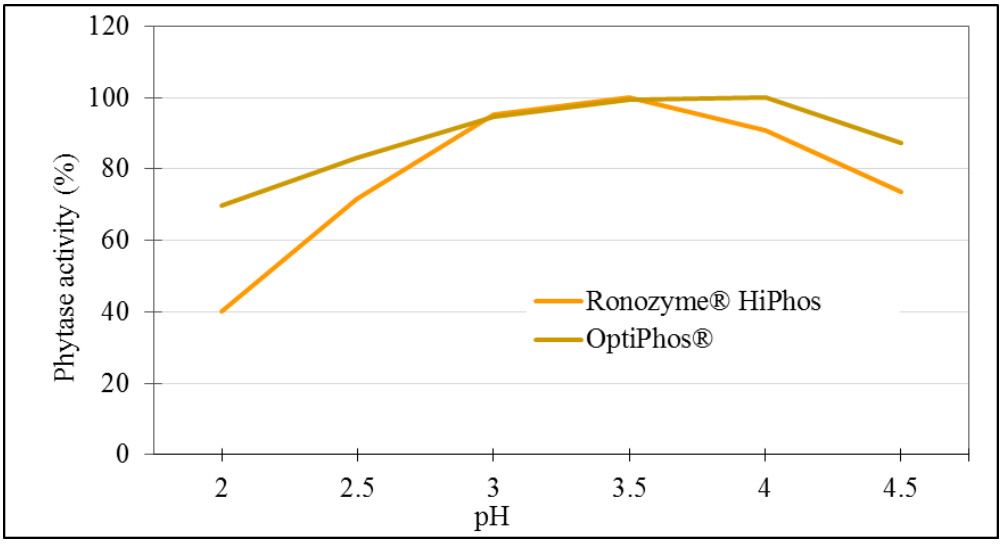

Fig. 1: *relative phytase activity at different pH levels after incubation during 10 minutes*

OptiPhos® was by far more resistant to pepsin, either when incubated with relative low doses of pepsin for a long time (2 h, Fig. 2) or when incubated with a higher pepsin/phytase ratio during a shorter period of time (data not shown).

The Kcat value of OptiPhos® was also higher at all pH levels. On an average basis, the Kcat value of OptiPhos® was 893 per second, compared to 668 per second for Ronozyme® HiPhos (+35 %, data not shown). The Vmax value of OptiPhos® was higher at most pH levels. In this view the mean Vmax value (in µmol P/min. per mg enzyme) of OptiPhos® was 1045 vs. 806 for Ronozyme® HiPhos (+23 %, Fig. 3).

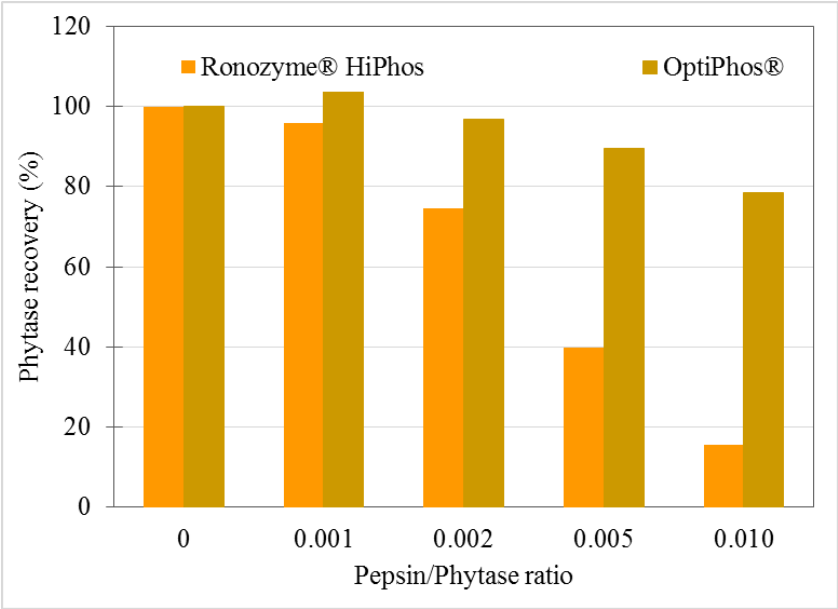

Fig. 2: *phytase recovery after prolonged exposure of OptiPhos® and Ronozyme® HiPhos to different low pepsin levels during a 2h period*

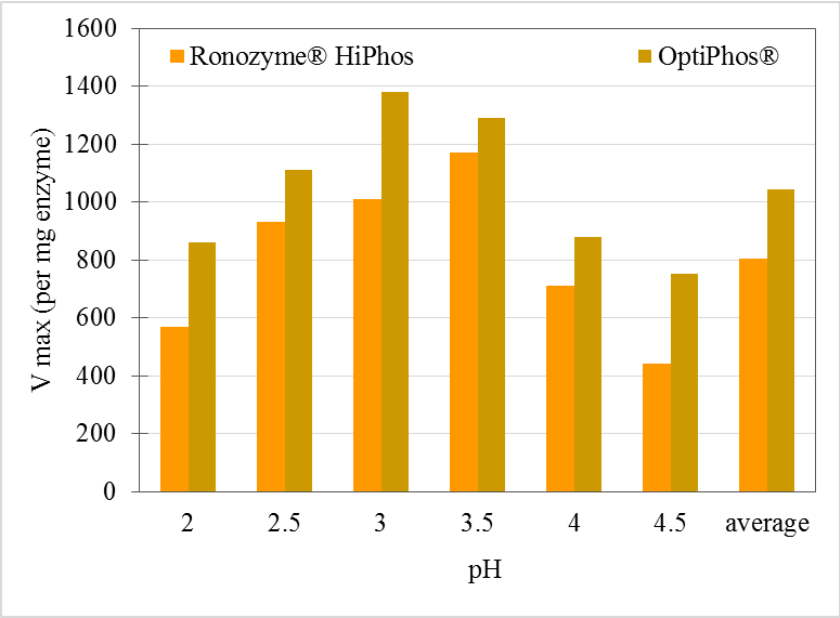

Fig. 3: *the Vmax values of OptiPhos® and Ronozyme® HiPhos at different pH levels*

Conclusions

OptiPhos® has a much broader pH working range with higher phytase activity than Ronozyme® HiPhos. OptiPhos® is far more resistant to pepsin breakdown, and works much faster in breaking down phytic acid at *in vivo*-relevant pH levels between 2.0 and 4.5.

P-113 (ID 69)

THE EFFECT OF OPTIPHOS® AND RONOZYME® HIPHOS ON TECHNICAL PERFORMANCE AND AVAILABLE P IN BROILERS

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Abstract

A broiler trial was set up at Southern Poultry Research, Athens (USA) using male broilers (Cobb x Cobb). Birds were distributed over 8 treatments with 8 replicates of 8 broilers each and fed with a corn/soy mash feed from 0-14 d (start) and 15-28 d (grower). Treatments consisted of one deficient control (0.20 % aP (available P)), three positive controls with increasing levels of added inorganic P (dicalciumphosphate, DCP) to yield an overall 0.30, 0.35 and 0.45 % aP in the feed, and two phytase treatments (OptiPhos® and Ronozyme® HiPhos) at 2 levels: OptiPhos® at 550 and 750 OTU/kg and Ronozyme® HiPhos at 1000 and 1500 FYT/kg). The technical performance data showed that the increase in weight gain and the decrease in FCR corresponded linearly with increasing levels of aP from DCP or by the inclusion of phytase. The technical performance at day 14 and 28 of OptiPhos® treatment was generally better compared to Ronozyme® HiPhos treatment, in particular at the lowest inclusion level regarding BW gain, and at the highest inclusion rate regarding FCR. Increasing the dose of OptiPhos® reduced FCR significantly at 28 days, demonstrating that a higher dose of OptiPhos® has a benefit on technical performance. These results also indicated that the commercially used exchange rate of 2 FYT Ronozyme® HiPhos for 1 OTU OptiPhos® is in fact an underestimation and should rather be situated between 2.5 and 3.

Keywords: phytase, poultry, available P, matrix values, OptiPhos®

Introduction

Phytases are used in commercial broiler nutrition to reduce the need of and dependency on inorganic P sources by liberating P from phytate, present in vegetal feed materials. The use of phytase thereby leads to a lower feed cost (optimizing technical performance) and to a reduced excretion of P, having a positive environmental effect. However, plural scientifically correct trials need to be set-up in order to verify if the claimed matrix value and inclusion level of different phytases are correct, and leading to a similar or even improved performance compared to a feed purely supplemented with inorganic P sources.

Material and methods

A broiler trial was set up at Southern Poultry Research, Athens (USA) in the period January/February 2014. Male broilers (Cobb x Cobb) were distributed over on Petersime batteries (8 birds per cage, 8 repetitions per treatment) which were fed a corn/soy based diet (0-14 d: starter feed and 15-28 d: grower feed). There were 8 treatments: one deficient negative control NC (0.2 % aP), three positive controls with increasing levels of added inorganic P (DCP) on the NC to yield 0.30, 0.35 and 0.45 % aP in the feed, and two phytase treatments (OptiPhos® and Ronozyme® HiPhos) at 2 levels: OptiPhos® at 550 and 750 OTU/kg and Ronozyme® HiPhos at 1000 and 1500 FYT/kg added on the NC. Body weight, daily growth, feed intake, and feed conversion (FCR) were measured at day 14 and 28.

Results

The weight gain increase and the FCR decrease corresponded linearly with increasing levels of aP (Table 1). The technical performance at day 14 and 28 of OptiPhos® was generally better than Ronozyme® HiPhos, in particular at the lowest level for BW gain, and at the highest inclusion rate for FCR (Table 1). This indicates that the used exchange rate of 2:1 of Ronozyme® HiPhos to OptiPhos®, is an underestimation of the aP value of OptiPhos® compared to Ronozyme® HiPhos. Increasing the dose of OptiPhos® reduced FCR significantly at 28 days, demonstrating that superdosing OptiPhos® does have a benefit on technical performance.

Table 1: effect of the inclusion of OptiPhos® and Ronozyme® HiPhos at two different levels on body weight gain and mortality-adjusted FCR at 14 and 28 days of age

	Day 14		Day 28	
Treatment	Weight gain (g)	Adj. FCR	Weight gain (g)	Adj. FCR
Deficient control (DC) (0.20% aP)	0.267[a]	1.402[a]	929[ab]	1.65[a]
DC + 0.10% aP	0.287[b]	1.382[ab]	939[abc]	1.62[ab]
DC + 0.15% aP	0.296[bcd]	1.348[cd]	948[abcd]	1.58[cd]
DC + 0.25% aP	0.316[d]	1.333[d]	993[abd]	1.55[de]
DC + OptiPhos® (550 OTU)	0.302[cde]	1.36[ce]	967[abcd]	1.61[b]
DC + Ronozyme® HiPhos (1000 FYT)	0.294[bcd]	1.38[be]	921[b]	1.62[ab]
DC + OptiPhos® (750 OTU)	0.303[cde]	1.34[cd]	986[abcd]	1.57[cde]
DC + Ronozyme® HiPhos (1500 FYT)	0.303[cde]	1.35[c]	976[abcd]	1.60[bc]

a-e: values in a column with different letter are significant different (P < 0.05).

Conclusions

OptiPhos® outperformed Ronozyme® HiPhos especially at lower inclusion levels, revealing OptiPhos® to be the more efficient phytase. OptiPhos® at 50 % of the Ronozyme® HiPhos dose (e.g. 750 OTU vs 1500 FYT) showed better performance, illustrating that the ratio OptiPhos®/ Ronozyme® HiPhos is, in fact, higher than 2.

P-114 (ID 68)

THE EFFICACY OF OPTIPHOS® AND RONOZYME® HIPHOS ON PERFORMANCE AND TIBIA ASH IN YOUNG BROILERS

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Abstract

A trial was conducted at Schothorst Feed Research (NL), to evaluate the effect of OptiPhos® and Ronozyme® HiPhos on growth performance and tibia ash content. Male Ross308 broilers in 6 replicates of 23 birds per treatment (reduced to 20 birds at day 5) were fed a starter feed which was equal for all birds from day 0 to 5. From day 5 to 21, trial feeds were fed to the different treatments (10 in total). Treatments used were one negative control (NC) with low Ca and low P, and three positive controls using the NC to which monocalciumphosphate (MCP) was added to increase the P level with 0.6, 1.2 and 2 g per kg of feed. Furthermore, there were also six NC + phytase treatments (OptiPhos® liquid dosed at 25, 50 and 100 mL/T providing 160, 320 and 640 OTU/kg, and Ronozyme® HiPhos dosed at 17.5, 35 and 70 mL?/T providing 490, 980 and 1960 FYT/kg). Feeds were pelleted (80°C, 3 mm) and enzymes were dosed after pelleting. Body weight, daily growth, feed intake, and feed conversion were measured during the trial period. At day 21, four tibias per pen were collected and pooled into one sample. The results showed that (a) increasing the dose of OptiPhos® above the recommended levels (250 OTU/kg) had a positive effect on body weight gain, (b) increasing the levels of OptiPhos® above the recommended dose (250 OTU/kg) had more effect on tibia ash than increasing levels of Ronozyme® HiPhos, and (c) the real potential of OptiPhos® in replacing P as MCP was 1.43 g aP per kg at 250 OTU.

Keywords: phytase, poultry, available P, matrix values, OptiPhos®

Introduction

Phytases are used in commercial broiler nutrition to reduce the need of and dependency on inorganic P sources by liberating P from phytate, present in vegetal feed materials. The correct estimation of the matrix value of the different phytases is therefore essential to allow a nutritionist to formulate feeds with optimal P levels. This correct estimation of the matrix values requires multiple trials which have to be set-up in a scientifically correct manner.

Trial description

A trial was set up at the Schothorst Feed Research, the Netherlands (January – February 2013) using male Ross308 broilers distributed over 10 treatments with 6 replicates of 23 birds per treatment (reduced to 20 birds at day 5). Broilers were fed a started diet till day 5 which was similar for all birds. From day 5 till day 21 the trials feeds were provided. There were 10 treatments including one negative control NC (corn/soy based: 57.6 and 30.3 %; 2900 kCal AMEn/kg, 21 % crude protein, 10.5 % dig. Lys, 0.65 % Ca, 0.44 % P and 0.25 % aP), and three positive controls adding P as mono-calciumphosphate (P-MCP) to the NC at 0.6, 1.2 and 2 g per kg. There were also six phytase treatments: OptiPhos® liquid dosed at 160, 320 and 640 OTU/kg and Ronozyme® HiPhos dosed at 490, 980 and 1960 FYT/kg (analyzed values). Feeds were pelleted (80°C, 3 mm) and enzymes were added in liquid form–post-pelleting. Body weight, daily growth, feed intake, and feed conversion were measured. At day 21, four tibias were collected per pen (day 21) and pooled into one sample (so 6 samples per treatment) for bone ash analysis.

Results

The negative and positive control (= not supplemented with phytase) were negative on phytase activity (Table 1). Increasing P availability by increasing phytase levels or adding MCP, increased the BW and tibia ash percentage while reducing FCR.

Table 1: Effect on technical performance and tibia ash

Tr.	Phytase	Phytase (per kg)	BW (g)	FCR	Tibia ash (g/kg fat free DM)
1	NC	< 100 FTU	677[a]	1.384[e]	354[a]
2	PC 1 (NC + 0.6 P-MCP)	< 100 FTU	708[b]	1.351[d]	396[b]
3	PC 2 (NC + 1.2 P-MCP)	< 100 FTU	849[c]	1.327[c]	454[de]
4	PC 3 (NC + 2.0 P-MCP)	< 100 FTU	922[fg]	1.305[ab]	486[g]
5	Optiphos®	160 OTU	851[c]	1.331[c]	440[c]
6	Optiphos®	320 OTU	890[de]	1.322[bc]	465[ef]
7	Optiphos®	640 OTU	905[efg]	1.303[a]	486[g]
8	Ronozyme® HiPhos	490 FYT	865[cd]	1.328 [c]	446[cd]
9	Ronozyme® HiPhos	980 FYT	896	1.321[bc]	472[f]
10	Ronozyme® HiPhos	1960 FYT	934[g]	1.3[a]	487[g]

[a-g] Mean values in a column with a different superscript letter are sign. different (P≤ 0.05).

From the results as indicated in Table 1, a response curve between tibia ash percentage and added P as MCP could be established. The obtained fitted line (linear equation) offers an opportunity to estimate a P value for a certain inclusion of phytase (OTU or FYT) and P as (Fig. 1).

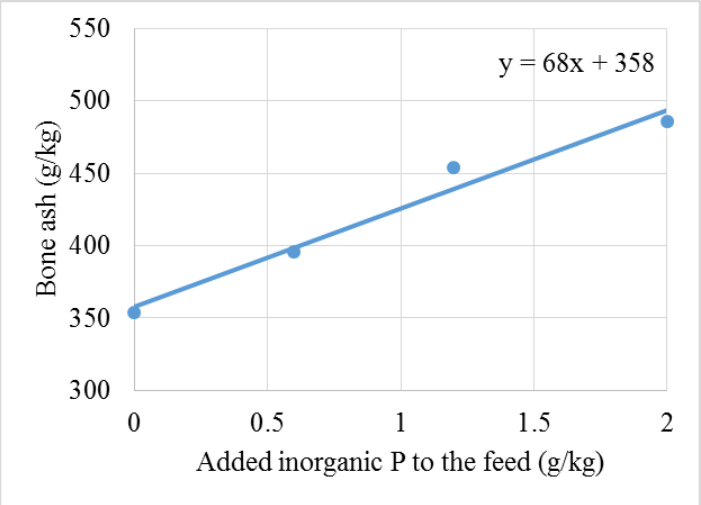

Fig. 1: the effect of increasing inorganic P on bone ash concentration.

From this regression curve, an equivalence between Ronozyme® HiPhos to OptiPhos® could be calculated for 125 to 750 OTU as shown in Table 2. It can be observed that the ratio between Ronozyme® Hiphos and OptiPhos® was on average 2.63, ranging from 2.4 at low dose to 2.9 at high dose. This indicates that levels of OptiPhos® above the standard recommended inclusion have more extra effect on tibia ash, than increasing levels of Ronozyme® HiPhos. In table 2 it is demonstrated that 250 OTU OptiPhos® is equal to 1.43 g P as MCP, which is close to the currently advised value of 1.45 g aP per kg of feed.

Table 2: equivalence between OptiPhos® and Ronozyme® HiPhos based on tibia ash and P regression

OptiPhos® (OTU/kg)	Ronozyme® HiPhos (FYT/kg)	P-MCP (g/kg)	Ratio Ronozyme® HiPhos/OptiPhos®
125	295	1.10	2.4
250	630	1.43	2.5
500	1375	1.77	2.8
750	2175	1.97	2.9

Conclusions

It can be concluded that increasing the dose of OptiPhos® above the normal recommended levels had an additional positive effect on body weight gain, and that the equivalence of 1 OTU OptiPhos® is on average 2.63 FYT of Ronozyme® HiPhos. It can also be observed that increasing levels of OptiPhos® above the recommended dose have more extra effect on tibia ash than increasing levels of Ronozyme® HiPhos, and that the real potential of OptiPhos® in replacing P as MCP is close to its claimed matrix value of 1.45 g aP/kg feed.

P-115 (ID 217)

ENERGY METABOLISABILITY AND ILEAL DIGESTA VISCOSITY OF GROWING TURKEYS FED DIETS CONTAINING MALTED SORGHUM SPROUTS SUPPLEMENTED WITH ENZYME OR YEAST

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Introduction

Nigerian livestock enterprise has suffered more than any other industry as a result of rising costs of production as feed alone accounts for up to 75% of total cost of broiler production (Ubosi, 2000) and short supply of essential raw materials such as maize, ground nut cake and soybean meal. The resultant effects include low production level, narrow profit margin and collapse of the once prosperous poultry industry. Moreover, the competition between man and livestock for these feed ingredients necessitate the use of alternative sources of ingredient. Example of such is Malted Sorghum Sprout.

Malted sorghum sprout (MSP) is a non - conventional feed stuff and a by-product of sorghum malting. MSP has been reported by Oduguwa *et al*. (2001) to have low nutritive value for monogastric animals and cannot be used as a main protein source. The low nutritive value may be due to its content of non-starch polysaccharide and fibre as well as tannin. Therefore, there is a need for the use of appropriate feed enzymes to improve the nutritive value of this cereal by-product. Bedford and Schulze (1998) documented that supplementation of diets with exogenous enzyme reduced the adverse effects of some of these compounds especially those produced by carbohydrates and proteins. Enzyme had been used to improve fibre digestion and reduced viscosity of digesta (Bedford *et al.,* 1991 and Atteh, 2001). Enzyme treatments have been used to improve performance of broiler chickens, increase weight gain, dry matter digestibility and nutrient digestibility, respectively (Sundu*et al.,* 2006).

Bakers' yeast has a relatively high lysine content and protein efficiency ratio. This yeast is very rich in essential minerals, crude protein (40 - 45 %) and vitamin B complex except B_{12} (Moyad, 2008). Yeast extracts have been widely reported as successful growth promoter in poultry industry. It helps in the metabolism of protein, lipids and carbohydrates (Spring, 2002). Yeast promoted growth of intestinal microflora (Spring *et al*., 2000) and increased growth (Parks *et al*., 2001). LutfulKabir (2009) noted that yeast improved digestion and stimulated the immune system.

The study reported below evaluated the response of turkeys fed diets containing MSP supplemented with enzyme and or yeast.

Materials and Methods

One hundred and twenty 28d old British United Turkeys (BUT) were brooded for 28d pre-experimental period. At 28th day, the birds were randomly allocated to 6 dietary treatments of 20 poults per treatment replicated four times with 5 poults each such that each treatment contained either enzyme or yeast at 200ppm in diets containing MSP at 0,50, or 100g/kg respectively to investigate the energy metabolisability and Ileal digesta viscosity of growing turkeys. The exogenous enzyme used is produced by (DSM Nutritional products Philippines, Inc.) is a commercial blend of xylanase (EC3.2.1.8), β glucanase (EC3.2.1.4) and protease (EC 3.4.2.1). The yeast used is the commercially available baker's yeast produced by LALLEMAND Canada Inc. At day 84, 6 groups of 6turkeys each were allocated to 6 experimental diets while an additional group of 6 turkeys were used for estimation of endogenous losses. All the turkeys were housed individually in a two tier steel metabolic cage. Three days of acclimatization were allowed before the commencement of the digestibility study. Known weights of feed (based on the daily requirement) were given to respective birds in the metabolic cage. Leftovers were measured and discarded on a daily basis. Excreta collection was done daily for a period of three days, unlike the former group of birds only water was administered to this group of birds so as to determine the endogenous losses. At the lapse of 24 hours, excreta was collected quantitatively on daily basis for two days. Apparent metabolisable energy (AME), AME corrected for nitrogen retention (AMEn), true metabolisable energy (TME) and TME corrected for nitrogen retention were determined according to the method of Sibbald (1979).

At day 112, 2 birds per replicate, 48 turkeys in all were selected based on final live weight of 8175g to determine the ileal digesta viscosity. The turkeys were slaughtered and digesta content was collected from the Merckel's diverticulum to the ileo-caecal junction. The ileal content of each replicate were emptied into labeled sample bottles. Uniform weights of samples were taken with a sensitive scale and were diluted to a volume of 400ml. The ileal digesta viscosity was determined using a BROOKFILED DV-E viscometer. The readings were taken at 50, 60 and 100 revolution per minute. Data generated were analysed using ANOVA. Polynomial contrast (Linear and quadratic) was used to determine effects of enzyme or yeast inclusion using SPSS (1999).

Results

Birds fed 50 and 100g/kg MSP had higher (P < 0.05) AME, AMEn and TMEn, while the least values for all the measurements were recorded for birds fed control diet. The TME values showed no effect (P > 0.05) following dietary inclusion of MSP. Dietary supplementation with enzyme or yeast showed no effect (P > 0.05) on apparent and true metabolisable energy values. MSP inclusion showed significant effect (P < 0.05) on ileal digesta viscosity of birds for all revolution per minutes used. At 50rpm, ileal viscosity quadratically reached its peak (P < 0.0001) and reduced when the MSP inclusion was increased to 100g/kg. At 60 revolutions per minute, ileal viscosity linearly increased (P <0.01) in all birds fed diet containing MSP. Similar values were recorded for birds fed 50 and 100 g/kg MSP. At 100 rpm, ileal viscosity significantly (P < 0.01) increased for birds fed 100 g/kg MSP. Lower but similar values of viscosity were recorded for turkeys fed control diet and diet containing 50g/kg MSP.

Table1: Effects of MSP inclusion and Enzyme or Yeast Supplementation on the Apparent and True Metabolizable energy of growing Turkeys.

Measurements	0	50	100	SEM	L	Q	Enzyme	Yeast	SEM	P value
AME (MJ/kg)	15.29^{b}	15.63^{a}	15.77^{a}	0.08	0.019	0.025	15.57	15.56	0.12	0.14
AMEn (MJ/kg)	15.23^{b}	15.57^{ab}	15.70^{a}	0.08	0.019	0.055	15.52	15.48	0.13	0.15
TME (MJ/kg)	16.43	16.75	16.78	0.07	0.057	0.112	16.66	16.65	0.13	0.17
TMEn (MJ/kg)	16.38^{b}	16.93^{a}	16.71^{ab}	0.08	0.086	0.010	16.78	16.57	0.13	0.16

^{abc} Means on the same row having different superscript differ significantly (P < 0.05)

Table 2: Effects of MSP inclusion and enzyme or yeast supplementation on Viscosity of growing Turkeys.

	MSP Inclusion (g/kg)				P value		Additives (mg/kg)			
Speed	0	50	100	SEM	L	Q	Enzyme	Yeast	SEM	P value
50	297.8^{b}	329.9^{a}	308.8^{b}	4.56	0.232	0.000	313.1	311.2	3.72	4.12
60	250.7^{b}	277.4^{a}	274.1^{a}	4.38	0.008	0.003	267.7	267.1	3.74	4.23
100	216.2^{b}	222.6^{b}	230.5^{a}	2.25	0.004	0.019	218.3	227.8	1.83	2.10

^{abc} Means on the same row having different superscript differ significantly (P < 0.05)

Conclusion

Turkeys were able to metabolise MSP at 50 g/kg and100g/kg for improved AME, AMEn and TMEn irrespective of the additive used. Supplementation with 200mg/kg enzyme or yeast did not significantly affect ileal digesta viscosity.

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P-117 (ID 104)

EFFECT OF MULTI-ENZYME SUPPLEMENTATION ON THE UTILIZATION OF MALTED SORGHUM (*SORGHUM BICOLOR*) SPROUTS BY BROILER CHICKENS

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Abstract

A feeding trial was conducted to evaluate the utilisation of Malted Sorghum Sprouts (MSSP) supplemented with Maxigrain® (an enzyme cocktail containing α-amylase, Xylanase, β-glucanase, Exo-Cellulase, Pectinase, Protease, Phytase and Lipase) by broiler chickens. Standard diets were formulated for 270 starter and finisher chickens with graded levels of MSSP. Birds were distributed in completely randomised design into 5 treatments with 3 replicates each. MSSP was included at 0%, 10% without maxigrain, 10 + maxigrain; 15% without maxigrain and 15 + maxigrain. Birds were supplied with treatment diets and water *ad libitum*. Data collected included the initial and final weight for each phase while weight gain and feed consumption were recorded weekly. Feed/gain ratio and feed cost/Kg gain were computed. Data were subjected to ANOVA and means were compared using DMRT. At the starter phase, birds fed 10% MSSP with Maxigrain® had significantly ($P < 0.05$) highest final body weight and daily weight gain, and also had the best feed/gain ratio and lowest feed cost per Kg gain. At the finisher phase, all the treatments had similar ($P > 0.05$) final weight and daily weight gain of birds. Feed conversion was however best for birds fed 15 % MSSP with Maxigrain®, which also had the best and lowest feed cost per Kg gain. Broiler Chickens effectively utilised and obtained best performance with 10 % and 15% MSSP in diet at starter and finisher phase respectively with Maxigrain® supplementation.

Keywords: Moulted Sorghum Sprouts, Broiler Chickens, Maxigrain® Enzyme, Growth Performance

Introduction

Sorghum bicolor (L) Moench is widely grown in the semi-arid and arid savannah regions of Nigeria. MAUNDER (2002) reported that sorghum is a traditional crop of much of Africa and Asia and an introduced and hybridized crop in the western hemisphere. Sorghum is the world's fifth most important cereal and is grown in semi-arid regions of Africa being well adapted to the harsh climate and naturally resistant to many pests (BELTON *et al*., 2003).

It benefits from an ability to tolerate drought, soil toxicities and temperature extremes effectively than other cereals. In terms of the nutritive value, cost and availability, sorghum grain is the next alternative to maize in poultry feed (SUBRAMANIAN AND METTA, 2000).

Malted sorghum sprout (MSSP) is a by-product of sorghum malting. The separated roots and shoots which are left after malt extraction from the young germinating sorghum seedlings are collectively called sorghum sprout (ALETOR *et al*., 1998). MSSP has a lot of prospect as a feed stuff of the livestock industry. It is rich in organic nitrogen. It contains (g/kg); 226 crude protein, 48 crude fibre, 33 ether extract, 16 ash, 522 nitrogen free extract and 16.26 MJ/kg DM gross energy. Magnesium was reported to be the most abundant mineral while potassium was the least in MSSP. Among the trace minerals, Zinc is the most abundant while copper is the least. Sorghum sprout is reported to contain a considerable number of amino acids with low level of methionine, lysine and threonine (ANING *et al*., 1998; IKEDIOBI, 1989).

Materials and Methods

Experimental site: The experiment was conducted at the Teaching and Research Livestock Farm of the Department of Animal Science, Ahmadu Bello University, Zaria, Nigeria. Zaria is located within the Northern guinea savannah zone on latitude 11^0 9' 45''N and longitude 7^0 38' 8''E, with an altitude of 610m above sea level (OVIMAPS, 2014).

Malted sorghum sprouts: MSSP was sourced from Nigerian Brewery Company (NBC) Kaduna Nigeria. It was dried in the sun and then incorporated into broiler diets.

Experimental Design and management of birds: Two hundred and seventy (270) day-old broiler chicks were used. The birds were assigned in a completely randomized design (CRD) to five dietary treatments with three replicates consisting of 18 birds each. Five diets were formulated; Diet 1, was maize based diet as the control, Diets 2 and 4 had 10% and 15% MSSP respectively; Diets 3 and 5 had 10% and 15% MSSP respectively, both supplemented Maxigrain® - an

enzyme cocktail - at 0.01%. Diets were adjusted to meet standard requirements of birds at the starter and finisher phases each of which lasted for 28 days. Feed and water were provided *ad libitum*. Records on weight gain, feed intake, feed conversion ratio, cost per kg weight gain and mortality were taken at both phases.

Results

The effect of MSSP on growth performance of starter and finisher Broiler chickens is presented in table 1. For the starter broiler chickens, the final weights were significantly ($P < 0.05$) higher for enzyme supplemented diets both at 10 % and 15 % MSSP. Best performance was obtained for birds fed 10 % MSSP with Maxigrain® supplementation in terms of final weight, weight gain, feed to gain ratio and the cost of producing 1 Kg of meat.

At the finisher phase, all the treatments had similar ($P > 0.05$) final weight and daily weight gain of birds. Feed conversion was however best for birds fed 15 % MSSP with Maxigrain®, which also had the best and lowest feed cost per Kg gain.

Enzyme supplementation of diets containing MSSP resulted into better utilization of this feed resource.

Conclusion

- Broiler starter chickens can efficiently utilise 10 % moulted sorghum sprouts in the diets with optimum performance when supplemented with Maxigrain enzyme cocktail.
- Broiler finisher chickens can efficiently utilise 15 % moulted sorghum sprouts in the diets with optimum performance when supplemented with Maxigrain enzyme cocktail.

Table 1: Effect of graded levels of MSP on the performance of broiler chickens

Parameters	T1	T2	T3	T4	T5	
			% MSSP			
	0	10	10+Max	15	15+Max	SEM
Starter Phase						
Initial wgt (g/bird)	85.7	85.0	85.7	85.33	86.33	2.13
Final wgt (g/bird)	797.3^{a}	630.6^{b}	897.10^{a}	550.17^{b}	613.69^{b}	53.48
Wgt gain(g/bird/day)	25.4^{a}	19.5^{b}	28.98^{a}	16.60^{b}	18.83^{b}	1.89
Feed intake (g/bird/day)	54.4^{a}	42.3^{bc}	43.17^{b}	40.79^{bc}	39.43^{c}	1.58
Feed/gain ratio	2.14^{b}	2.18^{b}	1.53^{a}	2.47^{c}	2.13^{b}	0.13
Feed cost/kg gain (₦)	209.4^{c}	196.26^{bc}	138.76^{a}	213.79^{c}	185.69^{b}	11.59
Mortality	0.00	0.00	0.00	0.00	0.00	--
Finisher Phase						
Initial wgt (g/bird)	753.3	750.0	750.0	753.3	753.3	5.16
Final wgt (g/bird)	1833.3	1850.0	1916.7	1833.3	1923.3	45.19
Wgt gain(g/bird/day)	38.7	39.3	41.7	38.6	41.8	1.65
Feed intake (g/bird/day)	108.8^{a}	92.8^{b}	89.5^{b}	82.8^{c}	81.6^{c}	2.01
Feed/gain ratio	2.82^{c}	2.37^{b}	2.15^{a}	2.15^{a}	1.97^{a}	0.09
Feed cost/kg gain (₦)	216.6^{d}	168.1^{c}	154.4^{b}	146.7^{ab}	135.7^{b}	6.09
Mortality	0.00	0.00	0.00	0.00	0.00	-

a, b, c: Means in the same row having different superscript are significantly different ($P<0.05$).
SEM: Standard error of means. Max: Maxigrain enzyme

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P-118 (ID 22)

EFFECT OF RONOZYME® PHYTASE ON THE UTILIZATION OF TWO POPULAR VARIETIES OF SORGHUM (*SORGHUM BICOLOR*) IN NIGERIA BY BROILER CHICKENS

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Summary

The effect of feeding 300 broiler chickens with two common varieties of sorghum in Nigeria, *Jandawa* (Red sorghum) and *Kaura* (SAMSORG-17) with or without phytase was evaluated in a Completely Randomised Design of five dietary treatments, each replicated thrice with 20 chicks. Treatment 1 was maize- T2, *Jandawa*- T3, Kaura- T4, *Jandawa* with phytase- and T5, *Kaura* with phytase- based standard diets were formulated for starter and finisher chickens. Water and feed were offered to the birds *ad libitum* for the eight-week period. Collected data were initial and final weight while weight gain and feed consumption were recorded weekly. Feed/gain ratio and feed cost/Kg gain were computed. At 8 weeks, three birds per treatment were used for carcass and bone mineral analyses. Serum Ca and P were determined. Data were subjected to ANOVA and means were compared using DMRT. Birds fed *Kaura* with phytase had significantly ($P < 0.05$) highest final body weight and weight gain at the starter phase, non-significant ($P > 0.05$) differences at the finisher phase but had significantly ($P < 0.05$) lowest cost of production for the eight weeks period. Diets with phytase inclusion had significantly ($P < 0.05$) higher tibia Ca and P but non-significantly higher serum Ca and P than other treatments. Phytase in *Kaura* based diets significantly improved growth performance of broiler chicks, reduced cost of production and enhanced bone mineralization than *Jandawa* and maize based diets.

Keywords: Jandawa, Kaura, Ronozyme Phytase, Growth Performance, Bone Minerals

Introduction

Grain sorghum is the third most important cereal crop grown in the United States and the fifth most important cereal crop grown in the world. In 2010, Nigeria was the world's largest producer of grain sorghum, followed by the United States and India (FAO, 2012). MAUNDER (2002) reported that sorghum is a traditional crop of much of Africa and Asia and an introduced and hybridized crop in the western hemisphere.

Enzyme inclusion in poultry feeds is one of the major nutritional advances in the last fifty years. Some of the enzymes that have been used over the past several years and have potential for use in the feed industry include cellulase (β-glucanases), xylanases and associated enzymes, phytases, proteases, lipases, and galactosidases (KHUSHEEBA AND SAJID, 2013). The addition of phytase to feed improves phosphorus utilization in both ruminant and monogastric animal; thus reducing the need for supplemental inorganic phosphate and helps in reducing the environmental problems that arise from organic phosphate excretion. Commercial phytases are available and are effective in improving phytate phosphorus utilization in poultry diets (WU *et al*., 2003; DILGER *et al*., 2004).

Amongst cereal grains, sorghum contains relatively high phytate concentrations (SELLE *et al*., 2003) and it follows that broilers on sorghum-based diets should be advantaged by phytate-degrading enzymes.

Materials and Methods

Experimental site: The experiment was carried out at the Poultry Unit of Animal Science Departmental Teaching and Research farm, Ahmadu Bello University, Zaria, Nigeria. Zaria, located in the Northern Guinea Savannah zone is on latitude 11^0 9' 45''N and longitude 7^0 38' 8''E, with an altitude of 610m above sea level. The climate is characterized by a well-defined dry and wet seasons with a mean annual rainfall of 700-1400mm (OVIMAPS, 2014)

Experimental Design and management of birds: Three hundred broiler chickens were allotted to five dietary treatments with 3 replicates of 20 birds each in a completely randomized design (CRD). The birds were housed in deep litter pens and managed with all necessary routine management practices with feed and water supplied *ad libitum*. Five treatment diets were formulated to meet standard requirements. Diet 1: (Control) Maize based diet. Diet 2: Jandawa based diet; Diet 3: Kaura based diet. Diet 4: Jandawa based diet with phytase. Diet 5: Kaura based diet with phytase

Growth Study: Initial and final weights of birds were taken at the beginning and at the end of both phases. Weight gain and feed intake were measured weekly while feed/gain ratio and cost per Kg gain were computed for both phases. Mortality was recorded as they occurred.

Carcass Evaluation and Bone Mineral Analysis: Three birds were selected from each treatment at the end of the feeding trial, fasted of feed overnight, weighed, slaughtered for carcass evaluation. Carcass weight, cut parts and organ weights were recorded. The prime cuts and organ weights were expressed as a percentage of dressed weight and live weight respectively. The tibia bones were removed carefully from the thigh. The fresh bones were oven dried at 100°C overnight to obtain a constant weight. The dry bones were ashed at 550°C for 6 hours. The bone ash was analysed for calcium content using Atomic Absorption Spectrophotometry (AAS) and the phosphorus content using flame photometry. Serum Calcium and Phosphorus were also determined using standard procedures.

Data Analysis: All data obtained were statistically analysed using the GLM Procedure of SAS and significant difference between treatment means were determined using Duncan's Multiple Range Test (SAS, 2002).

Results

Growth Performance of broiler chickens

Table 1 shows the performance of broiler chickens. Starter birds fed Kaura with phytase had significantly ($P<0.05$) higher final body weight and weight gain, which agrees with KHAN *et al*., (2013) who reported that phytase addition in broiler feed greatly enhanced the growth rate and weight gain of chicks. The maize and Jandawa diets (T1 and T2) had significantly lower feed consumption than all the other treatments which were at par. The Kaura diet with phytase increased feed consumption but with a significant increase in weight gain. Therefore, birds efficiently utilized Kaura diets.

Kaura diet with phytase had the least cost of production (₦148.96) compared to other treatments with 158.44, 167.67, 159.01 and 176.33 for T1-T4 respectively. The use of phytase with Kaura significantly reduced the cost of producing broiler chickens than the traditional maize based diet.

Broiler finishers showed no significant ($P > 0.05$) difference in the final body weight, weight gain, feed intake and feed to gain ratio across all treatment diet.

The feed cost required to produce one kilogram of meat was lowest and similar for Kaura with enzyme and maize diet. This was followed by Kaura without enzyme and then Jandawa with enzyme. The highest cost of production was recorded for Jandawa without enzyme.

Phytase inclusion resulted into a general improvement in nutrient digestibility.

Bone mineral contents of birds

Table 2 shows the result of mineral analysis of the tibia bones of broiler chickens fed Kaura and Jandawa with and without enzyme inclusion.There were significant ($P<0.05$) differences across treatments. Phytase inclusion increased tibia calcium of birds fed Jandawa diet with phytase (up to 6%) and tibia phosphorus (up to 3%) and Kaura diet with phytase (up to 7%) and tibia phosphorus (up to 3%). This is a good indication of increased availability of Phosphorus from phytate mineral complex by the action of phytase as also earlier reported by SABESTIAN *et al*., (1997) and MONDAL *et al*., (2007).

Conclusion

- Complete replacement of maize with Kaura and Jandawa variety of sorghum did not impair body weight, feed intake, feed conversion ratio, nutrient digestibility, carcass and organ weights and bone mineral content in the diets of broiler chicken.
- Phytase inclusion in Sorghum based broiler diets significantly improved growth performance and mineral contents of bones.

Table 1: Performance of broiler birds fed sorghum based with or without phytase enzyme

Parameters	T1 Maize	T2 Jandawa	T3 Kaura	T4 Jandawa+ phytase	T5 Kaura+ phytase	SEM	LOS
Starter Phase (0-4 weeks)							
Initial wgt (g/bird)	43.53	43.40	43.53	43.40	43.30	0.16	NS
Final wgt (g/bird)	722.10^{b}	755.50^{b}	755.60^{b}	755.53^{b}	820.57^{a}	21.38	*
Wgt gain(g/bird)	678.57^{b}	712.10^{b}	712.07^{b}	712.13^{b}	777.27^{a}	21.46	*
Feed intake (g/bird)	1347.63^{b}	131.23^{b}	1412.4^{a}	1369.97^{a}	1440.87^{a}	42.63	*
Feed/gain ratio	1.98	1.84	1.99	1.92	1.85	0.05	NS
Feed cost (₦/Kg)	80.02^{b}	91.18^{a}	80.02^{b}	91.68^{a}	80.52^{b}	2.89	*
Feed cost/kg gain (₦)	158.44^{b}	167.67^{c}	159.01^{b}	176.33^{d}	148.96^{a}	4.27	*
Finisher Phase (5-8 weeks)							
Initial wt (g/b)	722.10	755.50	755.60	755.53	820.57	21.38	NS
Final wt (g/b)	2272.2	2339.8	2257.9	2282.5	2395.2	83.86	NS
Wt gain (g/b)	1550.12	1584.28	1502.33	1526.99	1574.67	70.17	NS
Feed intake (g/b)	3902.50	4040.00	3883.20	4036.10	3999.50	143.58	NS
Feed/gain ratio	2.52	2.53	2.59	2.64	2.54	0.40	NS
Feed cost (₦/Kg)	74.05^{a}	84.85^{c}	74.05^{a}	85.35^{d}	74.55^{b}	0.01	*
Feedcost/kg gain	186.60^{a}	216.65^{c}	191.54^{b}	225.61^{d}	189.36^{a}	3.19	*

a, b,c Means with different superscripts on thesame row are significantly different (P<0.05)
LOS: Level of significance. NS: Non-significant difference (P>0.05) *: Significant difference (P<0.05)
SEM: Standard error of means

Table 2: Calcium and phosphorus contents of tibia bone and blood serum

Parameters	T1 Maize	T2 Jandawa	T3 Kaura	T4 Jandawa+ phytase	T5 Kaura+ phytase	SEM	LOS
Bone							
Calcium (%)	29.11^{a}	23.39^{b}	24.69^{ab}	29.13^{a}	31.33^{a}	3.42	*
Phosphorus (%)	13.27^{a}	10.52^{b}	11.11^{ab}	13.11^{a}	14.10^{a}	1.55	*
Serum							
Calcium (mmol/l)	1.81	1.78	1.76	1.84	1.88	0.09	NS
Phosphorus (mmol/l)	2.02	2.14	2.27	2.61	2.50	0.24	NS

a,b,c, Means with different superscripts on the same row are significantly different (P<0.05)
LOS: Level of significance. NS: Non-significant difference (P>0.05). *: Significant difference (P<0.05)
SEM: Standard error of means

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P-119 (ID 121)

SOLUBILISATION AND VISCOSITY REDUCTION EFFECTS ON CEREAL CELL WALLS MEDIATED BY XYLANASE AND ß-GLUCANASE

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Abstract

Viscosity reduction of three feed enzymes was measured in a high throughput pressure sensing system using commercial dosages on purified polysaccharides (arabinoxylan and ß-glucan). Fluorescence and antibody microscopy techniques were also used to visualize effects on the solubilisation of endosperm cell walls of wheat and barley.

Viscosity data clearly demonstrated depolymerisation of mixed-linked ß-glucans by the two multicomponent enzymes. The two multicomponent enzymes also reduced the viscosity of the arabinoxylan solution, while the monocomponent xylanase most effectively depolymerised the arabinoxylan. Microscopy data revealed that the multicomponent enzyme chosen for the studies could solubilize both wheat and barley cell walls by targeting both the ß-glucan strutures as well as the arabinoxylans. The xylanase could effectively solubilise wheat and barley cell walls by merely attacking the arabinoxylans.

It's concluded that viscosity measurements provides information on differences in hydrolytic capacity of feed enzymes. Additionally the study has shown that microscopy techniques that visualise cell wall solubilising effects of feed enzymes can be useful for understanding enzyme mode of action and efficacy on different feed ingredients.

Keywords: xylanase, ß-glucanase, solubilisation, viscosity

Introduction

Even after milling of feed ingredients some nutrients will remain within intact cell walls built up by indigestible fibre components. Commercial exogenous feed enzymes, such as mixed-linked ß-glucanases or arabinoxylanases, are used to degrade fibre components in cereals by de-polymerising soluble fibres. This may reduce digesta viscosity and thereby facilitate nutrient absorption. Exogenous microbial enzymes may also mediate solubilisation of the insoluble cell wall matrix, thereby releasing captured nutrients.

Materials and Methods

Three commercial enzymes; RONOZYME WX (monocomponent xylanase), RONOZYME MultiGrain (a multienzyme product containing mainly xylanase and ß-glucanase) and RONOZYME VP a commercial multicomponent enzyme containing over 40 different activities mainly targeting other components besides arabinoxylan and mixed-linked ß-glucan.

The substrates used for the viscosity study, arabinoxylan and -glucan, were obtained from Megazymes, Ireland.

Viscosity studies were conducted using a pipetting workstation, Hamilton, with eight microprocessor-controlled pipetting channels that are analogous to manual pipettes. It is capable of pressure sensing during aspiration and dispensing of solutions. The viscosity pressure (ViPr) assay displays a linear correlation between pressure measured (Pa) and kinematic viscosity (ABEL & PETTERSSON, 2011). During the complete aspiration and dispensing process the air displacement is continuously monitored in intervals of 10 ms by a pressure sensor to verify an uninterrupted substrate flow. Pressure measured in this work is defined as viscosity determined in relation to the ViPr assay.

Sections of barley and wheat (10 µm) were treated for 3h with RONOZYME MultiGrain or RONOZYME WX. Enzyme or buffer droplets were applied directly on the glass slide carrying the sample. Approximately 600 µL was applied to each glass slide.

Autofluorescence, as well as histochemical staining, was used to visualize the endosperm structures. Fluorescence microscopy was performed using a Nikon Eclipse 80i Microscope (Nikon Corporation, Japan) to visualize structures in both wheat and barley using Calcofluor white.

Confocal images of immunolabeled samples were acquired from sections of barley and wheat by detecting the Alexa-555 fluorescein-signal from the LM11 immuno-label using a Leica CLSM SP2 microscop.

Results

Within 30 minutes a distinct viscosity reduction was observed when adding the commercial products to a viscous arabinoxylan solution (Fig. 1). There was no noticeable viscosity reduction observed for the ß-glucan solution when adding the monocomponent xylanase, as expected, while the two multicomponent products (RONOZYME Multigrain and VP) caused a marked viscosity reduction (Fig. 1) due to their ß-glucanase activity.

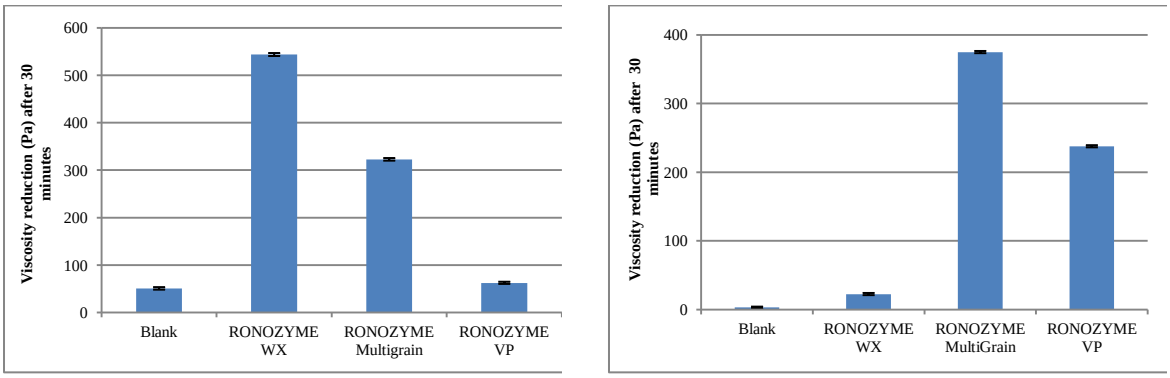

Figure 1. Viscosity reduction of an arabinoxylan solution 1g/100 mL (left figure) and a ß-glucan solution 0.5 g/100 mL (right picture) when adding commercially related dosages of the respective enzymes.

Staining with calcoflour white revealed the mixed linked ß-glucan structures present in the cell walls of barley (HESSELMAN & ÅMAN, 1986) and on enzyme addition with the Ronozyme Multigrain the cell wall structures were disrupted and no longer visible (Fig. 2). A similar effect was not observed for the monocomponent xylanase (data not shown). Both the multicomponent and the monocomponent product efficiently degraded the arabinoxylan present in the cell walls (Fig.3), as indicated by immuno-labeling with the LM 11 label targeting arabinoxylans.

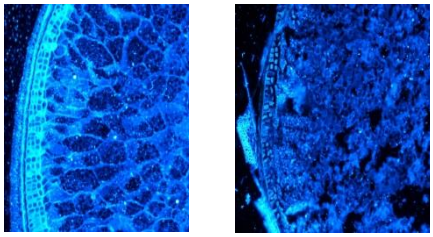

Figure 2. Barley stained with calcoflour white, left picture (control), and the same sample after 3 h of enzyme incubation with RONOZYME Multigrain.

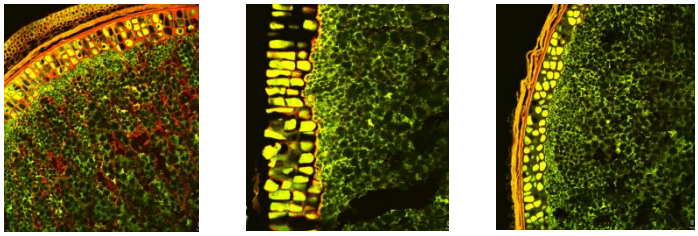

Figure 3. Barley labelled with the LM 11 immuno label (left picture) and after 3 h when treated with RONOZYME Multigrain (middle picture) or RONOZYME WX (right picture).

Conclusions

The results obtained in this study demonstrates the strong hydrolytic capacity of xylanases and ß-glucanases that results in efficient viscosity reduction of soluble xylans and ß-glucans. By using microscopy techniques it was also possible to demonstrate the cell wall degrading efficacy of commercial feed enzymes commonly used in animal production. A notable effect demonstrated was the capacity of a monocomponent xylanase to degrade the cell wall constituents of barley. This finding indicates that a ß-glucanase is not necessarily essential for releasing nutrients encapsulated by insoluble cell walls partly made up by mixed-linked ß-glucans. However, in situations where high concentrations of soluble ß-glucans increase digesta viscosity to levels detrimental for nutrient absorption a dedicated ß-glucanase may provide an efficient solution.

In the present study the use of microscopy techniques to visualize cell wall solubilisation of cereal cell wall components in vitro by feed enzymes agrees with the viscosity reductions obtained by these enzyme products.

The methods employed in this study may be used as a valuable *in vitro* methodology to demonstrate the complexity of feed ingredient degradation and solublisation by commercial feed enzymes. Coupling of such results with actual animal performance data may provide an important tool for better understanding the relationship between cell wall degradation and viscosity reduction as predictors of the productive value of feed enzymes.

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P-120 (ID 107)

EFFECT OF A MICROBIAL 6-PHYTASE SUPPLEMENTATION ON PLASMATIC MYO-INOSITOL AND ON THE PECTORALIS MUSCLE TRANSCRIPTOME IN BROILERS

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Summary

A floor pen trial of five weeks was conducted to evaluate the effects of a 6-microbial phytase supplementation in broilers in terms of growth performance, *myo*-inositol plasma concentration, bone mineralization, breast meat weight, and *Pectoralis major* muscle transcriptome. Two diets were given *ad libitum*: A positive control diet (PC) and a negative control diet (NC) formulated with a moderate deficiency of phosphorus compared to PC. The NC diet was distributed with or without phytase supplementation at 1000 U / kg. Phytase supplementation led to a significant improvement of WG, FCR, *myo*-inositol plasma concentration, tibia ash percentage, and muscle weight compared to NC. A transcriptomic study showed that several genes involved in muscle development were up-regulated in chickens fed phytase compared to NC, involving transcription factors MEF2 activation via calcineurin A, and muscle differentiation through IGF with PI3 kinase. This study suggests that the addition of this phytase increases the concentration of *myo*-inositol, the final product of phytate hydrolysis, and influences muscle development in broilers.

Keywords: poultry, nutrition, muscle, *myo*-inositol, gene expression.

Introduction

Phytate is considered an anti-nutritional compound due to its ability to bind to cations, proteins and starch generating insoluble aggregates that lead to reduced bioavailability in poultry (Olukosi, 2012). Supplementation with microbial phytase has been shown to improve phytate-P digestibility in farm animals and to reduce environmental P pollution. Increased retention of phytate-P and the associated beneficial effects on Ca solubility has a range of beneficial effects on feed intake and bone mineralisation (Aureli *et al*., 2011). In addition, phytase improves growth performance, energy and amino-acid digestibility (Guggenbuhl *et al*., 2012). Additionally, the involvement of *myo*-inositol in the improved growth efficiency of poultry has recently been implied (Cowieson *et al*., 2014). The aim of the present work was to investigate physiological effects of phytase supplementation on plasma *myo*-inositol concentration and breast muscle transcriptome.

Material and Methods

The experiment was done at facilities of the Research Centre of Animal Nutrition (CRNA) of DSM Nutritional Products, France. On the day of arrival, broilers were divided by weight into 2 groups of 20 birds per treatment. Body weight gain (WG) and feed consumption for the whole period (d1-36) were determined for each cage.

The basal diets contained maize and soybean meal as main ingredients. The respective negative control diet was formulated with moderate deficiency of phosphorus (P total = 5.1 g/kg ; Av.P (available P) = 2.01 g/kg for the starter and and P total = 5.2 g/kg ; Av.P = 2.14 g/kg for the grower) ; and calcium (Ca) (Ca = 6.0 g/kg for the starter and Ca = 6.2 g/kg for the grower) and fed as such (negative control, NC) or supplemented with 2.0 g/kg non phytate-P from dicalcium phosphate dihydrate (DCP) as a positive control (PC). NC was supplemented with bacterial 6-phytase (DSM Nutritional Products Ltd, Switzerland) at 1000 FYT/kg (Phy1000). Biological samples were collected on d36 for plasma *myo*-inositol determination, tibia ash percentage, and breast muscle weight. Muscle RNA extraction was done by the phenol-chloroform method and microarray analysis was performed using the WT PLUS reagent kit and CHIGENE-1.0-ST arrays by Affymetrix (Affymetrix UK Ltd., High Wycombe, UK). Validation of microarray results was done by quantitative PCR (qPCR) on target genes.

Results

The analysed CP content in all diets were slightly higher than formulated, while ME, P, Ca concentrations were on or close to target. Phytase activity recovered in the feed after pelleting was in line with expectations and in accordance with the design of the experiment.

The addition of either dicalcium phosphate or phytase to the NC group significantly improved weight gain (WG) and bone mineralization. A significant increase in plasma myo-inositol concentration on d36 was recorded with phytase supplementation compared to NC diet.

From the comparison of the both groups PC and Phy1000 versus NC, a total of respectively 300 and 625 genes were modulated. Significant influenced pathways (FDR<0.01) were observed for animals fed phytase compared to NC and were mainly associated to development (Table 1).

Table 1: Functional analysis from microarray data compared to NC.

Treatment	Metabolic pathways	FDR
PC *vs* NC	Cytoskeleton remodeling_TGF, WNT and cytoskeletal remodeling	0.034
	Neurophysiological process_Netrin-1 in regulation of axone guidance	0.034
Phy1000 *vs* NC	Immune response_Function of MEF2 in T lymphocytes	0.002
	Development_Role of HDAC and calcium/calmodulin-dependent kinase (CaMK) in control of skeletal myogenesis	0.002
	Development_Non-genomic action of Retinoic acid in cell differentiation	0.002
	Development_Notch Signaling Pathway	0.004

A deeper analysis of the pathway "Role of Histone Deacetylase (HDAC) and calcium/calmodulin-dependent kinase (CaMK) in control of skeletal myogenesis" was performed. Differentially expressed genes associated with this network were up-regulated compared to negative control: Calmodulin (CALM1), calcineurin A (PPP3CB), Myocyte Enhancer Factors 2 (MEF2A and MEF2C), and P300/CBP associated factor (PCAF). The phospholipase C beta (PLCB1) expression was also up-regulated by qPCR. This enzyme enhances the effect of calmodulin/calcineurin A on muscle growth (Tokumitsu *et al*., 1999) but also the interactions between calcineurin and MEF2 (Li *et al*., 2013). The IGF/PI3K way seems to be affected since the phosphatidyl-inositide-3-phosphate kinase, regulatory subunit 1 (PI3KR1) was also significantly up-regulated, as the catalytic subunit (PIK3CA). These enzymes are involved in muscular development and differentiation (Wullschleger *et al*., 2006) via the binding of insulin or IGF on its receptor. Other studies showed insulin-mimetic effect of *myo*-inositol (Cowieson *et al*., 2014 and Józefiak *et al*., 2010) which was at a higher level in animals fed phytase compared to NC. The effect of *myo*-inositol on growth performance during grower period was also described by Cowieson *et al*. (2013). Results obtained could suggest a *myo*-inositol effect on IGF receptors, sensitive to insulin, and leading to modulation of gene expression linked to muscular development. Microarray data and confirmation of fold changes by qPCR are presented in table 2.

Table 2. Fold change in gene expression. (* : p<0.05, ** : p<0.01.)

Gene	Treatment	Fold change Microarray (n=4)	Fold change qPCR (n=16)	SEM (qPCR)
PI3KR1	PC *vs* NC	1.32	1.51	-0.65 / +0.46
	Phy1000 *vs* NC	2.02**	3.04*	-0.80 / +0.63
PI3KCA	PC *vs* NC	1.01	1.69	-0.66 / +0.47
	Phy1000 *vs* NC	1.37	2.84*	-0.93 / +0.70
PPP3CB	PC *vs* NC	1.18	1.88	-0.73 / +0.53
	Phy1000 *vs* NC	1.67*	2.67*	-0.59 / +0.48
MEF2A	PC *vs* NC	1.08	1.42	-0.21 / +0.19
	Phy1000 *vs* NC	1.44*	1.68*	-0.30 / +0.26
MEF2C	PC *vs* NC	1.17	1.73	-0.50 / +0.39
	Phy1000 vs NC	1.43*	2.14*	-0.50 / +0.41
PLCB1	PC *vs* NC	1.02	1.48	-0.50 / +0.37
	Phy1000 *vs* NC	1.43	2.66*	-0.79 / +0.61

Conclusions

Exogenous phytase has a range of effects that extend beyond P and Ca and that these effects exhibit profound changes at a molecular level in broiler chickens. Addition of phytase significantly increases plasma *myo*-inositol concentrations and up-regulates key metabolic pathways involved in skeletal muscle development. These results explain why the addition of phytase to poultry diets often results in performance responses that cannot be readily explained by effects on nutrient digestibility alone. As phytase dosing continues to increase more attention should be given to dietary phytate concentrations, the antinutritional effects of phytate and the anabolic advantages of *myo*-inositol.

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P-123 (ID 300)

EXOGENOUS PROTEASE IN MASHED AND PELLETED DIETS FOR BROILERS

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Abstract

The aim of this study was to evaluate the performance and carcass yield and cuts of broilers fed with mashed and pelleted diets with or without addition of protease. Seven hundred sixty eight birds were reared from 1 to 42 days old, distributed in a completely random design in a 2x2x2 > factorial arrangement (physical forms of feed, nutritional levels and with or without addition of protease) with eight treatments and eight replicates. For diets with low levels of nutrients, reduction of 4% was performed in crude protein (CP) and amino acids. The pelleted diets provided improvement in performance parameters of broilers at 42 days of age. The reduced levels of CP and amino acids increased feed conversion ratio. The addition of exogenous protease did not have main effect on the performance and carcass yield and cuts. However, there was interaction between the addition of protease and nutritional levels on carcass yield, and interaction between the physical forms of feed and addition of protease on breast yield.

Keywords: carcass yield, enzyme, pelleting, performance

Introduction

It is well known that the addition of exogenous enzymes in diets, as a nutritional strategy, has a practical goal of reduction of production costs and increase performance in poultry industry. There are many commercial enzyme products available, and research about use of phytase is well established in broiler nutrition. However, it is necessary research focused more on the other enzymes such as protease. Even though corn and soybean meal are widely used in diets for broilers because of their high nutritional value, it has been reported that considerable amount of protein pass through the digestive tract without complete digestion (Leme, et al. 2004). Because of this portion of protein no digested, the addition of exogenous proteases is a good opportunity to improve nutrient utilization (Freitas, et al. 2011). Ghazi et al, 2002 observed positive effects, in diets deficient or marginal in amino acids, when a single-component protease was used. Given the above, the aim of this study was to evaluate the performance and carcass yield and cuts of broilers fed with mashed and pelleted diets with or without addition of protease.

Material and Methods

All the experimental procedures used in this research were approved by the Ethics Committee on Animal Use of the School of Veterinary Medicine and Animal Science of the University of Sao Paulo. A total of 768 Cobb 500 male broiler chicks vaccinated for Marek's disease were obtained from a commercial hatchery. Broilers were reared from 1 to 42 days old, distributed in a completely random design in a 2x2x2 factorial arrangement (physical forms of feed, nutritional levels and with or without addition of protease) with eight treatments and eight replicates. Each pen was provided with nipple drinkers, one tube feeder and rice husk as litter. Feed and water were provided *ad libitum*. Live performance measurements (body weight gain, feed intake, and feed conversion) were determined at 21 and 42 days of age. At 43 days of age, two birds from each pen were randomly selected and processed for carcass yield and commercial cuts. Carcass weight was expressed as proportion of body weight, whereas carcass parts were expressed relative to the whole carcass. Corn and soybean meal were used as the major ingredients to formulate the feeds for a 4-phase program: 1 to 7 d, 8 to 21 d, 22 to 35 d, and 36 to 42 d of age. Dietary treatments were: T1 – mashed diet with reduced nutritional levels and without addition of protease; T2 – mashed diet with reduced nutritional levels and with addition of protease; T3 – mashed diet with recommended nutritional levels and without addition of protease; T4 – mashed diet with recommended nutritional levels and with addition of protease; T5 – pelleted diet with reduced nutritional levels and without addition of protease; T6 – pelleted diet with reduced nutritional levels and with addition of protease; T7 – pelleted diet with recommended nutritional levels and without addition of protease; T8 – pelleted diet with recommended nutritional levels and with addition of protease. Diets without reducing nutrient levels were formulated according to the requirements of Rostagno et al. (2011). For diets with low levels of nutrients, reduction of 4% was performed as suggested by the average of the manufacturer of the reduction in crude protein (CP) and amino acids (lysine, methionine

and threonine). The commercial protease used in this study is produced by fermentation of *Bacillus licheniformis* containing transcribed genes *Nocardiopsis prasina.* The results were analyzed using the statistical program SAS (2012).

Results

Live performance and carcass yield results are summarized in Table 1. Birds fed pelleted diets had improved all parameters performance when compared with those fed mashed diets from 1 to 42 days of age. In addition, the reduced levels of crude protein and amino acids resulted in impairment in feed conversion ratio. However, protease supplementation did not have main effect in bird performance. For the factorial part of the experiment there were no interactions between the effects evaluated for performance. Concerning to carcass yield traits, the addition of exogenous protease did not have main effect on carcass yield and cuts. However, there was interaction between the addition of protease and nutritional levels on carcass yield (Table 2), and interaction between the physical forms of feed and addition of protease on breast yield (Table 3).

Table 1. Live performance and carcass yield of broilers fed diets with different physical forms, reduced or recommended nutritional levels and with or without protease.

Treatments		BWG (kg)	FI (kg)	FCR (kg:kg)	Carcass (%)	Breast (%)	Legs (%)
Form	**Mashed**	2,70[b]	4,880[b]	1,87[a]	72,91	31,35	32,93
	Pelleted	2,82[a]	5,350[a]	1,75[b]	73,16	30,98	33,37
Protease	**Without**	2,75	5,104	1,81	73,15	31,00	33,19
	With	2,77	5,106	1,80	72,91	31,31	33,12
Nutritional	**Reduced**	2,73	5,220	1,86[a]	72,92	30,85	33,46
Level	**Recommended**	2,80	5,012	1,76[b]	73,14	31,46	32,85
Mains		2,6	5,105	1,81	73,03	31,15	33,16
SEM		0,022	0,057	0,022	0,105	0,191	0,161
Effects		***P*-value**					
Physical form		0,0068*	<0,0001*	0,0047*	0,2503	0,3739	0,1844
Protease		0,6893	0,9870	0,5783	0,2475	0,4168	0,7835
Nutricional level		0,1002	0,0698	0,0156*	0,2714	0,1535	0,0589
Form*Protease		0,6326	0,2791	0,1952	09021	0,0108*	0,1521
Form*Nutritional		0,3493	0,1225	0,4922	0,1976	0,6230	0,5730
Protease*Nutritional		0,4142	0,5922	0,5783	0,0172*	0,5301	0,4844
Form*Protease*Nutritional		0,8375	0,1498	0,1156	0,3969	0,7595	0,2078

[a,b] Means within a column not sharing a common superscript differ significantly based on test F (0,05)

Table 2. Carcass yield of broiler fed diets with different nutritional levels and supplemented or not with protease.

Carcass yield (%)		Nutritional level	
		Reduced	**Recommended**
Protease	**Without**	73,09	73,11
	With	72,74b	73,17a
Mean		72,92	73,14
SEM		0,148	0,149

[a,b] Means within a row not sharing a common superscript differ significantly based on test F (0,05)

Table 3. Breast yield of broiler fed diets with different physical forms and supplemented or not with protease.

Breast yield (%)		Physical form	
		Mashed	**Pelleted**
Protease	**Without**	31,67	30,32
	With	30,96	31,52
Mean		31,35	30,98
SEM		0,266	0,273

Conclusions

Results demonstrated that pelleted diets improved broilers performance and the addition of protease in diets with recommended levels increased carcass yield.

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ENERGY AND NUTRIENT UTILIZATION RESPONSES OF BROILERS FED DIETS SUPPLEMENTED WITH AN α-AMYLASE

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Abstract

A study was conducted to evaluate the effects of an α-amylase (RONOZYME® A, DSM Nutritional Products) supplemented in corn-soybean meal diets fed to a total of 480 slow-feathering Cobb 500 male broilers. Birds were randomly distributed to 10 treatments (8 replicates of 6 birds each). Birds were fed with a basal diet from 14 to 25 d (21% crude protein (CP), 1.15% dig. Lys, 0.86% dig. TSAA, 0.75% dig. Thr, 3,100 kcal apparent metabolizable energy (AME)/kg and 1% Celite), and to a basal diet replaced with corn at 40% (Test). basal and Test diets were supplemented with 40, 80, 120 and 160 kilo-Novo α-amylase units (KNU)/kg of feed. Total excreta were collected from 21 to 24 d whereas all birds were slaughtered at 25 d for ileum content collection. Feed, excreta and ileum samples were submitted to analysis for total tract retention and apparent ileal digestibility determinations. Amylase added to the basal diet resulted in quadratic increases ($P < 0.05$) in dry matter (DM), AME_n ($Y = - 0.020x^2 + 3.096x + 2,943$; max = 3,063 kcal/kg; $r^2 = 0.20$), and ileal digestible energy (IDE; $Y = - 0.023x^2 + 4.870x + 3,044$; max = 3,302 kcal/kg; $r^2 = 0.28$). Amylase added to the Test diet led to quadratic increases ($P < 0.05$) in AME_n ($Y = - 0.026x^2 + 3.662x + 3,475$; max = 3,601 kcal/kg; $r^2 = 0.20$), DM and CP digestibility of corn. In conclusion, when the amylase was tested in a complete diet 78 and 105 KNU/kg maximized AMEn and IDE release effect, respectively, whereas a maximum energy release of corn was estimated with 70 KNU/kg.

Keywords: amylase, broiler, metabolizable energy

Introduction

Broiler diets have been formulated with corn and soybean meal, which supply the majority content of dietary energy and protein. The nutritional value of these ingredients can be variable depending on the content of protein, oil, starch, non-starch polysaccharides and antinutritional factors such as phytate, resistant starches and enzyme inhibitors (Cowieson, 2005). Although corn and soybean meal are considered highly digestible ingredients, there are possibilities for improvement of their nutritional value because some nutrients are not completely digested in the small intestine (Cowieson et al., 2011). In this context, studies with supplemental enzymes targeting the degradation of substrates that release energy for poultry have been increasing. Exogenous carbohydrases such as xylanases, amylases and glucanases, have been reported to improve energy utilization and the performance of broilers (Olukosi et al., 2008; Vieira et al., 2015). These enzymes may improve the access of endogenous enzymes to cell contents due to hydrolysis of cell wall arabinoxylans (Kocher et al., 2003) as well as to augment endogenous amylase in young animals (Gracia et al., 2003). Decreases in endogenous amino acids losses may also contribute to the beneficial effects of supplemental enzymes, possibly associated with a reduction in the antinutritional effect of some polysaccharides and/or through feedback mechanisms that reduce the need for endogenous enzyme synthesis and secretion (Jiang et al., 2008). Thus, the objective of the present study was to evaluate the effects of adding an exogenous α-amylase-β-glucanase complex on energy utilization, nutrient digestibility and total tract retention of corn or corn-soybean meal-based diets in broiler chickens.

Material and Methods

A total of 480 one-day-old, slow-feathering Cobb × Cobb 500 male broiler chicks, vaccinated for Marek's disease at the hatchery, were randomly placed in 80 wire cages (0.9 × 0.4 m^2). Each cage was equipped with one feeder and one drinker. Birds had ad libitum access to water and mash feeds. Birds were allocated to 10 experimental diets with 8 replications in a completely randomized design. A standard corn-soybean meal-based broiler starter was fed from 1 to 14 d (3,050 kcal/kg AME_n, 21.7% CP, 1.05% Ca, and 0.53% non-phytate P). Chicks were individually weighed into groups of 6 birds per cage before placement and the experimental diets were provided from 14 to 25 d. Birds were fed with a

basal diet (Basal) and a basal diet replaced with corn at 40% (Test). Basal and Test diets were supplemented with 40, 80, 120 and 160 kilo-Novo alpha-amylase units (KNU)/kg [Ronozyme A (CT)] of feed. Each gram of the α-amylase + β-glucanase complex product corresponds to 200 kilo-Novo α-amylase units and 350 fungal β-glucanase units (Novozymes A/S, Bagsvaerd, Denmark). All experimental diets had 1% Celite as indigestible marker.

Excreta were collected twice daily on wax paper at 21, 22, 23 and 24 d being immediately mixed and pooled by cage and stored at −20°C until analysis. Previous to calorimetry, excreta were dried in a forced air oven at 55°C and ground to pass through a 0.5-mm screen. Intestinal contents were collected from all birds at 25 d after euthanasia by electrical stunning. Jejunal and ileal contents from all birds were flushed with distilled water into plastic containers, pooled by cage, immediately frozen in liquid nitrogen, and stored in a freezer at −20°C until lyophilized. Data were submitted to a 2-way ANOVA using the GLM procedure of SAS Institute (2009). Significance was accepted at $P < 0.05$ and mean differences were separated using Tukey's HSD test.

Results

Amylase added to the basal diet resulted in quadratic increases ($P < 0.05$) in dry matter (DM), AME_n ($Y = - 0.020x^2 + 3.096x + 2{,}943$; max = 3,063 kcal/kg at 78 KNU/kg; $r^2 = 0.20$), and ileal digestible energy (IDE; $Y = - 0.023x^2 + 4.870x + 3{,}044$; max = 3,302 kcal/kg at 105 KNU/kg; $r^2 = 0.28$) as shown in Table 1.

Table 1. Energy and nutrient utilization responses of broilers fed corn-soybean meal diets supplemented with an α-amylase + β-glucanase complex

Item	Total tract collection					Ileal collection	
	DM, %	AME, kcal/kg	AME_n, kcal/kg	Crude protein, %	Ether extract, %	DM, %	IDE, kcal/kg
Amylase, KNU/kg							
0	70.4	3,421	2,934	65.7	83.0	61.7	3,016
40	75.4	3,563	3,036	68.8	85.6	64.8	3,266
80	75.9	3,638	3,107	69.2	85.4	65.1	3,271
120	72.9	3,521	2,955	68.1	85.1	65.4	3,278
160	71.8	3,505	2,939	68.0	84.6	66.0	3,281
SEM	0.535	24.364	20.573	0.592	0.755	0.469	29.365
P-value[2]							
L	0.9571	0.4726	0.6317	0.3631	0.6424	0.0042	0.0074
Q	0.0002	0.0115	0.0066	0.1032	0.3353	0.1552	0.0274

[2]Linear or quadratic regression effects ($P < 0.05$).

The effects of an α-amylase + β-glucanase complex supplementation on total tract retention and ileal digestibility of corn in broiler chickens are shown in Table 2. Amylase added to the Test diet led to quadratic increases ($P < 0.05$) in AME_n ($Y = - 0.026x^2 + 3.662x + 3{,}475$; max = 3,601 kcal/kg at 70 KNU/kg; $r^2 = 0.20$), DM ($Y = - 0.0004x^2 + 0.087x + 77.1$; max = 82% at 109 KNU/kg; $r^2 = 0.16$), and CP digestibility ($Y = - 0.0005x^2 + 0.117x + 52.4$; max = 59% at 117 KNU/kg; $r^2 = 0.20$) of corn.

Table 2. Ileal digestibility and total tract utilization of nutrients and energy of corn supplemented with an α-amylase + β-glucanase complex

Item	Total tract collection					Ileal collection	
	DM, %	AME, kcal/kg	AME_n, kcal/kg	Crude protein, %	Ether extract, %	DM, %	IDE, kcal/kg
Amylase, KNU/kg							
0	77.4	3,561	3,493	52.0	73.1	72.3	3,512
40	79.9	3,607	3,545	57.0	75.5	77.2	3,525
80	80.8	3,681	3,597	57.9	74.9	79.1	3,772
120	81.5	3,653	3,466	58.9	73.4	78.8	3,586
160	79.2	3,474	3,560	58.0	70.6	73.8	3,540
SEM	0.587	32.111	29.539	0.882	0.749	0.993	42.137
P-value[2]							
L	0.1811	0.5800	0.2473	0.0977	0.1878	0.5217	0.6759
Q	0.0321	0.0397	0.0129	0.0254	0.0653	0.0076	0.1158

[2]Linear or quadratic regression effects ($P < 0.05$).

Conclusions

Corn-soybean meal-based diets supplemented with α-amylase had a beneficial impact on energy utilization for broilers. When the amylase was tested in a complete diet 78 and 105 KNU/kg maximized AME_n and IDE release effect, respectively, whereas a maximum energy release of corn was estimated with 70 KNU/kg.

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EFFECT OF PHYTASE SUPPLEMENTATION UP TO 4000 U/KG IN BROILERS FROM 22 TO 42 DAYS POST-HATCH GIVEN DIETS SEVERELY LIMITED IN AVAILABLE PHOSPHORUS

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Abstract

An experiment was conducted from 22 to 42 days post-hatch to evaluate the effectiveness of high concentrations of supplemental phytase in maize–soya bean meal-based diets severely limited in available phosphorus (P). Growth performance, plasma P and tibia ash (TA) were measured. The experiment used 220 21-d-old male broilers in 20 pens with 11 birds per pen. Dietary treatments included a positive control [PC, 4.3 g/kg nonphytate P (NPP)], negative control (NC, 1.4 g/kg NPP) and NC plus 1000, 2000 or 4000 phytase U/kg of the diet. Birds fed on the PC diet had higher average daily gain (ADG), gain to feed ratio (G:F), plasma P and TA than those fed on the NC. ADG and G:F values of the NC plus 4000 phytase U/kg reached those of the PC; nevertheless, plasma P values of the NC diets did not come up to that of the PC. While TA values of the NC, NC + 1000 or NC + 2000 did not reach that of the PC, TA of the NC + 4000 was greater than that of the PC. Overall mortality was approximately less than 3%, and not related to dietary treatments. Results of this study showed that, in the diets with 1.4 g/kg NPP, 4000 phytase U/kg can be sufficient to obtain a comparable performance in broilers to those given diets adequate in available P.

Keywords: broiler, performance, phosphorus, phytase

Introduction

Microbial phytase has been used as a feed additive in the poultry industry since 1990. While the standard use of exogenous phytase as a source of dietary P often limits the inclusion in broiler diets to approximately 500 U/kg, some research has been conducted on the effects of high phytase supplementation. Shirley and Edwards (2003) showed improvements in broiler performance, bone ash and phytate P utilization with each additional dose of phytase supplied to a diet severely deficient in available P. Although most studies have examined dietary inclusion of phytase from 1000 to 2500 U/kg, there have been a few trials that have focused on the effect of large and graded concentrations of supplemental phytase (U/kg) up to 4000 (Han et al., 2009) or 12000 (Shirley and Edwards, 2003) on broiler performance.

There are three main reasons that justified the present study: (1) with the exception of the works of Shirley and Edwards (2003) and Han et al. (2009), nearly all of the previous studies have not examined the effect of phytase doses with the absence of the inorganic P source in the diets. Even, Shirley and Edwards (2003) have not eliminated the whole amount of inorganic P source from the negative control (NC) diets; (2) Shirley and Edwards (2003) have evaluated the effect of phytase superdoses on broiler chicks at 1 to 16 d of age, while the broilers have the highest feed intake and P excretion in the finisher phase and may respond to phytase doses differently; (3) Han et al. (2009) have evaluated the effect of graded inclusions of phytase up to 4000 U/kg, but they have not shown consistent trends and responses to phytase doses in terms of feed efficiency and plasma P. In addition, the PC has had 3.5 g/kg NPP in their study, but this concentration of NPP is lower than that (4.2 g/kg) recommended to Ross 308 (ROSS, 2009). We concluded that a research with higher NPP concentration in the PC must be compared with the NC diets supplemented with phytase superdoses. Therefore, in the present study, phytase was included at 0, 1000, 2000 or 4000 U/kg at minimum concentrations of NPP (1.4 g/kg) in the diets from 22 to 42 d post-hatch.

Material and Methods

All diets were maize–soya bean meal-based and formulated to meet Ross 308 broiler nutrient requirements of finisher phase (Ross, 2009) with the exception of NPP in the NC diets. Experimental diets consisted of PC (with 4.3 g/kg NPP), NC (1.4 g/kg NPP), and NC plus 1000, 2000 or 4000 phytase U/kg of the diet. The Natuphos phytase (BASF, Mt. Olive, NJ) contained an enzyme activity of 1000 phytase U/g were used in the corresponding treatments.

The trial used 220 21-d-old Ross 308 male broilers in 20 pens from 22 to 42 d of age. All the birds had received a regular maize–soya bean meal-based diet with 5.0 and 4.5 g/kg NPP, respectively, from 1–10 and 11–21 d of age. Each treatment had 4 replicates and each replicate was assigned to a pen with 11 broilers (1.5 × 1.5 m). Birds were reared in

floor pens and in an environmentally controlled house; they were exposed to a 23:1 light:dark cycle. The experimental birds were given ad libitum access to water and mash diets.

Birds were weighed before placement (d 21) and on d 42 to calculate ADG. Feed intake was also measured from d 22 to 42 and used to calculate ADFI and G:F. On d 42, two birds/pen (8 birds/treatment) were randomly selected to obtain blood samples via wing vein. The concentrations of inorganic P in the plasma samples were analysed in duplicate using a spectrophotometer following the instructions of corresponding reagent kits (Pars Azmoon, Iran). On d 42, one bird/pen (4 birds/treatment) was randomly slaughtered to obtain tibia samples. TA percent was calculated as [TA weight/dry defatted tibia weight] × 100.

Data were analysed using the GLM procedure of SAS (2003). The pen was the experimental unit, except for TA content in which the individual bird was the experimental unit. Ash results presented as percentages were transformed to their arcsine square root before statistical analysis, and the nontransformed data are presented in the table. Contrast statement was performed to compare the mean of treatments. Orthogonal polynomial contrasts were used to determine significant linear and quadratic responses to phytase supplementation.

Results

The objective of the trial presented was to evaluate the efficacy of a microbial phytase on growth performance, plasma P and TA of broiler chicks fed on a maize–soya bean meal-based diet severely limited in available P from 22 to 42 d post-hatch. Results showing the effect of phytase superdoses on growth performance are presented in table 1. Overall (d 22–42), mortality was less than 3%, and not related to dietary treatments (P > 0.05; data not shown). Birds fed on the PC had higher ADG, G:F, plasma P and TA than those given the NC. Supplementation of phytase to the NC diets significantly improved ADG and G:F. ADG exhibited linear and quadratic responses to phytase supplementation of the NC diets. G:F showed a linear response to phytase supplementation to the NC diets. ADG and G:F values of the NC plus 4000 phytase U/kg reached that of the PC. Improvements in ADG and G:F were, respectively, 20 and 19% with phytase supplementation of 4000 U/kg. The lack of differences in ADFI between treatments may indicate that young birds may be more susceptible to a NPP deficiency than the older birds which were used in the trial.

Table 1. Efficacy of graded level of phytase on growth performance[a], plasma P (on d 42) and tibia ash (TA, on d 42) of male broiler chicks.

Diets[b]	ADG (g)	ADFI (g)	G:F (g/g)	P (mg/dl)	TA (%)
PC	101.5	231.8	0.438	9.3	39.5
NC	81.4	219.4	0.371	2.8	33.0
NC + 1000 U/kg	88.6	221.7	0.400	4.9	36.3
NC + 2000 U/kg	91.3	230.3	0.396	4.3	37.6
NC + 4000 U/kg	97.5	221.1	0.441	5.6	41.1
SEM	2.42	5.21	0.010	0.56	0.85
Source of variations (P > F)					
Treatments	<0.001	0.34	0.001	<0.001	<0.001
Linear response	<0.001	0.52	<0.001	<0.001	<0.001
Quadratic response	0.01	0.20	0.08	0.02	0.003
PC vs. NC	<0.001	0.11	<0.001	<0.001	<0.001
PC vs. NC + 1000 U/kg	0.001	0.19	0.02	<0.001	<0.001
PC vs. NC + 2000 U/kg	0.01	0.84	0.01	<0.001	0.03
PC vs. NC + 4000 U/kg	0.27	0.17	0.80	<0.001	0.05

[a]Broiler chicks were on trial from 22-to 42-d-post-hatch. ADG = average daily gain; ADFI = average daily feed intake; G:F = gain to feed ratio.
[b]PC = positive control (4.3 g/kg nonphytate phosphorus); NC = negative control (1.4 g/kg nonphytate phosphorus).

Plasma P exhibited linear and quadratic responses to phytase addition to the NC diets (table 1). Plasma P values of the NC diets did not come up to that of the PC, indicating the amount of P release from phytate was not sufficient to boost plasma P in the absence of inorganic P source from the diet. TA exhibited a quadratic response to phytase supplementation to the NC diets (table 1). TA values of the NC, NC + 1000 or NC + 2000 did not reach that of the PC, however, TA was greater in broilers given 4000 phytase U/kg compared with those fed on the PC. There was approximately 4% increases in TA of the NC + 4000 compared with that of the PC.

Conclusions

The results of this study showed that in diets with 1.4 g/kg NPP, 4000 phytase U/kg can be sufficient to obtain a comparable performance in broilers to those fed on diets adequate in available P.

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EVALUATION OF A NEW, HIGHLY EFFICIENT PHYTASE IN BROILERS

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Abstract

For economic and environmental reasons use of phytase (500 FTU/kg) is common in monogastrics' feeding. Our aim was to evaluate a new, bacterial 6-phytase in corn-soybean meal diets with a maximum of 250 FTU/kg.

A total of 960 ROSS-308 broilers were allocated into 4 treatments (trt). Positive control feed was formulated without added phytase, its P and Ca content was in accordance with the recommendations (PC; P: 7.1 and 6.3, Ca/P: 1.3 and 1.2, in starter and grower phase, resp). Negative control feeds were formulated with reduced P and Ca (NC; P: 4.7 and 4.4, Ca/P: 1.3 and 1.2, in starter and grower phase, resp.) and supplemented with 0, 125, and 250 FTU/kg phytase.

Supplementation of 250 FTU/kg of phytase sufficiently compensated the negative effect of P deprivation on BW, FI, and ADG. Tibias were the weakest in NC group (187.2 N) significantly differed from PC and 250 FTU/kg phytase supplemented trts (mean of 238.4 N). The contrast in dietary P level resulted in consistent differences in bone ash, Ca, and P content (PC vs. NC; $P<0.05$) and the phytase supplementation increased the concentration of chemical components in tibia bone. However, added 250 FTU/kg to NC feed was not enough to achieve the same chemical composition as in PC group likely due to narrow Ca/P ratio and thus absolute lack of Ca.

In conclusion, P content of a corn-soybean broiler diet can be reduced by 30% without detrimental effect on performance and bone characteristics, if 250 FTU/kg new phytase is applied.

Keywords: new-phytase, broiler, performance, bone mineralization

Introduction

The bioavailability of P in grains is limited for monogastric farm animals. It is known that digestibility of dietary P can be improved by releasing phytate bound P with phytase. In broilers the recommended dose of commercial enzyme products in corn-soybean diets is 500 FTU/kg or more. Recently a high efficient, microbial 6-phytase produced by *Aspergillus niger* has been developed, and our aim was to evaluate this new phytase with maximum dose of 250 FTU/kg based on growth rate and bone characteristics of broilers.

Material and Methods

A performance and a bone mineralization study were conducted with identical 4 dietary treatments. The performance study was carried out with 960, 1-day-old ROSS-308 broiler cocks. A 2-phase feeding was used (starter phase: d 1-21, grower phase: d 22-38) and experimental diets were formulated on corn-soybean basis. Diet A was the positive control without added phytase, its P and Ca content was in accordance with NRC (1994) recommendations (PC; total P: 7.1 and 6.3, Ca/P: 1.3 and 1.2, non phytate P (NPP): 4.3 and 3.8 g/kg, in starter and grower phase, respectively). Diets in treatment B were formulated with reduced P and Ca (negative control: NC; total P: 4.7 and 4.4, Ca/P: 1.3 and 1.2, NPP: 2.0 and 1.8 g/kg, in starter and grower phase, resp.) and without added phytase. In treatments C and D the diet composition was the same as in diet B, but was supplemented with 125 FTU and 250 FTU new phytase (treatment C and D, respectively) per kg of feed. The birds were fed ad libitum from self-feeders during the trial. Drinking water was freely available from round drinkers. In the performance trial feed intake (FI), average daily gain (ADG), and the feed conversion (FCR) as well as mortality rate were studied.

The effect of the dietary P and phytase level on bone characteristics was evaluated with 10 birds taken from each dietary treatment at the end of the performance study (at 38 days of age). The assigned birds were slaughtered and the left tibia was dissected. The bone mineralization was determined considering the length, diameter, cross section area, and maximum breaking strength, as well as weight, ash, Ca, and P content of left tibia. The nutrient (dry matter, crude protein, crude fat, crude fiber, crude ash, Ca, P) content of the diets, as well as dry matter, Ca and P content of the tibia were determined in accordance with the AOAC (1989). The activity of the phytase products and feed samples were

determined according to the method of Engelen et al. (2001). The effect of dietary treatments was analyzed with one way ANOVA (SAS, 2004).

Results

The effect of dietary treatments on the growth performance of 38 days old broilers are presented in Table 1. Reduction of dietary P without enzyme supplementation resulted in 394 g (16.3%) lower final body weight due to the lower feed consumption (14,2 g/d identical with 16,2%; treatment A vs B; P<0.05). With the addition of phytase the body weight, the daily gain as well as the feed intake increased. Supplementation of 250 FTU per kg of the new phytase to the negative control diet was sufficient to compensate the detrimental effect of P deprivation on BW and ADG (treatment A vs D, P>0.05). Feed conversion, however, was independent on P supply or enzyme supplementation. The mortality rate was low regardless of dietary treatments in the course of the performance trial ranged from 2.1 to 4.6%.

Table 1. Effect of different dietary phytase supplementation on growth performance of broilers determined at 38 days of age

Parameters	TREATMENTS*				RMSE	P value
	A	B	C	D		
Body weight (g)	2412.9[a]	2018.5[c]	2148.6[b]	2317.3[a]	105.88	<0.0001
Body weight gain (g/d)	61.4[a]	51.9[c]	55.5[b]	59.5[a]	3.09	<0.0001
Feed intake (g/d)	87.9[a]	73.7[d]	77.4[c]	81.5[b]	3.34	<0.0001
Feed conversion (kg/kg)	1.48	1.48	1.47	1.44	0.043	0.1696

***A**: Positive control (without phytase supplementation); **B**: Negative control (with reduced Ca and P content, without phytase supplementation); **C**: Negative control + 125 FTU/kg; **D**: Negative control + 250 FTU/kg
[a,b]: Means with different superscripts are significantly different (P ≤0.05)

The effect of dietary P supply and phytase supplementation on the bone characteristics are shown in Table 2. Bone strength was the weakest in the NC group (187.2 N) and significantly differed from the PC and 250 FTU/kg phytase supplemented treatments (mean of 238.4 N). The contrast in dietary P level resulted in consistent differences in bone ash, Ca, and P content (treatment A vs. B; P<0.05) and the phytase supplementation increased the concentration of studied chemical components in tibia bone. However, addition of 250 FTU/kg to NC diet was not sufficient to achieve the same chemical composition as in PC group (treatment A vs D, P<0.05) likely due to small Ca/P ratio and thus absolute lack of Ca.

Table 2. Effect of different dietary phytase levels on bone characteristics and breaking strength as well as chemical composition (weight, ash, Ca and P content) of tibia

Parameters	TREATMENTS*				RMSE	P value
	A	B	C	D		
Length (mm)	85.22	81.09	85.53	83.96	3.431	0.0776
Diameter (mm)	6.84	6.78	7.05	6.82	0.578	0.7393
Max. breaking strength (N)	248.2[a]	187.2[b]	216.8[ab]	228.5[a]	40.78	0.0195
Weight (g)	10.2	9.7	10.1	9.8	1.06	0.6744
Ash (g/kg bone)	213.8[a]	171.6[c]	185.1[b]	193.6[b]	14.48	<0.0001
Ca (g/kg bone)	67.4[a]	53.4[c]	59.4[b]	62.3[b]	4.89	<0.0001
P (g/kg bone)	36.4[a]	28.3[c]	30.3[bc]	32.2[b]	2.76	<0.0001

***A**: Positive control (without phytase supplementation); **B**: Negative control (with reduced Ca and P content, without phytase supplementation); **C**: Negative control + 125 FTU/kg; **D**: Negative control + 250 FTU/kg
[a,b]: Means with different superscripts are significantly different (P ≤0.05)

Conclusion

In conclusion the P content of a corn-soybean broiler diet can be reduced by 30% without detrimental effect on performance and bone characteristics if 250 FTU/kg of a new phytase is applied.

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P-127 (ID 136)

STARCH DIGESTIBILITY AND ENERGY UTILIZATION OF BROILERS FED CORN-SOYBEAN BASAL DIETS SUPPLEMENTED WITH AMYLASE AND XYLANASE

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Abstract

A study was conducted to evaluate the effects of dietary α-amylase and β-xylanase supplementation of corn-soy diets formulated with or without supplemental phytase on energy utilization and starch digestibility in broiler chickens. A total of 336 slow feathering, Cobb × Cobb 500 male broilers were randomly distributed to 6 treatments having 8 replicates of 7 birds each. Birds were fed a common starter diet to 14 d. The experimental diets were provided afterwards until 25 d. A 2 × 3 factorial arrangement of 2 control diets (Basal = corn-soy diet without added phytase or PHY = corn-soy diet formulated with 1,000 phytase units/kg) and 3 carbohydrase supplementations (0, 80 kilo-Novo α-amylase units/kg, or 80 kilo-Novo α-amylase units/kg + 100 fungal β-xylanase units/kg) was used from 14 to 25 d. Excreta were collected from 21 to 24 d and all birds were euthanized at 25 d for jejunum and ileum content collection. Samples of feed, excreta, jejunal and ileal digesta were analyzed for determination of total tract retention and ileal apparent digestibility. No interactions between diet and carbohydrase were observed. Relative to the basal diet, AME_n was increased ($P < 0.01$) by 70 kcal/kg and 99 kcal/kg when birds were fed the diet supplemented with amylase and amylase + xylanase, respectively. Starch digestibility in jejunum and ileum was increased ($P < 0.05$) by 3.5% and 2.4% when birds were fed the diet supplemented with amylase + xylanase. Results from this experiment show that corn-soy diets having phytase and supplemented with amylase and xylanase led to increased AME_n, and starch digestibility in broilers. Furthermore, the efficacy of exogenous amylase and xylanase was independent of the presence of microbial phytase.

Keywords: amylase, broiler, phytase, starch, xylanase

Introduction

Nutrient and energy digestibility of plant feedstuffs by poultry is limited by the proportion of their components for which there are no corresponding endogenous digestive secretions. This is the case of the non-starch polysaccharides that are present in and within the cell walls of soybean meal and corn (Bach Knudsen, 1997; Caffall and Mohnen, 2009). They are either indigestible or of very low digestibility when fed to poultry. Therefore, the energy derived from them is restricted by a lower organic combustion of these components as well as by the physical barrier represented by the cell wall itself that prevents enzyme access to substrates (Slominski et al., 1993). Additionally, phytate (myo-inositol hexaphosphate) is the main form of stored phosphorus in plant seeds. Besides the limitation in the use of phosphorus by poultry, phytate has also been reported to reduce energy utilization (Ravindran et al., 2006). Phytate and lower inositol phosphates can be hydrolyzed by the enzyme phytase (myo-inositol hexaphosphate phosphohydrolase), which has become a standard enzymatic additive in poultry diets (Cowieson et al., 2011).

As increasing numbers of enzyme products become available, the assembly of strategically optimized admixtures becomes a challenge in terms of targeting the complex mixture of components that have low digestibility. Whilst different enzyme classes hydrolyze different substrates and generate different products, a net effect on the increase in the digestibility of starch, protein/AA and fat with a cumulative energetic consequence seems to be an expected outcome of their action. While enzymes that target different substrates do not compete in terms of substrate degradation itself, they tend to overlap in nutrient digestion and performance, delivering sub-additive outcomes (Cowieson and Adeola, 2005). Increases in energy utilization from plant feedstuffs by broilers may derive from a large variety of components; however, since starch is quantitatively the largest energy supplier in broiler diets, any improvement in its degradation would lead to a significant benefit.

The objective of the present study was to evaluate the effects of an α-amylase alone or in combination with a β-xylanase on energy utilization and starch digestibility of broiler chickens fed corn-soy-based diets. These effects were

assessed both in a phytase-free diet and in a diet that included phytase in order to explore the possibility that carbohydrase efficacy may be muted by the presence of phytase.

Material and Methods

A total of 336 one-day-old, slow-feathering Cobb × Cobb 500 male broiler chicks, vaccinated for Marek's disease at the hatchery, were randomly placed in 48 wire cages (0.9 × 0.4 m2). Each cage was equipped with one feeder and one drinker. Birds had ad libitum access to water and mash feeds. Birds were allocated to 6 experimental diets with 8 replications of 7 birds each in a completely randomized design. A standard corn-soybean meal-based broiler starter was fed from 1 to 14 d (3,050 kcal/kg AME_n, 21.7% CP, 1.05% Ca, and 0.53% non-phytate P). A 2 × 3 factorial arrangement of 2 control diets (Basal = corn-soy basal diet without added phytase or PHY = corn-soy diet formulated with phytase) and 3 carbohydrase supplements (0, α-amylase, or α-amylase + β-xylanase) were provided from 14 to 25 d. The supplemental enzymes used in the present study are commercially available (Novozymes A/S, Bagsvaerd, Denmark). Their inclusion per kg of diet were: phytase [Ronozyme HiPhos (GT)] 1,000 phytase units (FYT), alpha-amylase [Ronozyme HiStarch (CT)] 80 kilo-Novo alpha-amylase units (KNU), and beta-xylanase [Ronozyme WX (CT)] 100 fungal xylanase units (FXU). All experimental diets had 1% Celite as indigestible marker.

Excreta were collected twice daily on wax paper at 21, 22, 23 and 24 d being immediately mixed and pooled by cage and stored at −20°C until analysis. Previous to calorimetry, excreta were dried in a forced air oven at 55°C and ground to pass through a 0.5-mm screen. Intestinal contents were collected from all birds at 25 d after euthanasia by electrical stunning. Jejunal and ileal contents from all birds were flushed with distilled water into plastic containers, pooled by cage, immediately frozen in liquid nitrogen, and stored in a freezer at −20°C until lyophilized. Data were submitted to a 2-way ANOVA using the GLM procedure of SAS Institute (2009). Significance was accepted at $P < 0.05$ and mean differences were separated using Tukey's HSD test.

Results

There were no interactions between diet and carbohydrase for ileal digestibility, total tract retention and starch digestibility in 25-d-old broilers (Table 1). Differences in AME_n and ileal digestibility of DM were found between PHY and basal diets ($P < 0.01$) with values, respectively of 3,483 kcal/kg and 65.2% for PHY and 3,412 kcal/kg and 63.4% for basal. In the present study, AME_n was improved ($P < 0.01$) by 70 and 99 kcal/kg when amylase and amylase + xylanase were supplemented, respectively. Amylase and xylanase supplementation provided an increase ($P < 0.01$) in AME_n of 2.8% when compared to the diet without carbohydrases. The digestibility of total starch in the jejunum and ileum was higher ($P < 0.01$) for broilers fed PHY or diets supplemented with amylase + xylanase. The digestibility of total starch in the jejunum and ileum was increased ($P < 0.05$) by 3.5% and 2.4%, respectively in broilers fed amylase + xylanase diet compared to broilers fed the diet without carbohydrases.

Table 1. Energy and nutrient utilization response, and apparent digestibility of starch by chickens fed experimental diets[1]

Item	Ileal collection		Total tract collection		Jejunal starch, %		Ileal starch, %	
	DM, %	IDE, kcal/kg	DM, %	AME_n, kcal/kg	Digestible	Total	Digestible	Total
Diet								
Basal	63.4	3,187	70.2	3,412	91.1	89.8	91.1	89.8
PHY	65.2	3,267	71.2	3,483	92.5	91.3	92.5	91.3
Carbohydrase								
0	62.9[b]	3,150	69.6[b]	3,391[b]	91.1[b]	89.6[b]	91.1[b]	89.6[b]
Amylase	64.4[ab]	3,234	70.9[ab]	3,461[a]	91.6[b]	90.2[ab]	91.6[b]	90.2[ab]
Amylase + xylanase	65.6[a]	3,297	71.5[a]	3,490[a]	92.8[a]	92.0[a]	92.8[a]	92.0[a]
Mean	64.3	3,227	70.7	3,448	91.8	90.6	91.8	90.6
SEM	0.576	18.798	0.266	12.392	0.297	0.347	0.297	0.347
Main effect *P*-value								
Diet (D)	0.0192	0.1213	0.0458	0.0010	0.0188	0.0138	0.0188	0.0138
Carbohydrase (C)	0.0260	0.1683	0.0093	0.0007	0.0567	0.0129	0.0567	0.0129
D × C	0.6747	0.9702	0.4889	0.9714	0.8159	0.2075	0.8159	0.2075

[a-b]Means with different superscript letter differ ($P < 0.05$) based on Tukey's honestly significant difference test.

Conclusions

Corn-soybean meal-based diets formulated with 1,000 phytase units/kg or supplemented with 80 kilo-Novo α-amylase units/kg and 100 fungal β-xylanase units/kg had a beneficial impact on energy utilization for broilers. AME_n was also improved when birds were fed amylase + xylanase diet compared to the diet without carbohydrases. Finally, the digestibility of starch in the jejunum was lower than in the ileum, and corn-soy diets formulated with phytase or supplemented with amylase + xylanase improved starch utilization.

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COMPOSITE ANALYSIS OF TEN BROILER TRIALS EVALUATING STANDARD AND SUPERDOSES OF A MICROBIAL PHYTASE IN THE PRESENCE OR ABSENCE OF A XYLANASE

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Abstract

In broiler diets the use of phytase to reduce inorganic phosphorus supplementation and of xylanase to improve nutrient utilisation is common practice. The objective of this analysis was to evaluate the impact of standard or superdoses of phytase, without or with a xylanase. A total of 10 broiler trials were conducted at research sites worldwide from 2012 to 2015 using diet, nutrient, husbandry and environmental conditions typical to the region. The dietary treatments included a diet with 500 FTU/kg of phytase and a reduction in available P and Ca by 0.15 and 0.16%, respectively (CON), the CON diet with superdoses (1000 to 1500 FTU/kg) of phytase (SD), the CON + xylanase (XYL), and SD + XYL. This resulted in a 2 x 2 factorial design and a total of 76 data points used in the model. In 4 of the trials, in addition to available P and Ca, the CON diet had a reduction in ME by 50 to 100 kcal/kg. The dosage of xylanase ranged from 8000 to 32000 BXU/kg. Body weight corrected FCR was calculated from broilers >35 days of age, and means were analysed using the fit model platform in JMP v. 11.2.1. Supplementation of broiler diets with phytase superdoses (1.552 vs 1.580) or xylanase (1.551 vs 1.581) improved ($P < 0.0001$) BW corrected FCR compared with broilers fed standard phytase or diets without xylanase. There was no phytase x xylanase interaction ($P = 0.89$), indicating phytase superdoses and xylanase work independently to improve the feed conversion ratio of broilers.

Keywords: poultry, phytase, xylanase, feed conversion ratio

Introduction

It has been estimated that approximately 95% of poultry diets contain an exogenous xylanase and approximately 90% an exogenous phytase. Supplementation of poultry diets with xylanase is reported to improve growth performance and nutrient digestibility through reduction of viscosity or improvements in energy availability through the breakdown of non-starch polysaccharides (NSP). While the content of NSPs in maize is not as high as that of wheat or rye, Masey O'Neill et al. (2012) reported beneficial responses on growth performance and nutrient digestibility when xylanase was supplemented in maize-based broiler diets. This may be associated with cell wall degradation (Meng and Slominski, 2005) or the production of oligosaccharides which exert a pre-biotic effect (Courtin et al., 2008) and thereby improve broiler performance and feed conversion ratio.

Phytase can hydrolyse phytate, thereby releasing phytate-phosphorus (P) and improving P digestibility. The use of exogenous phytase allows for reductions of inorganic phosphate in the diet while maintaining growth performance and bone ash (Zeller et al., 2015). Phytate is considered a source of P but also can be described as an anti-nutrient, and significantly reduces growth performance and nutrient digestibility in broilers. Recently, the use of superdoses of phytase has resulted in near complete phytate destruction and significant improvements in broiler feed conversion (Walk et al., 2014).

Both phytase and xylanase enzymes have different substrates and modes of action in broilers. However, broiler diets are commonly supplemented with both a xylanase and phytase. Therefore this composite analysis was conducted to evaluate the impact of standard or superdoses of phytase, without or with a xylanase, on broiler feed conversion ratio using 10 broiler trials conducted globally.

Material and Methods

Ten broiler trials were conducted from 2012 to 2015 in Brazil (n = 2), Mexico (n = 1), EU (n = 5), Thailand (n = 1) and India (n = 1). Trial diets were formulated to contain predominantly maize and soybean meal (Brazil, India, EU n = 1, and Thailand), sorghum and soybean meal (Mexico), or wheat and soybean meal (EU n = 4). The experimental diets were formulated to be nutrient adequate, with the exception of calcium and available P (avP), which was decreased by 0.16 and 0.15%, respectively, from requirement at each feeding phase. In addition, in four trials ME was reduced by 50 (India and Thailand), 80 (Brazil n = 1) or 100 (EU n = 1) kcal/kg from the requirement at each feeding phase. Phytase

(Quantum Blue, AB Vista, Marlborough UK) was formulated in the experimental diets at the standard dose (CON) of 500 FTU/kg or superdose (SD) of 1000 or 1500 FTU/kg. Xylanase (Econase XT, AB Vista, Marlborough UK) was added at 0 (- XYL) or variable doses which ranged from 8000 to 32000 BXU/kg (+ XYL; Table 1). Therefore, the experimental design included the CON - XYL, SD - XYL, CON + XYL or SD + XYL arranged as a 2 x 2 factorial of treatments. Only experimental means were used in the composite analysis (n = 84).

Table 1. Descriptive factors of each trial included in the composite analysis evaluating standard or superdoses of phytase, without or with a xylanase

Location	n	Diet composition	Phase 1	Phase 2	Phase 3	Xylanase,
			ME, kcal/kg			BXU/kg
India[1]	8	Corn and soybean meal	2950	3050		0 or 16000
Brazil	16	Corn and soybean meal	3050	3154		0 or 8000-16000
Mexico	4	Sorghum and soybean meal	3000	3100	3200	0 or 16000
Germany[2]	4	Wheat and soybean meal	2916	3100		0 or 16000
UK	4	Wheat and soybean meal	3000	3100		0 or 16000
UK	12	Corn and soybean meal	3000	3050	3100	0 or 8000-32000
UK	4	Wheat and soybean meal	3060	3150		0 or 16000
Brazil[3]	16	Corn and soybean meal	3050	3120	3170	0 or 8000-24000
Thailand[1]	8	Corn and soybean meal	2950	3000	3050	0 or 16000
Poland	8	Wheat and soybean meal	2919	3101	3149	0 or 16000

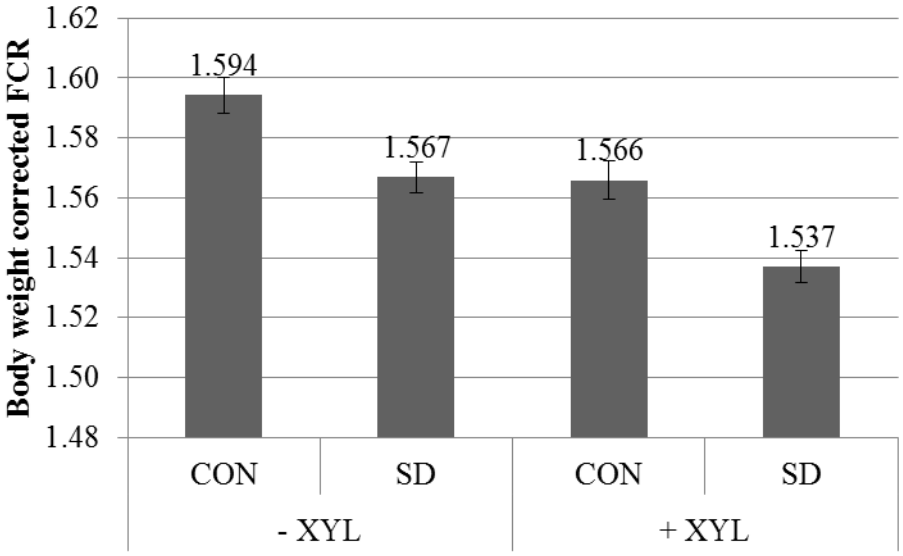
[1] Reflects a reduction in formulated ME by 50 kcal/kg.
[2] Reflects a reduction in formulated ME by 100 kcal/kg.
[3] Reflects a reduction in formulated ME by 80 kcal/kg.

Body weight corrected feed conversion ratio was calculated from body weight and feed conversion ratio of broilers greater than 35 days of age. Means were analysed using the fit model platform in JMP v. 11.2.1. The model included phytase dose, xylanase and the interaction. Trial site was included in the model as a covariate. Significance was accepted at $P < 0.05$. Significant means were adjusted using t-tests.

Results

The final model included 76 data points and had an $R^2 = 0.97$. The mean BW corrected FCR was 1.576 with an RMSE = 0.023. Phytase dose × xylanase interaction was not significant ($P = 0.89$; Figure 1). Superdoses of phytase significantly ($P < 0.0001$) improved body weight corrected FCR from 1.580 to 1.552, regardless of the presence or absence of xylanase. Similarly, supplementing broiler diets with xylanase significantly ($P < 0.0001$) improved body weight corrected FCR of broilers from 1.581 to 1.551, regardless of the level of phytase supplied in the diet.

Figure 1. Body weight corrected feed conversion ratio (FCR) of broilers fed standard (CON) or superdoses of phytase (SD) in the absence (- XYL) or presence (+ XYL) of xylanase



Conclusions

A composite analysis of 10 broiler trials conducted globally, using dietary and husbandry factors typical to the region, various ME matrices and grain-types indicated superdoses of phytase or the inclusion of xylanase into broiler diets results in significant improvements in body weight corrected feed conversion ratio. The lack of significant interaction would indicate phytase and xylanase work independently to improve FCR.

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Feed raw materials

P-130 (ID 110)

THE EFFECT OF DIFFERENT LEVELS OF EXTRUDED HEMPSEED (*CANNABIS SATIVA* L.) ON PERFORMANCE, PLASMA LIPID PROFILE AND IMMUNE RESPONSE OF BROILER CHICKS

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Abstract

The aim of this study was to evaluate the effect of extruded hempseed (EHS) on the performance, plasma lipid profile and immune response in broiler chicks during 1 to 42 days of age. A total of 250 one-day-old male broiler chick (Ross 308) were randomly assigned to 5 treatments. Each treatment was comprised of 5 replicates of 10 broiler chicks in each. The treatments were of: 1) Control diet (EHS0, diet contained no EHS); 2) diet contained %5 EHS (EHS5); 3) diet contained %10 EHS (EHS10); 4) diet contained %15 EHS (EHS15); 5) diet contained %20 EHS (EHS20). Diets provided similar metabolizable energy (ME) and crude protein (CP). Data were analyzed as a completely randomized design using of the PROC GLM procedure of SAS. The results of this study showed that inclusion of EHS into diet had no significant effect on performance parameters. The plasma Cholesterol (CHO), Triglyceride (TG), High density lipoprotein (HDL) and low density lipoprotein (LDL) did not significantly altered. Groups supplemented with EHS had higher IgG titres than control group. In conclusion, use of EHS into diet of broiler chicks could improve bird immune system and health.

Keywords: Extruded hempseed, Performance, Immune system, Broiler chick

Introduction

Hempseed (*Cannabis sativa L.*) have been used as an important source of food, fiber and medicine for thousands of years (Pringle, 1997). Whole hempseeds contain approximately 25% proteins, 30% fat (Callaway, 2004) and high percentage of ash and cellulose (House et al, 2010). This ratio is considerably high when compared to some traditional feeds such as corn, wheat and soybean meal. High crude ash and cellulose levels in diets may cause lower BW due to decreasing of energy and digestibility of feeds. High levels of the fiber in diet has negative effects on nutrients digestibility and then bird performance (Krogdahl, 1986). A method for decreasing harmful effects of anti-nutritional factors is extruding process that improve nutritional value of feed (Allan et al., 2004). Hempseed oil contains over 80% polyunsaturated fatty acids (PUFA) and also it is a rich source of two essential fatty acids Linoleic acid (LA) and alpha-Linolenic acid (ALA) which is considered to be optimum for human health (Callaway, 2004). The oil of hempseed is comparable to black currant oil which improves immunity (Barre, 2001). The researchers reported that hempseed significantly reduced serum LDL, but had no significant effect on TG, CHO and HDL concentration (Konca et al., 2014b). The aim of this study was to evaluate the effect of extruded hempseed (EHS) on the performance, plasma lipid profile and immune response in broiler chicks.

Material and Methods

A total of 250 one-day-old male broiler chick (Ross-308) were used in the study. All of the

birds were weighed and distributed into 5 treatment groups with 5 replicates and 10 birds per cage as replicate. The dietary treatments were: 1) Control diet (EHS0, diet contained no EHS); 2) diet contained %5 EHS (EHS5); 3) diet contained %10 EHS (EHS10); 4) diet contained %15 EHS (EHS15); 5) diet contained %20 EHS (EHS20). The diets were isocoloric and isonitrogenous. Performance parameters were measured in 10, 24 and 42 day of age. In 42 day of age, two birds were randomly selected from each cage and weighed individually and eviscerated. For determinating serum lipid profile and (sheep red blood cell) SRBC test, 2 blood samples per selected bird were taken and were kept in ice and were sent to laboratory. SRBC test was carried out according to Nelson et al.(1995) method. Data were analyzed as a completely randomized design using General Linear Models procedure (PROCC GLM) of SAS (2002) and means were compared by Tukey test.

Results

The results of this study showed that inclusion of EHS into diet had no significant effect on performance parameters (Table 1).

Table 1. The effect of different levels of extruded hempseed on performance of broiler chicks

Item	Treatment						
	EH0	EHS5	EHS10	EHS15	EHS20	[1]SEM	P-value
BW (g/bird)							
10 old-day	172.98	172.48	179.84	188.52	177.86	5.03	[2]NS
24 old day	771.71	767.62	763.01	752.20	740.24	22.65	NS
42 old-day	2379.92	2348.57	2248.94	2256.07	2304.53	60.56	NS
FI (g/bird/day)							
(1-10) day period	220.80	210.60	228	226.80	214.47	4.49	NS
(11-24) day period	1087.50	1128.48	1114.81	1141.60	1135.63	17	NS
(25-42) day period	2704.7	2683.1	2523.3	2487.4	2546.9	144.31	NS
(1-42) day period	4013	4020.2	3866.1	3855.8	3897	154.71	NS
FCR							
(1-10) day period	1.28	1.27	1.22	1.20	1.21	0.02	NS
(11-24) day period	1.41	1.48	1.46	1.52	1.54	0.05	NS
(25-42) day period	1.84	1.81	1.86	1.88	1.77	0.1	NS
(1-42) day period	1.65	1.65	1.67	1.70	1.66	0.06	NS

[1]SEM: Standard error of means

[2]NS: Non-significance

EHS supplemented diets did not significantly affect the plasma Cholesterol (CHO), Triglyceride (TG), High density lipoprotein (HDL) and low density lipoprotein (LDL) concentration (Table 2).

Table 2. The effect of different levels of hempseed on plasma lipid profile in broiler chicks

Parameter (mg/dl)	Treatment					SEM	P-value
	EH0	EHS5	EHS10	EHS15	EHS20		
CHO	152.50	134.45	145.20	126.20	139.66	11.12	NS
HDL	51.10	63.35	68.40	60.65	66.25	5.90	NS
LDL	92.19	74.24	71.11	57.52	66.25	6.72	NS
TG	46.05	29.30	28.45	40.10	35.80	3.85	NS

The results showed that although the broilers fed with EHS supplemented diets hadn't significant difference in terms of humeral immune response, but they had higher IgG content compared to control group (EHS0) (Table3).

Table 3. The effect of extruded hempseed on anti body titre against SRBC in broiler chicks

parameter	Treatment					SEM	P-value
	EH0	EHS5	EHS10	EHS15	EHS20		
SRBC	8.66	9.33	9.00	9.33	8.00	0.63	NS
IgG	2.00	2.66	4.00	3.66	2.33	0.69	NS
IgM	6.66	6.66	5.00	5.66	5.67	0.01	NS

Conclusions

The obtained results of the study showed that adding extruded hempseed into broiler chicks diet hadn't significance effects on performance, serum lipid profile and immune response, but overall there is good potential in EHS to improve bird health and performance.

Acknowledgements

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P-131 (ID 54)

CRUDE FIBRE IN LAYER FEED INFLUENCES PERFORMANCE AND PLUMAGE OF LSL HYBRID

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Abstract

The influence of a high crude fibre content (7% CF) in a layer feed was compared to a control feed (3.5% CF) under Swiss growing conditions on the research farm of Aviforum Foundation regarding the production performance and plumage quality of 2860 non-beak-trimmed Lohmann Selected Leghorn hybrids. The 17 week old hens were evenly distributed to 8 compartments and randomly allocated to the two diets described above, allowing 4 compartment replications. Both groups showed an equally high laying performance of 94% at the age of 64 weeks. Hens fed the high CF (HCF) diet ate 2.6g per day and animal more and their FCR (g feed/g egg) was 0.03 units higher during the first 6 laying periods (LP) than compared to the control group (LCF), and they ate less during the last 5 LPs, thus lowering their FCR towards that of the control group. High dietary CF led to lighter birds (1804g for HCF; 1863g for LCF) which produced heavier eggs with less strong shells (HCF 64g, 41.7N; LCF 63.7g, 43.7N). The plumage covered the hens well and no significant difference was found in feather scoring. Hens eating the high CF diet suffered significantly less from cannibalism (HCF 0.4%; LCF 1.2%) which indicates a positive influence of high CF against pecking. This was also reflected by a slightly better feather score of the neck, back and cloaca area of hens fed a higher CF diet (HCF 3.05, 3.98, 2.45; LCF 2.90, 3.85, 2.13). In conclusion, a CF content of 7% in a layer diet had a positive influence on the welfare of non-beak-trimmed hens lowering cannibalism without jeopardizing laying performance.

Keywords: layers, crude fibre, plumage, performance

Introduction

An intact plumage is important for the hen to regulate its body temperature without losing much energy. A badly covered hen will eat more and is thus less economical. Fibres in the feed of layer hens have an influence on the quality of the plumage (Van Krimpen *et al.*, 2005). A high fibre and a standard diet were tested under Swiss growing conditions on the research farm of Aviforum Foundation focussing on plumage quality and production performance.

Material and Methods

2860 LSL hybrids were evenly distributed into 8 compartments. These were randomly allocated to a diet containing 7% crude fibre (HCF) or a standard diet with 3.5% fibre (LCF), allowing 4 compartment replications with 1430 hens per diet. Both diets were isoenergetic and isonitrogenic. All animals were kept under the same conditions and management in an aviary system using wood shavings as litter material. The hens had daily access to a winter garden. They received a prelayer and two layer diets produced by Provimi Kliba SA, Kaiseraugst, until the age of 64 weeks. Laying performance, feed and water consumption as well as mortality were measured on a daily basis at the farm. Egg weights of all laid eggs were evaluated on one day of each laying period (LP). Egg shell strength and thickness as well as the yolk colour were measured during the peak performance, after 6 LPs and at the age of 64 weeks. Hen weight and plumage was evaluated regularly once per LP, scoring 10 hens per compartment on the neck, breast, stomach, back, wings and tail areas based on a scale of 4 (feathers intact) to 1 (no feathers) described by Tauson et al. (2005). To monitor excreta for abnormalities, the humidity of faeces was evaluated visually in the barn every 2 weeks and dry matter content was measured in the lab towards the end of the trial. Data were analyzed by ANOVA and means compared with the test of Tukey-Kramer.

Results

Laying performance (94% at the age of 64 weeks) and number of floor eggs (laid outside the nests) were not affected by treatments. HCF led to a higher feed consumption during the first 6 LPs. Neither the feed conversion ratio (FCR) nor the humidity of the faeces showed any significant differences between treatments (Table 1).

Table 1. Laying performance, floor eggs, feed consumption, FCR and humidity of faeces

	LCF LP 1-6	HCF LP 1-6	P-Level LP 1-6	LCF LP 1-11	HCF LP 1-11	P-Level LP 1-11
Laying performance, %	94.0	93.8	0.798	94.0	94.0	0.908
Floor eggs, %	0.7	1.0	0.247	0.6	0.9	0.258
Feed per day and hen, g	118.7	121.3	0.076	122.6	123.7	0.449
FCR, kg feed/kg egg mass	2.056	2.082	0.324	2.047	2.056	0.768
Humidity of faeces, 1=dry to 3=very humid	1.383	1.520	0.431	1.459	1.534	0.692

HCF led to heavier eggs than the standard diet (61.4g LCF, 62.1g HCF over 6 LPs with P-Level 0.007 and 63.7g LCF, 64.0g HCF over 11 LPs with P-Level 0.058). During the whole trial, significantly more big sized eggs were laid with HCF compared to LCF. No significant differences could be shown between the treatments regarding egg shell quality. Due to the heavier eggs, egg shell strength was slightly lower with HCF at the age of 64 weeks. Yolk colour was different after 6 LPs only (Table 2). Hen weights showed no significant differences between treatments (1863g LCF and 1804g HCF).

Table 2. Egg weight and egg weight distribution

	LCF LP 6	HCF LP 6	P-Level LP 6	LCF LP 11	HCF LP 11	P-Level LP 11
Egg weight. g	64.2	64.8	0.203	67.2	67.5	0.192
Small eggs <53g, %	0.3	0.2	0.875	0.3	0.0	0.159
Medium sized eggs 53-70g, %	89.5	84.7	0.006	80.1	76.7	0.038
Big sized eggs >70g, %	10.1	15.1	0.001	19.6	23.3	0.029
Egg shell strength, N	49.6	50.8	0.552	43.7	41.7	0.318
Egg shell thickness, µm	408	405	0.524	387	392	0.370
Yolk colour	12.1	11.9	0.013	12.2	12.0	0.168

Mortality reached an average of 5,5% (HCF) and 7,8% (LCF) after 11 LPs. Incidence of cannibalism in the control group (1.2%) was three times higher than with HCF (0.4%) and thus significantly different between treatments (P=0.043). The plumage covered the hens well and no differences could be found in overall feather scoring (3.68 LCF and 3.69 HCF). Kalmendal and Wall (2012) confirm this finding using sun flower cake containing soluble fibres in the feed. In this trial feeding lignocellulose (unsoluble fibres), the neck, back and cloaca areas were in a better state with HCF than with LCF (Figure 1).

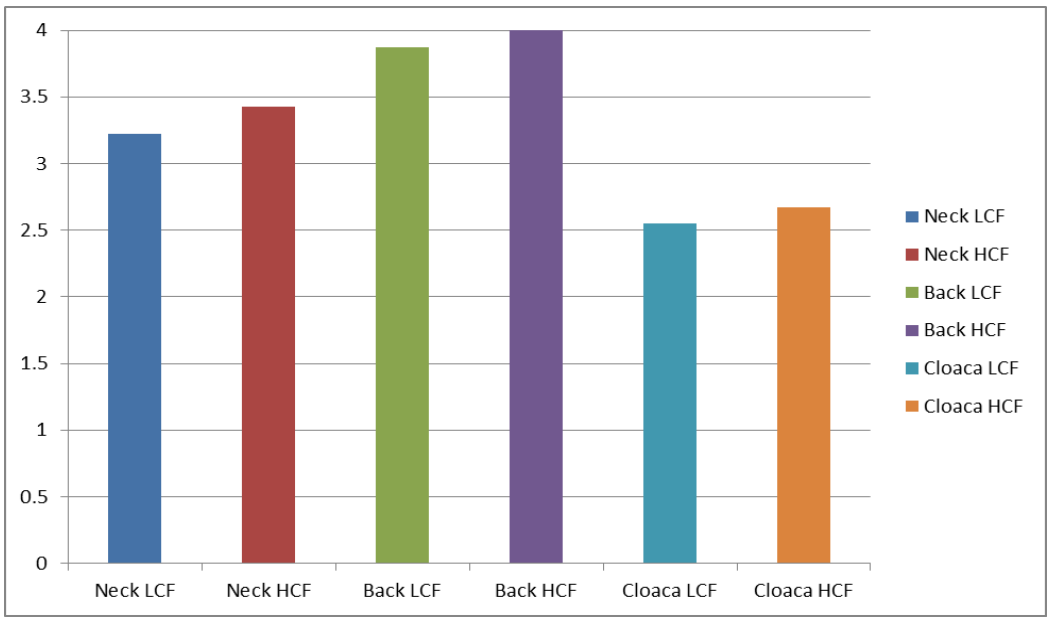

Figure 1. Feather scoring of neck, back and cloaca area in LP 11

Conclusions

HCF in the diet with oat, lucerne meal, sun flower cake and 2.5% lignocellulose as components reduced cannibalism, indicating a positive influence of crude fibre in the diet preventing feather pecking, without diminishing laying performance. The lignocellulose helps to stabilize the water content of the faeces as it contains unsoluble fibres and has a high water holding capacity, leading to dry faeces and a good litter quality (Neufeld *et al.*, 2014). Egg mass and percentage of big sized eggs were increased with HCF, favouring their commercialisation from the farm directly to the consumer. As Switzerland will abandon the trimming of the beak in the future, high crude fibre content in the feed should be tested on brown non-beak-trimmed hybrids, which are more likely to peck and suffer from cannibalism than LSL.

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P-132 (ID 154)

EFFECT OF TWO SOURCES OF ZINC ON BROILER PERFORMANCE

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Abstract

Various zinc sources are supplemented in poultry feeds, in order to fulfill animal requirements; zinc sulfate ($ZnSO_4$) and zinc oxide (ZnO) are among the most common. The aim of the study was to compare the effects of zinc from a new source of potentiated ZnO or from $ZnSO_4$ on broiler performance.

738 male broilers were divided into cages placed in a Colorado type house; 26 500 birds were raised in this building. The basal diet was based on wheat, corn and soybean meal. Experimental diets were supplemented with 60 ppm of zinc from $ZnSO_4$ or 60 ppm of zinc from potentiated ZnO (HiZox®). Zootechnical criteria (bodyweight, feed intake) were recorded at feed transition (12 days, 21 days, 28 days) and at the end of the trial (35 days). Litter score was evaluated at 28 days.

Broilers fed with potentiated ZnO showed a better start, with a significantly higher bodyweight at 12 days: +2.2% ($p < 0.05$). This difference could still be seen at 35 days (2251 g vs. 2231 g) but was not significant. Consumptions were equal between the 2 groups during the entire trial; the feed conversion ratio of the group fed with potentiated ZnO was better for the 0-12-days period after bodyweight standardization ($p < 0.05$). Litter quality was improved at 28 days for the group fed with potentiated ZnO, the difference between the 2 regimes is close to be significant ($p = 0.08$).

In conclusion, the new source of potentiated ZnO showed a positive impact on broiler growth performance and on litter quality, compared with $ZnSO_4$.

Keywords: zinc, broiler, performance

Introduction

Zinc (Zn) is an essential trace mineral that exists naturally in all plants, but its concentration is generally insufficient to meet animal requirements. In poultry, Zn deficiency leads to leg abnormalities, frizzled feathers and impaired growth performance. Consequently, Zn is usually supplemented in broilers diets, commonly in the form of zinc sulfate or the form of zinc oxide. Zn sources have different physicochemical characteristics and different effects on animal performance; in this study, zinc sulfate and a new source of potentiated ZnO (HiZox®) were compared.

Material and Methods

The trial took place in a Colorado type house, with dynamic transverse ventilation: 738 male broilers (Ross PM3) were divided into cages and raised among 26 500 birds. For each treatment, 9 cages of 41 broilers were used, with a density of 17 chicks/m². Animals were given *ad libitum* access to water and feed; there were one nipple line and one feeder per pen. Wheat straw was chosen for litter.

Basal diets, based on wheat, corn and soybean meal, were supplemented with 60 ppm of zinc from $ZnSO_4$ or 60 ppm of zinc from potentiated ZnO. Analysed values for total zinc in the two feeding programs were around 90 ppm.

Zootechnical criteria (bodyweight, feed intake) were recorded at feed transition (12 days, 21 days, 28 days) and at the end of the trial (35 days). Experimental unit was the pen of 41 birds. Litter score was evaluated at 28 days (feeder area, water system area and rest area), according to the Table 1.

Table 1. Litter score

Score	Description
1	Dry and crumbly
2	Crumbly but lightly wet
3	Crumbly but capping in some areas
4	Capping but we can find crumbly litter below
5	100% capping or completely wet

Results

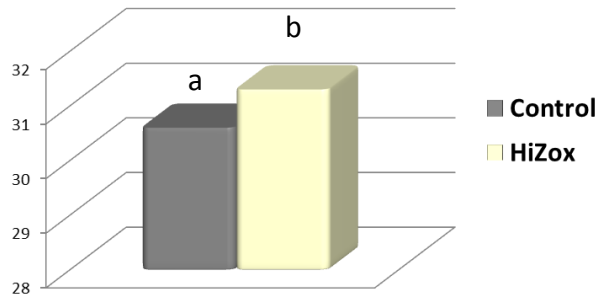
The purpose of the test was to evaluate efficiency of two different feed programs on broiler performance. Experiment was conducted with two groups of 369 male broilers that received different diets:

Control: basal diet + 60 ppm of Zn from zinc sulfate

Treatment: basal diet + 60 ppm of Zn from a source of potentiated ZnO.

In this trial, average cumulative mortality and culling rate was 4.2 %. There were no statistically differences between treatments on mortality or culling rate.

At day 12, after the starter period, birds fed with potentiated ZnO showed statistically higher bodyweight than the control group: 416 g vs. 407 g ($p = 0.037$) [Fig 1]. At any other age, there was no statistically significant difference between groups. However, at day 35, the broilers of the treatment group had a numerically higher bodyweight (2251 g vs 2231 g).



$p < 0.05$

Figure 1. Average daily gain (0-12 days)

Feed consumptions were equal with both sources of zinc, for any period.

There were no statistically significant feed conversion ratio (FCR) differences according to group for any period. However, using Aviagen method to standardize bodyweight, we obtained a significant difference between groups for 0-12-d-old period: 1.05 for $ZnSO_4$ > 1.03 for ZnO [Fig 2].

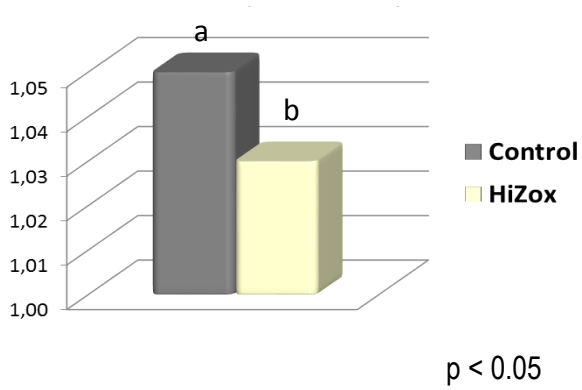


Figure 2. Feed conversion ratio (0-12 days)

In addition, the source of potentiated ZnO seemed to show a positive impact on litter quality. Thus, p-value was closed to be significant (p = 0.081), when the number of observation is limited (only 9 observations per treatment) [Fig 3].

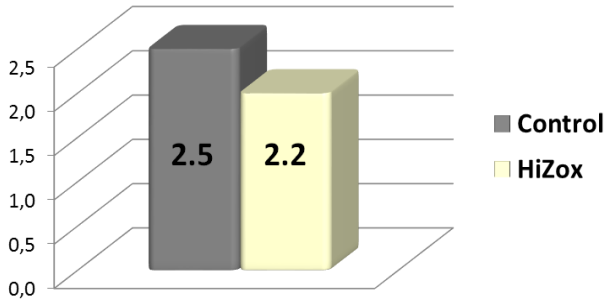


Figure 3. Litter score

Conclusions

The potentiated ZnO (HiZox®) improved broiler performance (BW, FCR) during the starter period and showed a positive effect on litter quality at 28 days.

P-134 (ID 175)

EFFECT OF DIETARY SUPPLEMENTATION WITH FERMENTED RICE BRAN ON GROWTH PERFORMANCE, BLOOD PARAMETERS, AND INTESTINAL MICROFLORA IN BROILER CHICKENS

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Abstract

The objective of this study was to investigate the effect of dietary supplementation with microbially-fermented rice bran on growth performance in broiler chickens. A total of one hundred and twenty 1-day-old broiler chickens, were randomly assigned to 5 dietary treatments in cages of 4 chicks each; each treatment had 6 replicate cages. A basal diet, formulated to be adequate in energy and nutrients, as well as four experimental diets, prepared by adding one of four ingredients 0.025% avilamycin, 1% Lactobacillus fermented rice bran, 1% Saccharomyces fermented rice bran, and 1% Bacillus fermented rice bran to the basal diet, were given to the birds over a 35-d period. The results indicated that BW gain during the first 21 d (starter period), growing periods (22 to 35d), and over the entire experimental period (0 to 35 d) was ($P<0.01$) different among treatments. Feed intake and feed conversion ratio varied significantly ($P<0.05$) across treatments in the starter period but not the growth period, or the overall experiment. The dietary supplements had no effect on any serum parameters except for total cholesterol, which was significantly lower for the supplements than the basal diet. Finally, intestinal flora Lactobacillus, Escherichia coli, and Salmonella did not vary across treatments. Our results suggest that the supplementation of poultry diet with fermented rice bran may be used to improve growth performance in broiler chickens.

Keywords: rice bran, fermentation, Bacillus, Saccharomyces, growth performance

Introduction

Antibiotics have been used as feed additives to improve growth performance and to control disease in animals. However, antibiotic use tends to produce antibiotic resistance, and leave residues in animal products. Since 2006, antibiotics have been banned from use as feed additives in the European Union. More recently, in July 2011, a full ban on the use of antibiotic growth promoters (AGPs) in animal feed came into effect in Korea in order to ensure the safety of livestock products for consumers. Therefore, it is necessary to find effective alternatives to antibiotic feed ingredients, such as prophylactic antibacterial substances and growth promoters. Rice bran, a by-product of rice milling, has high levels of important nutrients such as proteins, vitamins, minerals, complex carbohydrates, phytonutrients, phospholipids, and essential fatty acids (Ryan et al., 2011). However, the nutritional content of rice bran may vary widely because of differences in hull content, degree of polishing during milling, and differences in the variety of rice. It is abundantly available around the world, particularly in Asia, and Korea alone produces about 40 million tons of rice bran each year; this comprises 10% of the total weight of harvested paddy. Therefore, it a common feed supplement in animal production, including in poultry (Mu et al., 2011). Previous research on the supplementation of broiler chicken feed with rice bran has shown that it improves growth performance, however, studies on the usefulness of fermented rice bran for improving growth are scarce. The fermentation of materials with a microbial inoculum has been widely adopted in the development of novel functional ingredients because it may promote anti-flammatory activity (Kim et al., 2012). However, response of the growth performance, blood parameters, and intestinal microflora of animals to feeds with fermented rice bran is yet to be described. The objective of this study was to determine whether the dietary supplementation of broiler chicken feed with microbially fermented rice bran affects growth performance, blood parameters, and intestinal microflora.

Material and Methods

A total of one hundred and twenty 1-day-old male chickens (initial BW 36 ± 0.74 g) of the commercial broiler strain ROSS × ROSS 308, were raised for an experimental period of 35 d. All chicks were housed in a battery cage (86 cm × 57 cm × 35 cm, width×length×height) in an environmentally controlled room maintained at 30°C during the first week of the experiment and then gradually decreased to 24°C at the end of the experiment as recommended by the Ross manual; the room was lit throughout the experiment. Based on a completely randomized design, 6 replicate cages, each

containing 4 chicks, were assigned to 1 of 5 experimental treatments: (1) a basal diet formulated to meet or exceed the energy and nutrient requirements of broiler chickens (NRC, 1994), (2) basal diet plus 0.025% Avilamycin (AGPS), (3) basal diet plus 1% Lactobacillus-fermented rice bran (LFR), (4) basal diet plus 1% Saccharomyces-fermented rice bran (SFR), and (5) basal diet plus 1% Bacillus-fermented rice bran (BFR). A 2-phase feeding program with a starter diet from 0 to 21 d and a grower diet from 22 to 35 d was followed. Feed and water were provided ad libitum for 35 d. Body weight gain (g; BWG) and feed intake (g; FI) were measured weekly. A feed conversion ratio (FCR) was calculated as the ratio of BWG to FI; BWG, FI, and FCR were corrected for dead birds. All data were analyzed by ANOVA as a completely randomized design using the PROC MIXED procedure.

Results

Body weight gains during the starter period (0 to 21 d), growing period (22 to 35 d), and over the entire 35-day experimental period (0 to 35 d) were significantly higher for the experimental diets when compared to the basal diet (P<0.01). An orthogonal contrast revealed that birds fed with the antibiotic diet (AGPs) and fermented rice bran treatments (LFR, SFR, and BFR) displayed enhanced body weight gains of 14.1%, 9.5%, 8.9%, and 8.5% respectively, compared to the basal diet. Initial feed intake (during the 0 to 21-day starter period) was also significantly different across treatments (P<0.05) with SFR and BDR diets resulting in higher feed intake than the basal diet, AGPS, or LFR. However, feed intakes during the growth period and across the 35-day experimental period were similar across treatments. Similarly, initial feed conversion ratio (during the starter period), varied significantly across treatments (P<0.01) with higher initial FCR in birds that were fed SFR and BFR than in birds fed the basal diet, AGPS, and LFR; FCR did not vary across treatments during the growth period, or overall.

The positive effects of unfermented rice bran on the growth performance of broiler chicken may result from its high concentrations of protein, vitamin, minerals, complex carbohydrates, phytonutrients, phospholipids, essential fatty acids, and more than 120 antioxidants (Ryan et al., 2011). However, data explaining the positive effects of fermented rice bran products on growth performance in birds is unavailable, making it difficult to compare the present results with those of other studies. It is likely that the fermentation of plant materials increases the efficacy of their antioxidant and anti-inflammatory properties, when compared to those of unfermented materials (Cao et al., 2012; Kim et al., 2012). Therefore, the improved BWG and FCR reported here may be associated with increased antioxidants in microbially fermented rice-bran (MFR). This "increased antioxidants hypothesis" may also explain why supplementation of diets with relatively small amounts (1%) of MFR resulted in large positive effects on the growth performance of birds in this experiment.

Table 1. Growth performance of broiler chickens fed the diet containing fermented rice bran[1]

	Dietary treatments[2]					SEM	P-value
Items	Basal	AGPs	LFR	SFR	BFR		
BW gain, g/bird							
0 to 21 d	686.1[b]	763.7[ab]	773.4[a]	750.4[ab]	736.4[ab]	25.24	<0.01
22 to 35 d	858.1[c]	958.3[a]	950.9[ab]	945.8[ab]	913.5[b]	14.13	<0.01
0 to 35 d	1,544.3[b]	1,722.0[a]	1,724.2[a]	1,696.3[a]	1,649.9[a]	30.67	<0.01
Feed intake, g/bird							
0 to 21 d	975.0[b]	1,000.0[b]	980.6[b]	1,025.0[ab]	1,150.0[a]	44.37	0.02
22 to 35 d	1,686.1	1,713.4	1,645.6	1,671.1	1,606.5	164.94	0.99
0 to 35 d	2,661.1	2,713.4	2,626.2	2,695.1	2,756.5	170.31	0.93
FCR, feed/gain							
0 to 21 d	1.42[ab]	1.31[b]	1.28[b]	1.36[b]	1.56[a]	0.05	<0.01
22 to 35 d	1.97	1.79	1.74	1.77	1.75	0.18	0.88
0 to 35 d	1.72	1.58	1.53	1.59	1.67	0.10	0.70

[a-c]Values with different superscripts in the same row are significantly different (P<0.05).

[1]Data are least squares means of 6 observations per treatment.

[2]Basal = basal diet; AGPs = basal diet + 0.025% avilamycin; LFR = basal diet + 0.1% *Lactobacillus*-fermented rice bran; SFR = basal diet + *Saccharomyces*-fermented rice bran; BFR = basal diet + *Bacillus*-fermented rice bran.

The dietary supplements had no effect on any serum parameters except for total cholesterol, which was significantly lower for the supplements than the basal diet. Finally, intestinal flora Lactobacillus, Escherichia coli, and Salmonella did not vary across treatments.

Conclusions

Our results clearly show that the supplementation of the diets of broiler chickens with fermented rice bran increases body weight gain, and reduces total cholesterol. Consequently, dietary fermented rice bran may be a valuable functional ingredient in improving overall growth performance in broiler chickens, and important in poultry.

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P-135 (ID 111)

THE EFFECT OF HEMPSEED (*CANNABIS SATIVA* L.) ON PERFORMANCE, SOME BLOOD BIOCHEMICAL PARAMETERS AND IMMUNE RESPONSE IN BROILER CHICKENS

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Abstract

This study was conducted to investigate the effects of hempseed (HS) on performance, blood serum lipid profile and immune response in broiler chickens in during three growing periods (1-10; 11-24 and 25-42 day of age). A total of 250 one-day old male broiler chicks (Ross 308) were randomly distributed within 5 treatments with 5 replicates and 10 birds in each. The treatments were as follows: 1) Control diet (C, no HS in the diet); 2) 5% HS in the diet (HS5); 3) 10% HS in the diet (HS10); 4) 15% HS in the diet (HS15); 5) 20% HS in the diet (HS20). Data were analyzed as a completely randomized design using of the PROC GLM procedure of SAS. Overally, The results indicated that inclusion of HS over 10% to diet significantly reduced feed intake (FI), body weight (BW)($P<0.05$) but had no significant effect on feed conversion ratio (FCR). The serum cholesterol (CHO), triglyceride (TG) and high density lipoprotein (HDL) were not significantly altered; but low density lipoprotein (LDL) concentration HS supplemented diets were lower than that C diet. HS supplemented groups had higher IgG titres than C group. In conclusion, HS could be a potential feed in reducing plasma LDL and maitaing health of broiler chicks.

Keywords: Hempseed, Broiler, Performance, Blood, Immune system

Introduction

Hempseed (Cannabis sativa L.) is an annual, normally dioecious plant (Fortenbery and Bennet, 2004) known to have played a historically important role in food, fibre and medicine production (Callaway, 2004). It has been shown that whole hempseed contain over 30% oil, 25% protein and considerable amounts of fiber, vitamins and minerals. Hempseed oil contains over 80% polyunsaturated fatty acids (PUFA) and also it is a rich source of two essential fatty acids Linoleic acid (LA) and alpha-Linolenic acid (ALA) which is considered to be optimum for human health (Callaway, 2004). Consumers prefer foods rich in essential fatty acids, e.g. LA and ALA, due to their benefits for health. Linoleic acid and ALA are essential fatty acids for the human health and these should be taken with food (Goldberg et al., 2012). Some studies have shown that different levels of PUFA in the experimental diets of broiler had no effects on performance (Friedman and Sklan, 1995; Al-Khalifa et al., 2012; Konca et al., 2014a). The oil of hempseed is comparable to black currant oil which improves immunity (Barre, 2001). Konca et al. (2014b) reported that triglyceride, cholesterol and high density lipoprotein (HDL) were not significantly altered, however, low density lipoprotein (LDL) concentration in hempseed supplemented groups were lower than that of the control group. Also, hempseed may reduce LDL cholesterol and high blood pressure (Callaway, 2005). The objective of the present study was investigation of hempseed effects on performance, blood serum lipid profile and immune response in broiler chickens.

Material and Methods

A total of 250 one-day-old male broiler chicks (Ross-308) were used in the study. All of the birds were weighed and distributed into 5 treatment groups with 5 replicates and 10 birds per cage as replicate. The dietary treatments were: 1) Control diet (C, no HS in the diet); 2) 5% HS in the diet (HS5); 3) 10% HS in the diet (HS10); 4) 15% HS in the diet (HS15); 5) 20% HS in the diet (HS20). The diets were isocoloric and isonitrogenous. Performance parameters were measured in 10, 24 and 42 day of age. In 42 day of age, two birds were randomly selected from each cage and weighed individually and eviscerated. For determinating serum lipid profile and (sheep red blood cell) SRBC test, 2 blood samples per selected bird were taken and were kept in ice and were sent to laboratory. SRBC test was carried out according to Nelson *et al.*(1995) method. Data were analyzed as a completely randomized design using General Linear Models procedure (PROCC GLM) of SAS (2002) and means were compared by tukey test.

Results

The results indicated that inclusion of HS up to 10% into diet significantly decreased feed intake (FI), body weight (BW)(P<0.05) but had no significant effect on feed conversion ratio(FCR) (Table1).

Table1. The effect of different levels of hempseed on performance of broiler chicks

Item	Treatment						
	C	HS5	HS10	HS15	HS20	[1]SEM	P-value
BW (g/bird)							
10 old-day	190.35	186.22	179.68	181.64	181.07	2.94	[2]NS
24 old day	791.51a	782.89ab	698.50bc	659.30c	665.91c	20.07	*
42 old-day	2554.90a	2580.10a	2409.33ab	2247.04b	2282.08b	46.77	*
FI (g/bird/day)							
(1-10) day period	23.68a	23.22ab	22.90ab	22.44b	22.31b	0.25	*
(11-24) day period	82.54a	84.45a	80.83ab	75.21b	74.80b	1.50	*
(25-42) day period	172.83a	169.45a	158.42ab	147.98b	149.98b	4.59	*
(1-42) day period	103.27a	104.67a	100.02ab	92.67b	93.42b	103.27a	*
FCR							
(1-10) day period	1.24	1.25	1.28	1.23	1.23	0.02	NS
(11-24) day period	1.46	1.52	1.63	1.60	1.57	0.05	NS
(25-42) day period	1.89	1.86	1.85	1.87	1.86	0.04	NS
(1-42) day period	1.70	1.71	1.74	1.73	1.72	0.03	NS

[1]SEM: Standard error of means
[2]NS: Non-significance
a-c: Values with different superscript in a row differ significantly *p<0.05

The results of the study showed that there were no statistical differences among the treatment groups in terms of plasma TG, CHO, LDL and HDL., but LDL concentration in HS supplemented diets significantly were lower than C diet (P<0.05) (Table2).

Table2. The effect of different levels of hempseed on plasma lipid profile in broiler chicks

Parameter (mg/dl)	Treatment					SEM	P-value
	C	HS5	HS10	HS15	HS20		
CHO	165.59	144.40	148.40	143.63	142.95	10.49	NS
HDL	72.45	79.50	82.40	80.10	74.90	6.62	NS
LDL	83.58a	57.69b	55.69b	56.77b	57.50b	4.55	*
TG	47.80	36.05	51.55	33.80	52.75	4.22	NS

a-b: Values with different superscript in a row differ significantly *p<0.05

The effect of feeding hempseed on immune response is shown in Table3. There were no significant difference among treatments, but HS supplemented groups had higher IgG titres than C group. The table 3 shows the results.

parameter	C	treat-ment				SEM	P-value
		HS5	HS10	HS15	HS20		
SRBC	8.67	9.33	9.00	9.33	8.00	0.63	NS
IgM	6.67	6.67	5.00	5.67	5.67	0.69	NS
IgG	2.00	2.67	4.00	3.67	2.33	0.77	NS

Conclusions

The results of the study indicated that using hempseed into broiler chicks diet over 10% significantly reduced FI and BW, but it hadn't significant effect on FCR. Also the study showed that adding hempseed into diet significantly reduced LDL concentration in compared to C treatment. In conclusion, HS could be a potential feed in reducing plasma LDL and maintaining health of broiler chicks.

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P-137 (ID 86)

EFFECT OF DIFFERENT MAIZE BASED DIETS ON THE PRODUCTION PERFORMANCE AND CARCASS CHARACTERISTICS OF BROILER

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Abstract

A feeding trial was conducted for a period of 5 weeks with 160 day-old Starbro broiler chicks with a Randomized Block Design with 4 dietary treatments. Each treatment was replicated in 4 blocks with 10 chicks in each block. The dietary treatments were normal maize plus lysine and methionine, normal maize without lysine and methionine, Pacific 11 maize without lysine and methionine and Quality Protein Maize (QPM) without lysine and methionine. All diets were isocaloric and isonitrogenous that contained around 2975 Kcal ME/kg DM and 21.5% CP. At 5 weeks of age, 2 broilers from each block was randomly selected considering average body weight and slaughtered to analyze the meat yield traits. The QPM contained only slightly higher protein and lysine than Pacific 11. Other amino acid profiles of QPM were similar to that of Pacific 11. There were no differences in feed intake, water intake, total gain, feed conversion efficiency and mortality of broilers among the dietary treatments. Liveweight at slaughter ($p<0.01$), dressing percent ($p<0.05$) and breast meat ($p<0.001$) were highest, but heart ($p<0.05$) and digestive tract weight ($p<0.05$) were lowest in diet fed maize plus lysine and methionine. However, liveweight at slaughter and dressing percent did not differ ($p>0.05$) among the treatments without lysine and methionine. Morphological characteristics such as relationship between length of drumstick and carcass characteristics was promising.

Keywords: maize based diet, production performance, carcass characteristics, broiler

Introduction

Feed is a major cost of poultry production that accounts for 60-70% of the total cost of poultry production. Maize is a major feed ingredients in poultry diet which constitutes 50-60% of the total diet and can contribute up to 30, 60 and 90% of the diets protein, energy and starch respectively (Eshetie, *et al.* 2011). Although normal maize contains about 8-9% protein, the quality of two essential amino acids, lysine and tryptophan, is below nutritional requirements for poultry. However, utilization of quality protein maize (QPM) which can correct this deficiency because QPM has approximately twice the lysine and tryptophan contents of normal maize (DMR, 2001). Therefore, a study was conducted to investigate the effect of replacement of QPM by hybrid maize and normal maize with or without lysine and methionine on production performance and carcass characteristics of broiler.

Materials and Methods

A feeding trial was conducted for a period of 5 weeks with 160 day-old Starbro broiler chicks with a Randomized Block Design with 4 dietary treatments. Each treatment was replicated in 4 blocks with 10 chicks in each block. The dietary treatments were normal maize plus lysine and methionine (control diet), normal maize without lysine and methionine, Pacific 11 maize without lysine and methionine and Quality Protein Maize (QPM) without lysine and methionine. All diets were isocaloric and isonitrogenous that contained around 2975 Kcal ME/kg DM and 21.5% crude protein. Initial body weight of day old chicks was recorded immediately before the start of the trial and then weekly thereafter. FCR was recorded for whole of the period as total feed intake (Kg) per kg weight gain. Health status of birds was monitored every day and mortality of birds was recorded. At 5 weeks of age, 2 broilers from each block (total 32) was randomly selected considering average body weight and slaughtered to analyze the meat yield traits. Birds were slaughtered following “halal” method (Singh *et al.,* 2003) and allowed to bleed completely and then plucked and weight to determine blood and feather losses (Kotula *et al.,* 1960) and all data were recorded. Samples of feed ingredients and diets were analyzed for CP, CF and EE following AOAC, (1984). Amino acids of two maize hybrids, pacific 11 and QPM were determined by using HPLC. Data was analyzed by RBD with 4 blocks (Snedecor and Cochran, 1989) using the procedure

of SPSS 10 for windows (SPSS, 1998). Means compared for significant difference using Duncan's Multiple Range Test (Duncan, 1955).

Results and Discussion

The QPM contained relatively higher ash, CP and CF than pacific11. QPM contained slightly higher Lysine, Threonine, Tryptophan, and Methionine than pacific 11(table 1). The CP content of QPM used in this study (9.6%) was within the range (7.4-9.5%) reported by DMR, 2001.

Table 1. Chemical composition and amino acid profiles (g/100g) of QPM and Pacific 11.

Parameters	Chemical composition					Amino acid										
	DM	Ash	CP	CF	EE	Gly	Thr	Arg	Ala	Trp	Met	Val	Phe	Ile	Leu	Lys
QPM	90.2	1.7	9.6	1.8	6.1	0.3	0.2	0.3	0.5	0.1	0.1	0.3	0.3	0.2	0.7	0.2
Pacific 11	88.4	1.5	9.0	1.6	-	0.3	0.1	0.3	0.6	-	-	0.3	0.4	0.2	0.9	0.1

The data on final body weight, total gain, feed intake, FCR and mortality of broiler fed on different maize based diets are presented in Table 2. None of these characteristics differed ($p<0.05$) among diets. However, final weight (936g) and per day gain (31g) of broiler from 28 to 38 days reported by Miyasaka *et al.* (1997) was similar to the data of present study.

Table 2. Performances of broiler fed on different maize based diet

Parameters	Treatments				F value and significance
	Normal maize + LM	Normal maize	Pacific 11	QPM	
Initial weight (g)	38.7	39.1	40.8	40.7	0.32, NS
Final weight (kg)	1.1	1.2	1.2	1.1	0.32, NS
Total gain (kg)	1.1	1.1	1.2	1.1	0.55, NS
Feed intake (kg)	2.1	2.2	2.2	2.0	0.54, NS
FCR	2.0	1.9	2.0	2.0	0.15, NS
Mortality (%)	0.5	0.3	0.3	0.0	0.44, NS

NS= Non- significant ($p>0.05$).

Although lysine and methionine in normal maize based diet had no effect on production performance, it attached on the length of thigh ($p<0.01$) and drumstick ($p<0.05$). However lysine and methionine in normal maize-based diet did not affect ($p<0.05$) the length of neck, shank and wing of broiler (Table 3). Ishibashi *et al.* (2001) reported that when dietary lysine is low, bodyweight gain increased with increased lysine level.

Table 3. Morphological characteristics of broiler fed on different maize based diet

Parameters	Treatments				F value and significance
	Normal maize + LM	Normal maize	Pacific 11	QPM	
Neck (cm)	11.2	10.2	10.56	11.69	1.07, NS
Thigh (cm)	7.5^{b}	6.4^{a}	6.31^{a}	6.25^{a}	6.53, $p>0.01$
Drumstick (cm)	9.5^{b}	8.1^{a}	7.98^{a}	8.44^{a}	4.48, $p>0.05$
Shank (cm)	6.8	6.6	6.08	8.54	0.64, NS
Wing (cm)	17.3	16.1	16.56	16.25	1.54, NS

^{ab}, with different letters in the same raw differ ($P<0.05$); NS= Non- significant ($p>0.05$).

Carcass characteristics of broiler fed of different diets based on maize are presented in Table 4. Dressing percent (p<0.05; 81%) and breast meat (p<0.05:15%) were highest, but heart (p<0.05; 0.58%) and digestive track weight (p<0.05; 11%) were lowest in birds fed maize plus lysine and methionine. Live weight of slaughter and dressing percent did not differ (p<0.05) between the treatment without lysine and methionine.

Table 4. Carcass characteristics of broiler fed on different maize based diet

Parameters (% of live wt.)	Treatments				F value and significance
	Normal maize + LM	Normal maize	Pacific 11	QPM	
Dressing percent	81.2[b]	80.8b	77.0a	77.0a	5.39, p<0.05
Heart weight	0.6[b]	0.7ab	0.7ab	0.8a	1.89, p<0.05
Breast meat weight	14.7[c]	13.0b	10.4a	10.7a	13.84,p<0.001
Shank weight	81.2[b]	80.8b	77.0a	77.0a	5.39, p<0.05

[abc], with different letters in the same raw differ (P<0.05 to 0.001); NS= Non- significant (p>0.05).

Conclusions

There was no clear advantage to using QPM over normal maize or another hybrid Pacific 11 was found in terms of feed intake, total gain, feed conversion and mortality except for dressing percent. It is almost impossible to conclude from only one trail as the final weight of the broiler was only around 1.2kg, which should have been around 1.5kg in 35 days. Therefore, sever batches of trials are needed to reach to a conclusion.

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P-138 (ID 117)

THE EFFECT OF FEED RESTRICTION ON CHANGES OF MUSCLE FIBRE CHARACTERISTICS OF *MUSCULUS* PECTORALIS MAJOR IN BROILER CHICKEN

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Abstract

The aim of study was to evaluate the effect of one week feed restriction on muscle fibre characteristics in broiler chickens. The effect of quantitative feed restriction (80% ADL and 65% ADL) in the second week of age on histochemical characteristics of *musculus pectoralis major* (PM) was studied. Cockerels of Ross 308 (1215 chicken) were divided into 3 groups: *ad libitum* fed (ADL; 3×135), restricted 80 % of ADL (R80; 3×135) and with limited feed intake 65 % of ADL (R65; 3×135). Eight cockerels per group and age were slaughtered from 14 days of age till the end of the experiment at 35 days in a week interval to determine changes in muscle fibre characteristics. The number of muscle fibres in PM decreased ($P \leq 0.001$) with increasing age. Fibre cross sectional area (CSA) was significantly affected (0.027) by interaction of group and age. After the end of feed restriction (14 d) all groups had similar CSA (519.4 – 538.6 μm^2), the differences between groups were observed at 35 days of age with the largest area in R65 chickens (2295.7 μm^2), while R80 did not differ from ADL (1727.5 μm^2 and 1666.8 μm^2). There was no effect of feeding regime on giant muscle fibre incidence.

Keywords: feed restriction, broiler chicken, muscle fibre, m. pectoralis major, giant fibre

Introduction

Growth performance has been increased in broiler chickens due to genetic progress, improvement of nutrition and controlled environment. This growth is associated with higher body fat deposition, mortality and increasing metabolic and skeletal disorders (BARBUT et al., 2008). The feed restriction is applied as a prevention of these problems (MENCH, 2002). Early feed restriction in poultry stimulates compensatory growth in the realimentation period (PLAVNIK and HURWITZ, 1990).

Meat quality depends on muscle fibre characteristics, from which number and fibre cross sectional area are the most important. In poultry muscle fibre formation and number of muscle fibres are complete before hatching (SMITH, 1963). Fast-growing chickens have larger fibre cross sectional area than the slow-growing ones. This increase can be associated with higher number of giant fibres, which have fibre cross-sectional area three to five times larger than normal muscle fibres (DRANSFIELD and SOSNICKI, 1999). The presence of giant muscle fibres is an indicator of interrupted metabolism and abnormal contraction of muscle fibres during *rigor mortis.* DALLE ZOTTE et al. (2001) reported that every normal muscle fibre can be converted into giant one. Increased occurrence of giant is indicator for the development of insufficient meat quality (DRANSFIELD and SOSNICKI, 1999; REHFELDT et al., 2004).

The aim of this study was to evaluate the effect of one week feed restriction applied from 8 days of age on basic muscle fibre characteristics and presence of giant muscle fibres.

Material and methods

In the experiment, male broiler chickens of genotype Ross 308 (n=1215) were divided into three groups with three replicates (3×135 per each treatment): the control group fed *ad libitum* (ADL), the second group (R80) received 80 % of ADL and the group R65 had feeding reduced on 65 % of ADL. The feed restriction was applied from 8 to 14 days of age. Before and following feed restriction chickens were fed *ad libitum*. Water was available free throughout the whole experiment. Birds were housed in 9 littered pens with environmental conditions corresponding with requirements for broiler chickens. Eight chickens from each group were slaughtered at 14, 21, 28 and 35 days of age. Immediately after slaughter samples of *musculus pectoralis major* (MPM) were taken for detection of histological characteristics of muscle fibres. The samples were frozen in 2-methylbutan cooled by liquid nitrogen and then stored at –80°C until analysis. Cross-sections (12 μm) of samples were cut using a cryostat Leica (Leica Microsystems, Nussloch GmbH, Nussloch, Germany) at –20°C. Subsequently, it was performed staining by haematoxylin and eosin for basic histological charac-

teristics of muscle fibres. Image analysis NIS Elements AR 3.1 (Laboratory Imaging, Nikon, Tokio, Japan) was used to detection number of muscle fibres per 1 mm^2 and fibre cross sectional area (CSA). The giant fibre percentage was calculated.

Data were processed by two-way analysis of variance, group and age using ANOVA procedure (SAS Institute Inc, 2003). P-value P≤0.05 was considered significant for all traits.

Results and discussion

The results of one week feed restriction affecting muscle fibre characteristics are shown in Table 1. There was no interaction of group and age in number of muscle fibres per 1 mm^2. Together with increasing age, number of muscle fibres significantly (P≤0.001) decrease, which corresponds with the data of RYU and KIM (2005).

Table 1. The effect of feed restriction on *pectoralis major* muscle fibre characteristics

Group	Age (days)	Number of muscle fibres per 1 mm^2	Fibre cross sectional area (μm^2)	Giant fibres percentage (%)
ADL	14	1629.1	519.4^{g}	0.09
	21	1018.5	777.2^{fg}	0.10
	28	593.7	1360.7^{cd}	0.05
	35	464.6	1666.8^{bc}	0.02
R80	14	1574.0	538.6^{g}	0.04
	21	944.0	852.9^{ef}	0.06
	28	650.5	1143.9^{de}	–
	35	441.0	1727.5^{b}	0.03
R65	14	1626.0	520.5^{g}	0.05
	21	981.7	815.0^{fg}	–
	28	576.6	1418.7^{bcd}	0.08
	35	392.0	2295.7^{a}	0.04
RMSE		209.7	303.3	0.09
Significance				
Group		0.830	0.026	0.334
Age		≤0.001	≤0.001	0.711
Group * age		0.966	0.027	0.460

RMSE: root mean square error
[a,b,c,d,e,f,g] P≤0.05; Group: ADL – *ad libitum*, R80 – 80% feed of ADL, R65 – 65% feed of ADL

The CSA of muscle fibres was significantly (P≤0.027) influenced by interaction of group and age. All groups had similar CSA at 14 days of age, but at the end of experiment group R65 with the most intensive feed restriction had largest CSA, while R80 group with moderate feed restriction did not differ from ADL group. In contrast with our results REHFELDT et al. (2004) stated that qualitative and quantitative feed restriction reduced CSA of muscle fibres. Also LI et al. (2007) determined that restricted chickens revealed smaller CSA. In our experiment, the CSA significantly (P≤0.001) increased with age, which corresponds with DRANSFIELD and SOSNICKI (1999) and CHOI and KIM (2009).

Some fibres from *pectoralis major* exhibited histological features of giant fibres and also CSA of these fibres was typical for giant fibres. However, there was not detected the excessive incidence of giant muscle fibres in restricted groups and also age did not have an effect on giant fibre percentage. The percentage of giant muscle fibres was very low and according to REHFELDT et al. (2004) giant fibre percentage less than 0.5% is not associated with impairment in meat quality.

Conclusion

The results of the current study indicated that early feed restriction in broiler chickens did not affect number of muscle fibres per 1 mm^2, but the fibre cross sectional area enlarged in restricted chickens. The CSA was affected by intensity of feed restriction. The excessive incidence of giant fibres was not detected in any group.

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P-140 (ID 178)

VARIABILITY OF STARCH DIGESTIBILITY IN BROILERS FED WITH WHEAT-BASED DIETS: EFFECTS OF AGE AND FEEDING BEHAVIOUR

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Abstract

The starch digestibility of wheat may be more or less variable. Chemical parameters of wheat or its fineness of grinding can only explain a little part of the variability of starch digestibility. Beyond results of nutrients digestibility trials in broilers, two extreme wheat profiles have been highlighted as for the starch digestibility (good: WheatSD+ or low: WheatSD-) and the individual variability. Total tract digestibility of starch, nitrogen and energy of diets with both wheat and one maize, with or without enzymes, were quantified in five ages (8, 13, 18, 23 and 28 days) on 4 pools of 3 animals by treatment. The feed intake was measured daily and even closer (all the 2 hours), the days matching 3 of the 5 collections (7, 17 and 27 days). With age, the starch digestibility decreased significantly ($P<0.001$), but only for WheatSD- and especially from 18 days of age. Whatever the diet, the digestibility of energy and nitrogen varied significantly and similarly ($P<0.01$ and $P<0.001$) with the highest values between 13 and 18 days. The starch excreta content, measured individually, was positively correlated with daily intake only for WheatSD- from 18 days ($0.30<R^2<0.52$). The speed of ingestion changed during the day ($P<0.001$) with maxima before lights out (two to three times faster). For the last two hours, the value was positively correlated ($R^2=0.56$) with starch excreta content but only for WheatSD-.

Keywords: wheat, starch, digestibility, age, broiler

Introduction

Wheat is an important raw material in the feeding of poultry and may lead variability in nutrients digestibility and in the available energy content, which can reduce the performances (Gutierrez *et al*., 2008). Different factors (viscosity, hardness) had been highlighted without explaining all the variability (Carré *et al*., 2005). Métayer *et al*. (2007) found contradictory results on the role of those two criteria and claimed a positive relation between starch digestibility (SD) and nitrogen corrected apparent metabolisable energy (AMEn). The results of a meta-analysis (Nugues *et al*., 2013) allowed us to quantify the importance of the starch quantity in the wheat energetic value and remove many physical and chemical criteria as possible predictors of energy and starch digestibilities. Most of all, the important role of the animal makes difficult the prediction of the digestibility only from wheat characteristics. Other studies have been led to determine if technological parameters (wheat) or physiological mechanism (animal) could explain the variability. In this article, the role of ingestion rhythm and enzymes will be studied on the nutrients digestibility of two wheat models.

Material and Methods

Two wheat models, defined from a previous study, with extreme values concerning the SD (WheatSD+, Starch well Digested; WheatSD-, Starch not well Digested) were studied and compared to a corn. The effect of enzymes with xylanasic and β-glucanasic activities was also measured (E-/E+). The treatments were untitled : WheatSD-E-, WheatSD-E+, WheatSD+E-, WheatSD+E+, CornE- and CornE+, according to the raw material (RM) maximized in the feed and the addition (E+) or not (E-) of enzymes. A growing-phase feed were formulated (AMEn=2750 kcal/kg; Gross protein=19.5%) with an indigestible marker (TiO_2). The wheat-based diets were compounded of wheat (55.5%), corn (10%) and soybean meal (28.5%) and the corn-based diets of corn (60%), WheatSD+ (5.5%) and soybean meal (30%).

Every diet was tested on 12 male broilers (ROSS PM3). Excreta were collected individually in order to measure the starch content. 4 pools of 3 broilers were made by treatment to determine the starch, nitrogen and energy digestibilities at five ages (D8, D13, D18, D23 and D28). The growing performances of broilers (daily feed intake, total weight gain and FCR) were followed from D6 to D27. Individual feed intakes were measured every day and even all the two hours (between 8:30am and 8:30pm – 12 hours of light) to determine the speed of ingestion (D7, D17, D27). Statistics were performed with the software R v.2.15.0. Data were analysed by ANOVA with 3 factors (Age, RM, E), except for total performances (RM, E).

Results

For the five ages, the results of ANOVA on the different digestibilities (nitrogen, starch and energy) got on the pools (4 pools per treatment of 3 chickens) are reported on the table 1.

For SD, the ANOVA showed effects of RM and Age (P<0.001) and an interaction of RM*Age (P<0.01) due to a decrease with age only for WheatSD- (-2.2 points for E- ; -3.2 points for E+). This result attested the hypothesis of the degradation of SD with age but only for certain wheat profiles.

For each age, average and variability of individual starch content in excreta, which is a good predictor of SD (Métayer *et al*., 2015), are shown on figure 1. Responses were similar to the ones got on the pools of excreta but the variability brought additional information. An interaction RM*E was observed (P<0.01) due to a negative effect of enzymes on WheatSD- (+2.2 points at D28; contradictory to the expected). The SD of corn-based diets was better and less variable than the wheat-based diets one. WheatSD- was significantly less valuable than Corn on this criteria, WheatSD+ is intermediary. The variability increased with age and was from 1.5 to 3 times more important for wheat than for corn-based diets. The purpose of these individual measures was to compare animal performances to the capacity for starch digestion. No relation could be established between feed intake and starch content in excreta all the diets considered. But, significant correlations were observed for WheatSD-E- at D18, D23 and D28 (R^2=0.30, 0.45, 0.52), for WheatSD-E+ at D18 (R^2=0.42) and for WheatSD+E+ at D18 and D23 (R^2=0.31, 0.43). In this study, enzymes did not improve SD of diets.

The apparent nitrogen digestibility varied only with age (P<0.01). All diets considered, the highest values were observed at D18, whereas Métayer *et al*. (2015) reported a linear decrease with the age (from D6 to D20). The range of variation was of 2 points, except for WheatSD-E+ (4.6 points), but the mean deviation between the different ages was of only 1.2 point.

The energy and nitrogen digestibilities variations were similar but the difference between energy digestibility values of two treatments at the same age is bigger. Both criteria were well correlated (R^2=0.58; P<0,001), in agreement with Métayer *et al*. (2015). The interactions RM*Age (P<0.01) and Age*E (P=0.05) did not allow a simple analyse of the results. Enzymes would tend to improve the energy digestibility in young chicks (from 8 to 18 days of age).

The study of performances did not show any effect of enzymes from D6 to D27. The FCR of corn-based diets are better (P<0.01) than those of wheat-based diets (respectively 1.44, *vs*. 1.50 and 1.50) except for WheatSD+E+, which is similar to corn.

The speeds of ingestion (SI) were calculated from the two-hour feed intakes at D7, D17 and D27. Whatever the age, the chickens eat faster (P<0.001) in the two hours before the lights out (respectively 5.0 g/h, 16.6 g/h and 30.5 g/h at D7, D17 and D27); twice or three times faster than the daily average of other periods, even at the lights on. No effect of the RM was highlighted, except to D17 (P<0.05). The correlation between SI at the lights out and starch content in excreta is significantly and positive only for WheatSD-E- (R^2=0.56; figure 2), which attested our suggestion. However, the relation is the opposite for WheatSD-E+ (R^2=0.47) with no explanatory hypothesis. It is in agreement with Svihus (2014) who reviewed a negative correlation between feed intake and SD.

Table 1. Results of ANOVA on Nutrients digestibility of WheatSD-, WheatSD+ and Corn-based diets without or with enzymes (E- or E+)

Variable / Digestibility	Starch	Apparent Nitrogen	Energy
RM	P<0.001	NS	P<0.001
Age	P<0.001	P<0.01	P<0.001
Enzymes	NS	NS	P<0.01
RM*Age	P<0.01	NS	P<0.01
RM*E	NS	NS	NS
Age*E	NS	NS	0,05
RM*Age*E	NS	NS	NS
RSD / RCV (%)	0.9 / 0.9	1.4 / 1.7	1.2 / 1.6

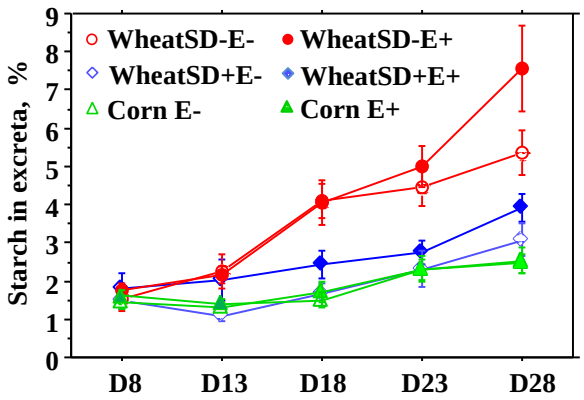

Figure 1. Starch content in excreta between D8 and D28 (averages and standard errors).

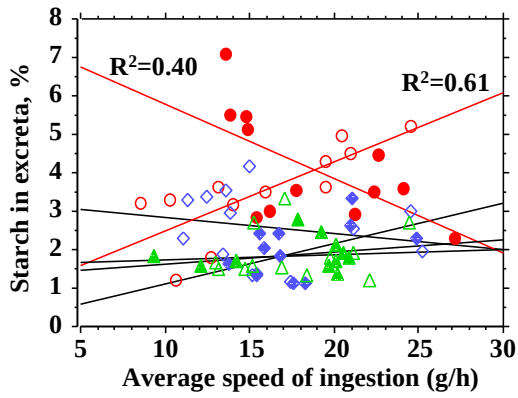

Figure 2. Relationship between starch content in excreta and SI (2 hours before lights out).

Conclusions

Whatever the cereal, wheat or corn, nitrogen as energy digestibilities vary with the age, with maxima around 13 or 18 days of age. Starch digestibility is variable from wheat to another, as high and homogenous as corn or lower and more variable. For this special kind of wheat, chickens have a good starch digestion before 18 days of age, and a higher intake at the end of the day increases the starch content measured in the excreta. In this context, starch digestibility is not improved by the tested enzymes.

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P-141 (ID 272)

THE EFFECTS OF FEEDING MAIZE OR WHEAT BASED DIETS WITH OR WITHOUT XYLANASE SUPPLEMENTATION ON THE GUT MORPHOLOGY AND MICROFLORA OF BROILER CHICKS

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Abstract

The aim of this trial was to get more understanding how maize and wheat based diets and using xylanase supplementation can modify the gut structure and the microbial fermentation in the caecum.

A total of 160 Ross 308 broiler cockerels were divided into four treatment groups and fed maize, wheat soybean meal based diets. Maize based diets contained only maize as the sole cereal. Wheat based diets contained beside maize 30, 40 and 50% wheat in the starter, grower and finisher phase respectively. Both maize and wheat based diets were fed with or without xylanase (0,15g/kg Axtra XB 201) supplementation (M+, M-, W+, W-). The most important difference in the morphology of jejunum was the significantly higher crypt depth and significantly lower villus:crypt ratio of treatment W+, compared with treatment W. Both W+ and W- treatments resulted higher viscosity in the ileum and higher Coliform bacteria concentration in the caecum compared with the M+ and M- groups. No significant changes in the number of Lactobacilli were found. Wheat based diets resulted higher concentration of short chain fatty acids (SCFA) and lower pH values in the caecum. Feeding wheat based diets decreased the caecal concentration of propionic acid and significantly increased the production of butyric acid.

From the results it can be concluded, that xylanase enzyme supplementation of wheat based diets results not only changes in the viscosity of the digesta, but modifies also gut morphology and the microbial fermentation in the caecum. Increasing the SCFA concentration and lowering the pH in the caecum can decrease the number of several pathogenic microbes and improve gut health.

Keywords: maize, wheat, xylanase, gut morphology, gut health

Introduction

One of the most important differences between maize and wheat is, that wheat contains higher amounts of water soluble none starch polysaccharides (NSP-s), mostly arabinoxylans. Soluble NSP-s increase the viscosity of the digesta, causing lower digestibility of nutrients and worse litter quality (CHOCT et al., 2004). In order to get around these negative effects, the exogenous NSP degrading enzyme, xylanase is widely used in the practice. Beside the effects of viscosity and digestibility there are not consistent information how the different NSP-s of the main cereals can affect gut morphology and microflora (FERKET, 2011). Therefore the aim of this trial was to get more understanding how maize and wheat based diets, with or without exogenous xylanase can modify the morphology of the small intestine and the microbial fermentation in the caecum.

Materials and methods

160 Ross 308 broiler cockerels were divided into four treatment groups. Maize based diets contained only maize as the sole cereal. Wheat based diets contained beside maize 30, 40 and 50% wheat in the starter (0-10d), grower (11-24d) and finisher (25-35d) phase respectively. Both maize and wheat based diets were fed with or without xylanase (0,15g/kg Axtra XB 201) supplementation (M+, M-, W+, W-). The soluble arabinoxylan contents of maize and wheat were 0.88 and 9.37 mg/kg respectively. Diets were isocaloric and isonitrogenous and were prepared to meet the nutrient requirements of Ross 308 hybrids. For the histomorphological analysis of the jejunum, the villus height, crypt depth, villus width, villus height:crypt depth ratio and thickness of muscle layer were measured. The viscosity of the ileal digesta was measured with a Brookfield DV II+ type viscometer. The number of Lactobacilli were counted on MRS agar after anaerobic incubation at 39°C for 48 h. Coliform bacteria were counted as red colonies on MacConkey agar, incubated aerobically at 39 °C for 24 h. For SCFA determination of caecal samples were measured by Gas Chromatograph, the pH with a portable pH meter. All data were analysed by two way analysis of variance by using SPSS 16.0 software.

Results

The most important difference in the morphology of jejunum was the significantly higher crypt depth and significantly lower villus:crypt ratio of the W+ group, compared with the treatment W. The villus height and the other parameters were not affected by the treatments ***(Table 1.)***.

Table 1. Histomorphologycal parameters of the jejunum (µm)

	Maize	Maize+enzyme	Wheat	Wheat+enzyme
villus height	825.0±22.0	791.5±35.3	812.5±31.3	823.4±21.1
crypt depth	177.7±6.3 ab	157.5±7.4 bc	150.8±5.1 c	183.4±7.3 a
apical transverse	121.0±6.5 b	143.3±6.1 a	129.7±5.1 ab	138.5±5.4 ab
villus/crypt ratio	5.0±0.2 ab	5.7±0.4 ab	5.6±0.2 a	4.8±0.2 b
muscle layer	119.3±3.8	131.9±23.8	115.0±4.0	122.7±3.9
basal transverse	175.0±9.5	191.6±6.9	185.5±8.8	199.0±8.8

Values of the table are means ±SEM; averages with different letter marks differ significantly ($p<0.05$)

The higher crypt depth in case of W+ treatment could cause increased mucus production which can affect the micro-flora of the small intestine content and the colonisation of the different bacteria. Due to the higher amount of soluble arabinoxylan content of wheat, significantly higher viscosity values were found in case of wheat based groups (W: 3.04; W+: 2.84 mPa·s**)** compared with the maize based diets (M: 2.31; M+: 2.56 mPa·s**)**.

In the caeca significantly higher numbers of Coliform bacteria were observed in chickens fed the wheat based diets with or without enzyme supplementation (W: 5,78; W+: 5.31 CFU/g) compared with the maize based treatments (M: 4.47; M+: 5.01 CFU/g). No significant changes in the number of Lactobacilli were found (W: 8.16; W+: 7.86; M:7.97; M+: 7.93 CFU/g).

Wheat based diets resulted higher concentration of short chain fatty acids (SCFA) and lower pH values in the caecum compared with the maize based treatments. In the case of wheat based diets, the enzyme supplementation resulted further decrease in pH ***(Figure 1.).***

Comparing the concentration of the different volatile fatty acids, feeding wheat based diets decreased the caecal concentration of propionic acid and increased the fermentation of butyric acid. The butyric acid content of caeca in W+ was significantly higher than those in the M and M+ groups ***(Figure 2.).***

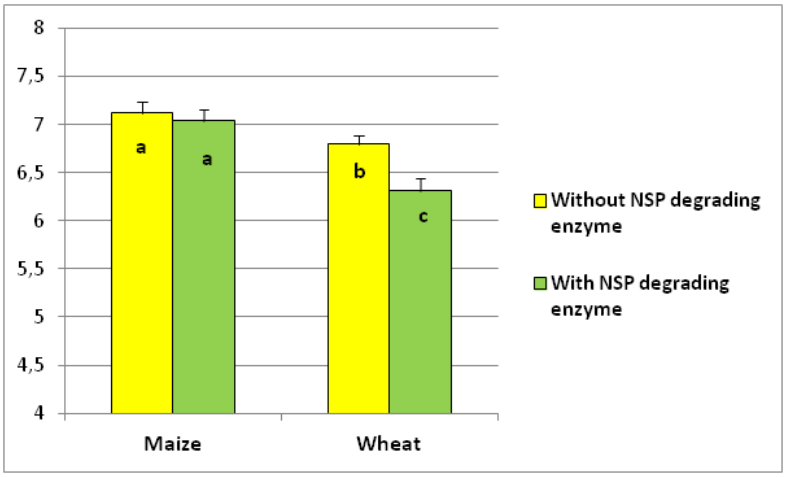

Figure 1. Caecal pH values

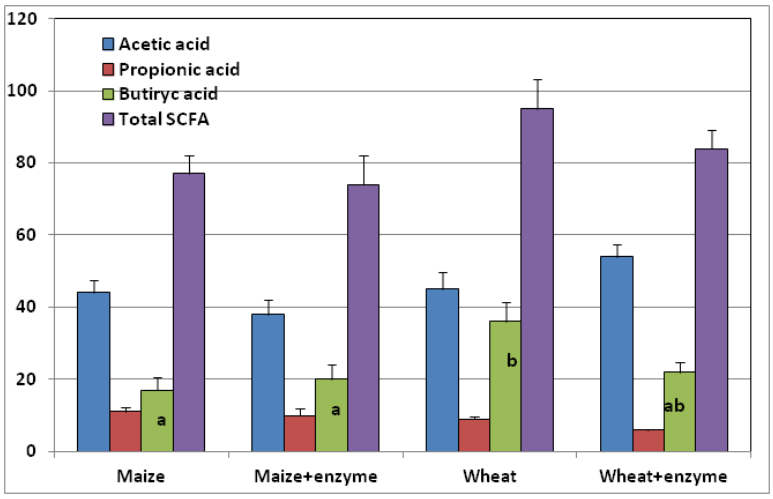

Figure 2. Caecal SCFA contents (µmol/g)

Conclusions

From the results it can be concluded, that xylanase enzyme supplementation of wheat based diets results not only changes in the viscosity of the digesta, but modifies also gut morphology and the microbial fermentation in the caecum. Increasing the SCFA concentration and lowering the pH in the caecum can decrease the number of several pathogenic microbes and improve gut health.

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P-144 (ID 246)

RESPONSE OF BROILER CHICKS FED ON STEAM- OR COLD-PELLETED DIETS CONTAINING RAW FULL-FAT SOYBEAN MEAL

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Abstract

A 2 x 4 factorial study was conducted to assess the response of broiler chickens fed steam- or cold-pelleted diets containing different levels (0, 10, 20 or 30 %) of raw full-fat soybean meal (RSBM). One half of each diet was steam-pelleted and the other half was cold-pelleted. Trypsin inhibitor contents of diets ranged from 1730.5 to 10484.4TIU/g. Each of the eight treatments was replicated six times, with 8 birds per replicate. Chicks were housed under an environmentally controlled cage system and fed to 14 days of age. Feed intake ($P < 0.05$) and body weight gain (BWG) ($P < 0.001$) declined with increase in RSBM in the diets. However, BWG was reduced ($P<0.001$) in chicks on the steam-pelleted diets. Feed conversion ratio was negatively affected ($P<0.05$) by increase in RSBM levels. There were no interactions between RSBM level and pelleting condition. An increase in RSBM content and feeding on steam-pelleted diets resulted in an increase in the weight of gizzard + proventriculus ($P<0.05$), pancreas ($P<0.001$), duodenum ($P<0.01$) and jejunum + ileum. The relative weight of the spleen, bursa, heart and liver was not affected by the two factors. There is a need to assess the response of chicks over a longer production cycle. However, it would appear that steam-pelleting was not adequate at reducing the antinutritional effects of RSBM.

Keywords: Cold-pelleting; commercial SBM; steam-pelleting; raw full-fat

Introduction

Soybean meal is generally expensive due to its position as the premier vegetable protein ingredient for poultry and its supply fluctuates due to events in the major producing areas of the world in North and South America (SHI et al., 2012). The meal can be fed as full-fat or after oil extraction. Full-fat meals are higher in energy, but lower in protein, and require heat treatment, in a bid to reduce the levels of protease inhibitors that are responsible for poor growth of birds on diets containing raw soybean meals (RSBM). It is not well known if changing the pelleting methods reduces effects of trypsin inhibitors. The objective of this study was to evaluate the response of chicks fed on steam- or cold-pelleted diets containing RSBM.

Materials and Methods

Four diets were formulated, in which 0, 10, 20 or 30 % of RSBM replaced commercial SBM. One half of each diet was steam-pelleted and the other half was cold-pelleted. A 2 x 4 factorial arrangement (4 RSBM levels x 2 methods of pelleting) was used to assess the response of broiler chicks to diets containing RSBM. Each diet was replicated 6 times, with 8 birds per replicate. The diets were fed from hatch to 14 days of age.

Results and Discussion

Feed intake (FI) declined with increase in level of RSBM but this was significant ($P<0.05$) only for the steam-pelleted diets (Table). The interaction between RSBM level and pelleting method tended ($P<0.07$) to be significant. Body weight gain (BWG) was also reduced ($P<0.001$) by increase in RSBM level while birds on the cold-pelleted diets gained ($P<0.001$) more than those on the steam-pelleted diets, especially at the higher RSBM levels. On both pellet types, FCR was reduced ($P<0.05$) as a result of increase in RSBM. The interaction between RSBM level and pelleting method was not significant in the case of BWG and FCR. The weight of proventriculus + gizzard (G+P) ($P<0.05$), duodenum ($P<0.01$) and pancreas ($P<0.001$), in relation to the body weight, increased with rising level of RSBM (data not shown). Feeding the steam-pelleted diets also resulted in an increase in weight of G+P ($P<0.01$) and jejunum + ileum ($P<0.001$).

The reduction in FI with increase in level of RSBM may be due to the presence of antinutrients in raw soybean. Weight gain may have also been adversely affected through the negative effects of protease inhibitors, illustrated by the noticeably large weight of the pancreas (MOGRIDGE *et al.,* 1996). Contrary to expectations, steam-pelleting resulted in poorer growth than cold-pelleting. It is not known if the high conditioning temperature reduced the nutritive value of the meal.

Table 1 Feed intake (g/b), live weight gain (g/b) and feed conversion ratio (FCR) of broiler chickens fed on different diets between hatch and 14 days of age

Pelleting method	RSBM[1] (%)	Feed intake	Body weight gain	FCR
Steam	0	582.4[a]	514.9[a]	1.13
	10	526.0[bc]	487.6[b]	1.15
	20	554.7[ab]	471.6[b]	1.17
	30	496.9[c]	426.1[c]	1.21
Cold	0	556.2[ab]	517.1[a]	1.11
	10	562.2[ab]	516.1[a]	1.14
	20	554.3[ab]	503.6[ab]	1.13
	30	544.0[ab]	479.8[b]	1.17
Pooled SEM		6.05	4.90	0.01
Sources of variation				
RSBM level		*	***	*
Pelleting method		NS	***	NS
RSBM x pelleting method		0.07	NS	NS

Conclusion

Further testing, including grower and finisher diets, containing such a levelled RSBM with a partial economic analysis might be required.

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P-145 (ID 183)

EFFECT OF EXTRUSION COOKING ON THE NUTRITIONAL VALUE OF FABA BEAN FOR BROILER CHICKENS

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Abstract

The aim of the investigations was to expand our knowledge regarding the effect of extrusion cooking on the nutrient value of currently cultivated Polish faba bean varieties. The experiment was conducted with 264 broiler male chickens, divided into eleven dietary treatments, 12 replications with 2 birds in each. The birds were kept (1-18d) individually in balance cages and received basal diets *ad libidum*. From day 19 to 23 birds were fed the basal diets which were mixed with faba beans meal in proportion 60:40. Five different cultivars of faba bean (Albus, Amulet, Kasztelan, Olga, Merlin) were used in the experiment. The birds from group one were fed only the basal diet. Diets of the remaining groups differed with regard to faba bean cultivars and form (raw or extruded). The following parameters were determined in the trial: ilea crude protein and amino acids digestibility and AME_N value. For this purpose the difference method of digestibility determination and ME was used. TiO_2 was added to diets in the amount of 0.3% to carry out necessary calculations. Comparing the groups without and with extrusion, higher values for ileal digestibility were determined when extrusion was applied (crude protein - 85.9 % vs. 89.5%; Lys - 89.7% vs. 94.7%; Thr - 81.6% vs. 85.0%). The same differences were determined in the case of AME_N (10.83 MJ/kg vs. 14.97 MJ/kg). The present trial demonstrated that extrusion exerted a beneficial effect on nutrient value of faba bean.

Keywords: faba bean, extrusion cooking, anti-nutritional factors

Introduction

Faba bean seeds can be used as a valuable source of protein, energy and other nutrients in broiler chicken nutrition. However, faba bean seeds also contain variable amounts of anti-nutritional factors such as tannins, protease inhibitors, oligosaccharides (raffinose, stachyose and verbascose) and non-starch polysaccharides (NSP), which can negatively affect nutrient utilization and animal performance. Current low-tannin faba bean cultivars are characterized by low concentrations of tannins, but they still contain other anti-nutritional factors, which can reduce nutrient digestibility. Results have so far shown that low-tannin faba beans are characterized by better amino acid digestibility than conventional faba beans. However, the digestibility coefficients of amino acids contained in low-tannin faba beans are still lower than those contained in soybean meal (Woyengo and Nyachoti 2012).

According to some researchers, extrusion cooking of faba bean seeds is a process which may improve the performance of broiler chickens. An appropriate processing temperature is critical for the elimination of heat-labile anti-nutritional factors found in legume seeds (Diaz et al., 2006). The effect of extrusion cooking on the nutritional value of faba beans is not well understood. Moreover, there is no comparative information on the influence of extrusion cooking on the nutritive value of low-tannin faba beans.

The aim of the investigations was to expand our knowledge regarding the effect of extrusion on the nutrient value of currently cultivated Polish faba bean varieties.

Material and methods

Raw and extruded faba bean seeds were analyzed in duplicate for crude protein (CP), starch (S), resistant starch (RS), phytin phosphorus, raffinose family oligosaccharides (RFO), tannin and trypsin inhibitor content. The experiment was conducted with 264 broiler male chickens, divided into eleven dietary treatments, 12 replications with two birds in each. The birds were kept (1-18d) individually in balance cages and received basal diets *ad libidum*. From day 19 to 23 birds were fed the basal diets which were mixed with faba bean meal in proportion 60:40. Five different cultivars of faba bean (Merlin, Olga, Albus, Amutet, Kasztelan) were used in the experiment. Birds belong to the first group were fed only the basal diet. Diets of the remaining groups differed with regard to faba bean cultivars and form (raw or extruded). The concentration of CP, S and amino-acids in diets and digesta were determined. The apparent metabolizable energy (AME_N) of faba bean and apparent ileal digestibility (AID) were determined using the difference method and TiO_2 was

used as a indigestibility marker. The obtained results were subjected to two-factorial analysis of variance (ANOVA). Means from experiments were compared using Duncan test and the significance of differences was assumed to be at the level of $p<0.05$.

Results and discussion

The analyzed composition of feed ingredients of raw and extruded low-tannin faba bean is presented in Table 1. The content of CP and S in DM was similar in raw and extruded faba beans and did not differ statistically significantly (Table 1). Faba beans in extruded form were characterized by lower concentrations of RS than faba beans in raw form ($p \leq 0.05$). The content of RFOs and tannins in DM was similar in raw and extruded faba beans and did not differ statistically significantly (Table 1). Extrusion cooking had a positive impact on the decrease in trypsin inhibitors and phytic P content in seeds and this decrease was statistically significant ($p \leq 0.05$). A similar relationship was confirmed by Alonso et al. (2000), who explained that during extrusion some molecules of inositol hexaphosphate were hydrolyzed to penta-, tetra- and triphosphates; therefore, the level of phytic P in seeds after extrusion was lower.

Table 1 Chemical composition of five faba bean cultivars in raw and extruded form

Item,% of DM	Merlin		Olga		Albus		Amulet		Kasztelan	
	–[1]	+	–	+	–	+	–	+	–	+
Crude protein	29.9[2]		31.8		32.6		32.0		28.5	
Starch	43.6		39.6		41.4		40.8		42.4	
Resistant starch	17.3	1.43	17.3	0.79	18.4	0.93	20.2	0.79	17.8	1.16
Oligosaccharides	2.55[2]		2.57		3.00		2.36		2.68	
Raffinose	0.13		0.18		0.24		0.18		0.22	
Stachyose	0.62		0.62		0.86		0.85		0.82	
Verbascose	1.80		1.77		1.90		1.33		1.64	
Tannins (mg/g)	0.060		0.062		0.064		0.060		0.068	
Trypsin inhibitors (mg/g)	0.60	0.30	0.50	0.50	0.60	0.20	0.50	0.02	0.60	0.30
Phytic P	0.38	0.12	0.32	0.35	0.50	0.19	0.34	0.29	0.40	0.21

[1] "–" faba bean in raw form; "+" faba bean in extruded form

[2] Two means are shown if the value of raw and extruded forms was statistically significantly different. If not – only one mean is presented

The AID of CP, S, amino acids and AME_N value is shown in Table 2. The Extrusion cooking of faba bean seeds had a statistically significantly positive effect on all determined parameters. There was no interaction between extrusion cooking and cultivars for CP. Moreover, in the trial carried out on broiler chickens, interactions were established between the faba bean cultivars and the form of seed with respect to AME_N values, AID of starch and amino acids (Table 2). The obtained interactions showed that the effect of extrusion cooking depended on low-tannin faba bean cultivars. Following the analysis of the examined cultivars, very high Pearson's correlations between phytic P contents in raw and extruded seeds and AME_N as well as starch and amino acid digestibility were found. These results confirmed that the effect of extrusion cooking depended on phytic P and RS concentrations in seeds (Table 3).

Table 2 Apparent ileal digestibility of crude protein, starch, amino acids and AME_N level in five faba bean cultivars in raw and extruded forms for broilers[1]

Cultivars	Extrusion	AME_N MJ/kg	CP %	Lys %	Thr %	Starch %
	–	10.83[b]	85.9[b]	89.7[b]	81.6[b]	77.2[b]
	+	14.97[a]	89.5[a]	94.7[a]	85.0[a]	96.9[a]
Merlin		13.96[a]	87.7[a]	92.0[a]	83.9[a]	86.8[bc]
Olga		12.86[b]	87.5[a]	92.4[a]	84.2[a]	87.0[b]
Albus		11.00[c]	84.0[b]	89.8[b]	77.2[b]	84.0[c]
Amulet		13.21[b]	89.4[a]	92.5[a]	85.7[a]	90.1[a]
Kasztelan		13.81[a]	90.0[a]	94.2[a]	85.4[a]	86.4[bc]
SEM		0.26	*0.58*	0.55	0.89	*1.52*
Cultivar		< 0.001	< *0.001*	0.002	0.002	*0.025*
Extrusion		< 0.001	< *0.001*	< 0.001	0.018	< *0.001*
Cultivar × Extrusion		< 0.001	*0.160*	0.004	0.028	< *0.001*

[1]n = 6

Table 3 Pearson's correlation coefficients between phytic P and resistant starch content in faba bean seed traits in raw and extruded forms and determined parameters (AID of starch and amino acids; n = 60, AME_N; n = 100)

Faba bean traits	Response	Correlation coefficient (r)	p
Phytic – P content	AME_N	– 0.89	< 0.001
	Starch AID	– 0.88	< 0.001
	Lys AID	– 0.70	< 0.001
	Thr AID	– 0.46	< 0.001
Resistant starch	AME_N	– 0.80	< 0.001
	Starch AID	– 0.93	< 0.001

Conclusions

Extrusion cooking is one of the processes which can improve the nutritional value of faba beans through a decrease in phytic acid, trypsin inhibitors and resistant starch content. Effect of extrusion cooking depend on the concentration of resistant starch and phytic P content in faba bean seeds.

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P-146 (ID 174)

EFFECT OF DIETARY SUPPLEMENTATION WITH RICE BRAN EXTRACTS ON THE GROWTH PERFORMANCE, BLOOD PARAMETERS OF BROILER CHICKENS

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Abstract

The objective of this experiment was to investigate the effect of rice bran extracts (RBE) as the dietary supplementation on growth performance, blood parameter, and immune response in broiler chickens. Two hundred forty 1-day-old ROSS 308 male broilers were allotted to 4 dietary treatments with 6 replicated pens consisting of 10 chicks. Three additional diets were prepared by adding 5, 10 or 20 g/kg of RBE to the basal diet. The experimental diets were fed on an *ad libitum* basis to the birds during 35 d. Consequently, increase in the inclusion level of RBE in diets improved BW gain by 9.0, 7.8, or 7.3%, respectively. Feed conversion ratio of birds by 10.3, 11.4, or 14.3%, respectively, but affected neither the feed intake of birds nor erythrocytes of birds. However, heterophil, lymphocyte, and monocytes increased (linear and quadratic, P<0.01) with inclusion level of RBE in diets increased. As expected, increasing inclusion level of RBE in diets increased (linear, P<0.01) the concentrations of total cholesterol. AST, ALT, glucose, and albumin were not affected by inclusion of RBE in diets. Increasing inclusion level of RBE in diets increased concentrations of IgG (linear, P<0.01). There was no effect of inclusion level of RBE in diets on concentrations of IgM. These results suggest that dietary RBE may be used functional ingredient to improve growth performance, total cholesterol, and immune response of birds.

Keywords: *broiler, rice bran extracts, growth performance, total cholesterol*

Introduction

Rice is the principle cereal food in Korea, and the by-products from its milling are used as important feed resources. One such by-product is rice bran extracts (RBE); it is gaining commercial importance worldwide because of its many beneficial nutritive and biological effects. Rice bran oil contains 90-96% saponifiable lipids and about 4% unsaponifiable lipids. The saponifiable lipids include 68–71% triglycerides, 2–3% diglycerides, 5–6% monoglycerides, 2–3% free fatty acids (FFAs), 2–3% waxes, 5–7% glycolipids, and 3–4% phospholipids (McCaskill and Zhang, 1999), whereas the principle component of the unsaponifiable fraction is ɤ-oryzanol (Raghuram and Rukmini, 1991).

ɤ-oryzanol, a phytosteryl ferulate mixture extracted from rice bran oil, has a wide spectrum of biological activities, as well as antioxidant properties (Juliano et al., 2005). RBE has an excellent fatty acid profile. Three major fatty acids, palmitic, oleic, and linoleic account for 90% of the total fatty acids of rice bran oil (Amarasinghe and Gangodavilage, 2004). The crude form of rice bran extracts is known to improve the growth performance of broilers. Dietary supplementation of rice bran oil in broilers improved growth performance (Anitha et al., 2006; Purushothaman et al., 2005) and decreased cholesterol concentrations (Berger et al., 2005). However, data pertaining to the effects of dietary supplementation of RBE on the growth performance, blood parameters, and immune response of animals are limited. In this regard, we investigated the effect of dietary supplementation of RBE on the growth performance, blood parameters, immune response of broilers.

Material and Methods

A total of two hundred forty 1-d-old ROSS 308 (initial BW = 36 ± 0.74 g) were obtained from local hatchery (Samhwa Hatchery, Hongseong, Republic of Korea) and were housed in a battery cages (86 cm × 57 cm × 35 cm, width × length × height) in an environmentally controlled room. The experiment was performed as a completely randomized design with 6 replicate pens consisting of 10 chicks. A 2-phase feeding program with a starter diet from 0 to 21 d and a grower diet from 22 to 35 d was used in this experiment. Within each phase, a control diet was formulated to meet or exceed NRC (1994) requirements of broiler chickens for macro- and micronutrients. Three additional diets were prepared by adding 5, 10 or 20 g/kg of RBE to the basal diet. The RBE was added at the expense of the basal diet. The experimental diets were mash form. The diets and water were available *ad libitum*. The room temperature for both conventional and battery cages was maintained at 30°C during the first week of the experiment and then gradually decreased to

24°C at the end of the experiment as recommended by the Ross manual. A 24-h lighting schedule was used throughout the experiment. All data were analyzed by ANOVA according to completely randomized design (Steel et al., 1997) using the PROC MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Outlier data were identified according to Steel et al. (1997), using the UNIVARIATE procedure of SAS, but no outliers were detected. The battery cage was an experimental unit for growth performance data, whereas the individual bird was an experimental unit for blood parameter, serum enzyme activities and immune response data. Dietary treatment was fixed effect in all statistical models. The LSMEANS procedure was used to calculate mean values. The orthogonal polynomial contrast test was performed to determine linear and quadratic effects of increasing inclusion level of RBE in diets on each measurement. Significance and tendency for statistical tests were set at $P < 0.05$ and $0.05 \leq P \leq 0.10$, respectively.

Results

During the initial 21 d of the experiment, BW gain and feed conversion ratio increased (linear, P<0.05) with inclusion level of RBE, but feed intake was not influenced by inclusion of RBE in diets. During 22 to 35 d of the experiment, BW gain increased (linear and quadratic, P<0.05) with inclusion level of RBE and a tendency for improved feed conversion ratio (linear, P<0.10) was observed as inclusion level of RBE in diets increased. There was no effect of inclusion level of RBE in diets on feed intake of birds. For overall experiment, increasing inclusion level of RBE in diets increased BW gain (linear and quadratic, P<0.01), and improve feed conversion ratio (linear, P<0.05). There was no effect of inclusion level of RBE in diets on feed intake of birds. There was no effect of inclusion level of RBE in diets on erythrocytes of birds. However, heterophil, lymphocyte, and monocytes increased (linear and quadratic, P<0.01) with inclusion level of RBE in diets increased (Table 4). As expected, increasing inclusion level of RBE in diets increased (linear, P<0.01) the concentrations of total cholesterol (Table 5). AST, ALT, glucose, and albumin were not affected by inclusion of RBE in diets. Increasing inclusion level of RBE in diets increased concentrations of IgG (linear, P<0.01). There was no effect of inclusion level of RBE in diets on concentrations of IgM.

Table 1. Immune response of broiler chickens fed the diet containing rice bran extract (RBE)[a]

	RBE (g/kg)				Pooled	*P*-value	
Items	0	5	10	20	SEM	Linear	Quadratic
IgG (mg/mL)	1.26	1.56	1.56	2.51	0.51	0.01	0.27
IgM (µg/mL)	132.2	259.7	162.6	225.9	82.18	0.63	0.71

[a]Data are least squares means of 6 observations per treatments.

Conclusions

The results of this study indicate that dietary supplementation of rice bran extracts improves growth performance and decreases total cholesterol level in broilers. Therefore, dietary rice bran extracts is considered a valuable functional ingredient to improve the growth performance of birds.

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P-148 (ID 127)

USE OF SEMI-LEAFLESS PEAS (*PISUM SATIVUM* L) WITH ENZYME ADDITION IN DIETS FOR BROILERS

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Abstract

The trial was conducted to study the effects of dietary pea inclusion and the supplementation of combination of xylanase (XLS), amylase (AMS) and protease (PRT) on broiler performance, intestinal viscosity and organoleptic quality of meat. A total of 3000 1-d-old Ross 508 broiler chickens were reared. Birds were sexed and equal number of females and males were reared in each experimental unit. Eight experimental diets were arranged 4×2 factorially, the factors being dietary pea inclusion (0, 150, 300 and 450 g/kg) and the supplementation of combination of XLS, AMS and PRT (0.1 % in the diet). Pea inclusion had no effect ($P > 0.05$) on body weight, body weight gain and feed consumption. FCR of the birds fed XLS, AMS and PRT supplemented diets increased ($P \leq 0.042$) along pea inclusion. Pea inclusion improved ($P \leq 0.038$) the tenderness and juiciness of breast meat for the XLS, AMS and PRT supplemented diets. The use of XLS, AMS and PRT improved the nutrition value of pea diets as evidenced from the improvement in body weight especially for the birds fed control diet ($P < 0.001$) and diet included peas up to 150 g/kg ($P = 0.005$). The supplementation of XLS, AMS and PRT improved FCR and decreased ($P = 0.040$) feed consumption of the birds fed control diet ($P = 0.044$). These results suggest that XLS, AMS and PRT improved more the nutritional value of wheat than that of pea. In conclusion, 450 g/kg semi-leafless peas studied can be used in the broiler grower diets without negative effects on the bird performance. The use of combination of XLS, AMS and PRT improve bird performance.

Keywords: amylase, broiler, pea, protease, xylanase

Introduction

There is a strong interest in maximizing the use of locally produced vegetable protein sources, like peas (*Pisum sativum* L.), as a substitute for imported SBM. The different combinations of enzymes have been studied to improve nutritive value of peas (BRENES et al., 1993; IGBASAN & GUENTER 1996; COWIESON et al., 2003). However, there is a lack of studies where benefits of the addition of the combination xylanase (XLS), amylase (AMS) and protease (PRT) to wheat-pea based diets would have been evaluated. The aim of this study was to study the effects of dietary pea inclusion and the supplementation of combination of XLS, AMS and PRT on broiler performance, intestinal viscosity and organoleptic quality of the meat. A further aim of this study was to find an optimal inclusion level of peas (cv. Karita) in diets for broilers.

Materials and Methods

A total of 3000 1-d-old Ross 508 broiler chickens were reared in 48 floor pens with equal number of females and males. Eight wheat-SBM based experimental diets were arranged 4×2 factorially, the factors being dietary pea (cv. Karita) inclusion (0, 150, 300 and 450 g/kg) and the supplementation of combination of XLS, AMS and PRT (0.1 % in the diet). The exogenous enzyme combination used was Avizyme 1200 -enzyme (Danisco Animal Nutrition, Marlborough, UK). After slaughtering one breast meat of one bird per replicate was removed and frozen for organoleptic quality evaluation. The ileal digesta of one bird per replicate was collected from the distal part of ileum for viscosity determination. The experimental data was subjected to ANOVA using the GLM procedure of SAS (SAS Institute Inc., Cary, NC, USA). The treatment effects were separated into six orthogonal contrasts. In the current study, $P \leq 0.05$ was considered to be significant.

Results and Discussion

Dietary pea inclusion up to 450 g/kg had no effect ($P > 0.05$) on body weight, carcass weight, body weight gain or feed consumption. Bird performance was comparable to that presented in earlier studies, where pea inclusion ranged from 100 to 480 g/kg (IGBASAN & GUENTER 1996; LAUDADIO & TUFARELLI 2010; DOTAS et al., 2014). Pea inclusion impaired ($P \leq 0.042$) the FCR of birds on XLS, AMS and PRT supplemented diets in line with the results by COWIESON et al., (2003) (Table 1). The addition of XLS, AMS and PRT increased final body weight ($P < 0.041$) of the birds on control diet and diets with 150 g/kg and 450 g/kg of peas. Body weight gain ($P < 0.040$) of the birds on control diet and diets with 150 g/kg and 450 g/kg peas and FCR of the birds on control diet and diets with 150 and 300 g/kg peas improved ($P \leq 0.040$) by the supplementation of XLS, AMS and PRT. The addition of XLS, AMS and PRT only decreased ($P = 0.044$) control diet consumption. The beneficial effects of XLS, AMS and PRT combination on bird performance were most likely a result of the effects of the enzymes on backbone substituent (arabinoxylans) in wheat. The intestinal viscosity of the birds (age 20 day) decreased ($P \leq 0.021$) by the addition of XLS, AMS and PRT and for birds on unsupplemented diets along pea inclusion. Our results in the unchanged flavour of breast meat agree with the results of MC NEILL et al., (2004). Pea inclusion improved ($P < 0.038$) tenderness and juiciness of breast meat of the birds on s XLS, AMS and PRT supplemented diets.

Conclusions

When diets are balanced with regards to their amino acid content 450 g/kg semi-leafless peas can be used in broiler grower diets based on wheat and SBM. The use of enzyme combination of XLS, AMS and PRT enhanced the nutritive value of wheat-pea diets. The beneficial effects of this were however more likely a result of the effects of the enzymes on backbone substituent in wheat. The dietary pea inclusion improves the tenderness and juiciness of breast meat, but only for the XLS, AMS and PRT supplemented diets.

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Table 1. The effects of dietary pea inclusion level and enzyme supplementation on bird performance, ileal viscosity and the organoleptic quality of breast meat.

Treatment	1	2	3	4	5	6	7	8							
Pea inclusion,	0	150	300	450	0	150	300	450	SEM	P_l	P_l	E_{contol}	E_{15}	E_{30}	E_{45}
Avizyme 1200	-	-	-	-	+	+	+	+		-	+				
Body weight, g															
d 9	238	239	240	238	238	243	239	244	3.0	0.858	0.406	0.951	0.254	0.842	0.183
d 37	2263	2288	2267	2265	2313	2352	2300	2305	13.6	0.684	0.130	0.014	0.002	0.097	0.041
Body weight gain, g															
from d 1 to d 9	198	198	200	197	197	203	199	203	2.9	0.946	0.368	0.898	0.297	0.879	0.164
from d 10 to d 37	2025	2049	2027	2026	2075	2108	2060	2061	13.8	0.659	0.095	0.015	0.004	0.093	0.080
from d 1 to d 37	2223	2247	2226	2224	2272	2311	2259	2265	13.6	0.665	0.131	0.014	0.002	0.094	0.040
Feed consumption,															
from d 1 to d 9	25	25	25	25	25	25	25	25	0.3	0.677	0.340	0.116	0.847	0.468	0.833
from d 10 to d 37	131	129	129	130	128	130	128	131	1.0	0.434	0.370	0.057	0.458	0.295	0.405
from d 1 to d 37	104	103	103	103	102	103	102	104	0.8	0.516	0.305	0.044	0.783	0.288	0.625
FCR, g of feed/g gr															
from d 1 to d 9	1.15	1.14	1.14	1.13	1.13	1.12	1.13	1.12	0.013	0.427	0.831	0.205	0.260	0.657	0.334
from d 10 to d 37	1.85	1.79	1.82	1.83	1.77	1.75	1.77	1.82	0.011	0.567	0.002	<0.001	0.009	0.003	0.436
from d 1 to d 37	1.79	1.73	1.76	1.77	1.71	1.69	1.71	1.75	0.009	0.490	0.003	<0.001	0.005	0.003	0.309
Ileal viscosity[2], cPs	10.2	7.3	7.2	6.8	3.5	3.9	3.5	3.3	0.90	0.021	0.749	<0.001	0.011	0.006	0.008
Ileal viscosity[3], cPs	6.6	6.6	5.9	5.7	5.0	4.5	4.4	4.5	0.47	0.090	0.461	0.017	0.004	0.036	0.073
Tenderness	4.8	4.8	4.8	4.8	4.1	4.9	4.5	5.3	0.25	0.932	0.016	0.051	0.635	0.344	0.159
Juiciness	4.5	4.5	4.5	4.7	4.2	4.6	4.5	4.8	0.17	0.532	0.038	0.166	0.889	0.889	0.779
Flavour	4.6	4.8	4.7	4.7	4.3	4.9	4.4	4.7	0.19	1.000	0.614	0.262	0.802	0.262	0.900

P_l = the linear effect of pea inclusion

$E_{control}$ = the effect enzyme supplementation on control diet (treatment 1 vs. treatment 5)

E_{15} = the effect enzyme supplementation on diet containing peas 150 g/kg (treatment 2 vs. treatment 6)

E_{30} = the effect enzyme supplementation on diet containing peas up to 300 g/kg (treatment 3 vs. treatment 7)

E_{45} = the effect enzyme supplementation on diet containing peas up to 450 g/kg (treatment 4 vs. treatment 8)

[1] entire experiment

[2] at 20 d of age

[3] at 30 d of age

P-149 (ID 218)

NATURAL STABILIZATION OF RICE BRAN AND THE EFFECT ON BROILER PERFORMANCE AND CARCASS YIELD

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Abstract

Rice bran represents eight percent of the raw rice grain processing, and contains a considerable nutrient profile for broiler. However, due to natural enzymes present at the external part of the rice grain and consequently concentrated in the bran portion when rice is submitted to polishing, results is a very unstable feedstuff, with fast rancidity generation during storage, subsequent oxidation and consequently negative effect on poultry performance. Alternatives for rice bran stabilization are of interest, especially natural alternatives. In this sense, 1000 ppm soybean tocopherols, 1000 ppm neem extract (*Azadirachta indica*) and 250 ppm citric acid were evaluated straight or in combination (T1 – Control, T2 - Neem; T3 – Citric Acid; T4 - Neem + Citric Acid; T5 – Tocopherols; T6 – Tocopherols + Neem; T7 – Tocopherols + Citric Acid; T8 – Tocopherols + Neem + Citric Acid), for rancidity or oxidation prevention, during 90 days storage period. Subsequently, those rice brans were tested in broiler diets to measure the effect of additives/stabilization programs on performance and carcass yield. Experimental diets contained 6.5% rice bran, fed from one to 28 d of age. Feed intake, weight gain and feed conversion ratio were not affected by the different rice bran stabilization programs. At 28 d, two birds per replicate were slaughtered and breast, leg and liver weight measured. In terms of yields, no difference between treatments was observed.

Keywords: Bird, Nutrition, alternative feedstuff, oxidation, feed efficiency

Introduction

Rice bran is a by-product of rice milling and features nutrients such as oil, including essential fatty acids, protein, fiber, vitamins, and antioxidants, such as g-oryzanol. However, in Brazil this by-product has a low commercial value, enabling its use mainly as an ingredient in animal feed, but because of the highly unstable nature during storage, and high in lipids, results in its rancidity, thus requiring some care. The evaluation of natural antioxidants for rice bran stabilization is scarce, therefore, the aim of this study was to evaluate the effect of different natural antioxidant additives in stored rice bran and later incorporated into the chicken diet cut to evaluate the performance and carcass yield.

Material and Methods

Place of execution: The research was performed at Embrapa Swine and Poultry, Concórdia, Santa Catarina State, Brazil. The rice bran (FAI) was purchased directly from the rice mill and immediately transferred to Embrapa R&D Feed Plant Facility for preservative program implementation.

Rice bran treatments and storage: To avoid the rancidity or oxidation during storage, FAI was treated with 1000 ppm soybean tocopherols, 1000 ppm neem extract (*Azadirachta indica*) and 250 ppm citric acid were evaluated straight or in combination, like demonstration on Table 1 (T1 – Control, T2 – Neem; T3 – Citric Acid; T4 - Neem + Citric Acid; T5 – Tocopherols; T6 – Tocopherols + Neem; T7 – Tocopherols + Citric Acid; T8 – Tocopherols + Neem + Citric Acid). All application were done in a typical feed mixer, were liquid additives were sprayed using a membrane pump and a spray nozzle. The mixing time after ending application was 10 minutes, as SOP description for this machine. All brans were stored in raffia bags of 15 kg. All treatments and replicates were kept under same condition, for 90 days. The climate conditions during the storage period were, maximum and minimum, temperature of 32 and 5Cº and relative humidity 95 to 35%, respectively, and an average rainfall of 215mm / month.

Table 1. Description of the treatment for rancidity prevention applied to rice bran (FAI)

	Natural additive sources (ppm)		
Identification	Neem (N)	Citric acid (CA)	Tocopherols (T)
FAI Control	0	0	0
FAI N	1000	0	0
FAI CA	0	250	0
FAI CA+N	1000	250	0
FAI T	0	0	1000
FAI T+N	1000	0	1000
FAI T+CA	0	250	1000
FAI T+CA+N	1000	250	1000

Feed Production: Diet was produced according Table 2. All feed were produced in mash form. The nutritional profile followed the Brazilian standards for broiler (Rostagno et al., 2011). The experimental FAI was included at a 6.5% level, as recommended by same author.

Table 2. Experimental diet composition fed from one to 28 days of age, for broiler male, Cobb 500, containing rice bran (FAI) stored for 90 days, under distinct preservation treatments using natural additives (Neem - N, citric acid - CA e tocopherols - T).

Ingredient	T1	T2	T3	T4	T5	T6	T7	T8
Corn (7.5%)	50.55	50.55	50.55	50.55	50.55	50.55	50.55	50.55
Soybean Meal (45%)	36.07	36.07	36.07	36.07	36.07	36.07	36.07	36.07
FAI Control	6.50	0	0	0	0	0	0	0
FAI N	0	6.50	0	0	0	0	0	0
FAI CA	0	0	6.50	0	0	0	0	0
FAI CA+N	0	0	0	6.50	0	0	0	0
FAI T	0	0	0	0	6.50	0	0	0
FAI T+N	0	0	0	0	0	6.50	0	0
FAI T+CA	0	0	0	0	0	0	6.50	0
FAI T+CA+N	0	0	0	0	0	0	0	6.50
Soybean oil	2.92	2.92	2.92	2.92	2.92	2.92	2.92	2.92
Kaolin (Filler)	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Dical-Phosphate[1]	1.38	1.38	1.38	1.38	1.38	1.38	1.38	1.38
Limestone[2]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Common Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
DL-methionine	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27
L-lysine	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Vitamin Premix[3]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Mineral Premix[4]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Toxin binder	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
TOTAL	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
AMEn (kcal/kg)	2950	2950	2950	2950	2950	2950	2950	2950
Crude Protein (%)	21.05	21.05	21.05	21.05	21.05	21.05	21.05	21.05
Calcium (%)	0.820	0.820	0.820	0.820	0.820	0.820	0.820	0.820
P available (%)	0.390	0.390	0.390	0.390	0.390	0.390	0.390	0.390
P total (%)	0.720	0.720	0.720	0.720	0.720	0.720	0.720	0.720
Sodium (%)	0.210	0.210	0.210	0.210	0.210	0.210	0.210	0.210

FAI – rice bran as presented at Table 1; [1]Calcium: minimum 210g/kg, maximum 250g/kg; Phosphorus: 180g/kg; [2]Calcium: minimum 33%; [3]Content per kg of product: Vitamin A: 9000000.000 UI, Vitamin D3: 2500000.00 UI, Vitamin E: 20000.00 UI, Vitamin K3: 2500.00mg, Vitamin B1: 1500.00mg, Vitamin B2: 6000.00mg, Vitamin B6: 3000.00mg, Vitamin B12: 12000.00mcg, Pantothenic Acid: 12g, Niacin: 25g, Folic Acid: 800.00mg, Biotin: 60.00mg, Selenium: 250.00mg. [4]Specification per kg of product: Cupper: 20g, Iron: 100g, Manganese: 160g, Cobalt: 2000.00mg, Iodine: 2000.00mg, Zinc: 100g.

In vivo trial: With the aim to evaluate the FAI nutritional quality after 90 d storage period under different preservation strategies, a broiler performance trial was performed, using 960 male Cobb500 birds in vertical metallic cages, equipped with a feeder and nipple drinkers, up to 28 days age. The birds were allocated in a randomized block design with eight treatments and 10 replicates of 12 birds each. Diets previously described were delivered ad libitum. At 7, 14, 21 and 28 days of age the feed intake, body weight, body weight gain, and feed conversion was determined. At 28 days, two birds, selected based on the mean body weight of the replicate, were selected, identified, and submitted to carcass part yield. All parameters were submitted to analysis of variation and means were compared throughout t-Student Test at a 5% of significance level.

Results and conclusion

Feed intake, weight gain and feed conversion ratio were not affected by the different rice bran stabilization programs. In terms of carcass part yields, no difference between treatments was observed (Table 3).

Table 3. Broiler performance and carcass part yields, male, Cobb500, fed with diets containing rice bran (FAI) stored for 90 days and preserved with distinct natural additives (Neem – N, citric acid – CA and Tocopherols – T) (mean ± standard error).

Treatments	FI (g)	WG (g)	FCR (g:g)	BW (g)	BY (%)	LiY (%)	LeY (%)
FAI Control	2066±16.7	1434±22.0	1.450±0.010	1415±28.7	22.8± 0.32	2.100±0.055	12.8± 0.12
FAI N	2062±15.6	1439±10.4	1.434±0.007	1458±30.5	23.6± 0.29	1.987±0.046	13.0± 0.13
FAI CA	2047± 9.1	1426±19.9	1.436±0.016	1432±22.8	23.1± 0.41	1.977±0.031	13.0± 0.19
FAI CA+N	2077±18.9	1424± 8.6	1.460±0.015	1438±28.1	22.5± 0.29	1.965±0.039	13.1± 0.16
FAI T	2057±20.3	1437±24.0	1.431±0.015	1468±34.4	22.7± 0.31	1.984±0.040	13.0± 0.16
FAI T+N	2034±17.3	1436±17.6	1.416±0.017	1507±26.8	23.0± 0.61	1.987±0.061	12.9± 0.15
FAI T+CA	2058±15.6	1418±17.9	1.451±0.015	1467±51.9	23.3± 0.34	1.970±0.042	13.0± 0.15
FAI T+CA+N	2011±24.1	1413±18.8	1.429±0.012	1431±26.1	22.7± 0.36	2.018±0.045	12.9± 0.13
P-value	0.226	0.916	0.100	0.428	0.336	0.463	0.751
CV (%)	2.49	3.44	2.70	6.22	4.68	6.45	3.18

FI – Feed intake; WG – weight gain; FCR – feed conversion ratio; BW – body weight; BY – breast yield; LiY – liver yield; LeY – leg yield.

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P-152 (ID 141)

EFFECT OF ONION AND GRAPE SEED EXTRACTS ON MEAT QUALITY AND ANTIOXIDANT STATUS IN BROILER CHICKENS

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Summary

A 34-day experiment was conducted on 80 day-old female broiler chickens (4 treatments, 10 replication, Ross 308) to determine the effect of vitamin E and natural polyphenols (onion and grape seed extracts, Proviox) on meat quality and antioxidant status. The birds received basal diets (starter, grower) without added vitamin E (a) or diets supplemented with vitamin E at 50/25 (b), 100/50 (c), 50/25 mg/kg and Proviox, 50/25 mg/kg (d). The following criteria were monitored: performance, carcass, meat quality and blood indicators (GPx, SOD, TAS and *α*-tocopherol). There were no differences in performance, dressing percentage, breast meat yield and chemical composition of meat. The experimental diets had no effect on the meat quality, with the exception of colour. The *b** value (yellowness) of meat significantly increased in the group with polyphenol supplementation ($P \leq 0.05$). As the supplemental vitamin E level increased, the serum *α*-tocopherol concentrations and SOD activity increased ($P \leq 0.001$). The chickens that received diets with the polyphenol supplement (d) were characterized by similar (SOD) or better (TAS, GPx) antioxidant status compared to the group fed diets only with the addition of vitamin E (b). The antioxidant status (TAS, GPx) was similar in groups c and d.

Keywords: polyphenols, vitamin E, meat quality, antioxidant status, broiler chickens

Introduction

Oxidative stress can cause a number of diseases, including cancer and degenerative diseases, since free radicals damage cells, and react with DNA, RNA, proteins and lipids (Lykkesfeldt and Svendsen, 2007; Voljč et al., 2011; Yin et al., 2013). The functions of the immune system become impaired. Endotoxins produced as a result of oxidative stress cause liver dysfunctions and defects in meat. Excessive oxidative stress is also a reason for problems related to reproduction and inferior productivity (Fellenberg and Speisky, 2006; Durand et al., 2013; Gobert et al., 2013). The above-mentioned phenomenon results from impaired mechanisms regulating the production of free radicals (diseases, environmental pollution and stress). Another oxidative stress inducing factor may involve nutritional factors such as unbalanced feed rations, poor quality feed fat, large amounts of polyunsaturated fatty acids, feed contaminated with mycotoxins, heavy metals, deficiency of natural antioxidants (vitamin E and C). The results of the study indicate that diets rich in natural antioxidants: vitamin A, C and E, carotenoids and polyphenols, can significantly affect the growth of reactive antioxidant status in the body, and thereby reduce the risk of certain diseases and favourably influence the performance. An interesting scientific problem is a possibility of the partial supplementation of vitamin E with preparations containing plant polyphenols, which can support (or "save") antioxidative functions of vitamin E (Surai 2014). Such opportunities have been confirmed in studies on pigs and poultry (Brenes et al., 2008; Frankič et al., 2011). The aim of the study was to determine the effect of natural polyphenols (Proviox) on the performance, carcass and meat quality, and on antioxidant and vitamin E status in broiler chickens.

Material and Methods

A 34-day experiment was conducted on 80 day-old female broiler chickens (4 treatments, 10 replication, Ross 308) to determine the effect of vitamin E and natural polyphenols (onion and grape seed extracts, Proviox) on meat quality and antioxidant status. The birds received basal diets (starter, grower) without added vitamin E (a) or diets supplemented with vitamin E at 50/25 (b), 100/50 (c), 50/25 mg/kg and Proviox, 50/25 mg/kg (d). Water and feed were provided *ad libitum*. Broilers were fed commercial starter and grower diets. The following criteria were monitored: performance (BW, FCR, European Broiler Index - EBI), carcass (carcass dressing percentage, breast muscle), meat quality (chemical composition, pH_1, pH_{24}, colour measurement (L, a, b), water holding capacity, drip loss) and blood indicators (glutathione peroxidase-GPx, superoxide dismutase-SOD activity, total antioxidant status-TAS and *α*-tocopherol concentrations). The analysis of chickens for fattening after slaughter was conducted on the 34th day of their lives. In total, 80 broilers (females) were slaughtered. Their gizzards, hearts and livers were dissected and the weight of each was de-

termined. Samples weighing 25 g were collected from the breast muscles. The composition was determined in the sample using the standard methods (AOAC 2005). Measurements of pH_{15} and a 24h post-mortem of the breast meat were made (using Hanna Instruments 8314). Meat analyses were carried out using a colorimeter (Konica Minolta Chroma Meter), based on the CIELab colour system in which three parameters are determined: a* - redness; b* - yellowness, L* - lightness. Drip loss was determined using the suspension method according to Prangle et al. (1998). Water-holding capacity was determined according to Grau and Hamm (1952). To determine oxidative status, blood samples were collected from the broiler chickens in tubes with a lithium anticoagulant, and whole blood was used for the analysis. The content of TAS was determined using the Antioxidant assay kit (Cat. No CS0790), the activity of SOD was determined using SOD determination kit 19160, and GPx activity used assay Cellular Activity Kit Cat. No CGP1. The level of *α*-tocopherol was determined by reversed-phase high-performance liquid chromatography (HPLC) with a SHIMADZU chromatograph. The results were processed statistically by one-way ANOVA and Duncan's test. Arithmetic means (*x*), standard errors of the mean (SEM) and significance level (P) were determined. All calculations were performed using STATISTICA 10 software.

Results

The inclusion of vitamin E and Proviox in the experimental diets increased body weight. On day 34 (the end of fattening), the group D birds (vitamin E+Proviox; 1,785 g) were characterized by the highest body weight. The noted difference reached approximately 120 g when compared to the control group of birds (1,662 g), but it was not statistically significant. The feed conversion ratio was not affected by the inclusion of vitamin E and Proviox. All birds remained in good health throughout the trial. There were no differences in dressing percentage, breast meat yield or the chemical composition of the meat. Acidity (pH), water-holding and drip loss were measured in muscle samples as parameters of meat quality. In comparison with the control group, no significant differences were evident in the chickens fed vitamin E-enriched diets. The experimental diets had no effect on meat quality, with the exception of colour. The b* value (yellowness) of meat significantly increased in the group with polyphenol supplementation ($P \leq 0.05$). Serum *α*-tocopherol concentrations were higher ($P \leq 0.001$) when vitamin E was added to the diets (Table 1). The addition of various levels of vitamin E and Proviox modified the TAS. For TAS, there were differences between the control (no vitamin E) and experimental groups. TAS serum increased more in chickens fed vitamin E with Proviox than in chickens fed only vitamin E. SOD serum (superoxide dismutase) values ranged from 22.59 to 72.47 enzyme units (U/ml). The highest SOD activities were observed in chickens fed a high level of vitamin E. GPx activity in the blood from chickens in the control group was significantly lower ($P \leq 0.001$) than in chickens receiving vitamin E. The highest GPx activities were observed in chickens fed vitamin E and Proviox and in those fed a higher level of vitamin E ($P \leq 0.001$).

Table 1. Effect of dietary vitamin E level and polyphenols supplementation on *α*-tocopherol (mg/ml serum) and TAS (mmol/L serum) concentrations, the activity of SOD (U/ml of serum) and GPx (U/ml of blood) in the serum and in blood broiler chickens at 34 days

Specification	Groups				SEM	P
	K-	Vit. E 50/25	Vit. E 100/50	Vit. E 50/25 Proviox 50/25		
α-tocopherol	0.32^{C}	1.10^{B}	1.33^{A}	1.05^{B}	0.064	<0.001
TAS	0.26^{B}	0.28^{B}	0.34^{A}	0.33^{A}	0.007	<0.001
SOD	22.59^{C}	43.73^{B}	72.47^{A}	48.10^{B}	2.980	<0.001
GPx	30.51^{C}	40.25^{B}	68.87^{A}	69.20^{A}	2.863	<0.001

A, B - $P \leq 0.01$

Conclusions

Based on the results, it can be concluded that the chickens that received diets with the polyphenol supplement (Proviox) were characterized by an antioxidant status similar (SOD) or superior (TAS, GPx) in comparison to the group fed with diets with moderate level of vitamin E. The antioxidant status (TAS, GPx) was similar for groups fed with diets with high level of vitamin E and with vitamin E and polyphenols supplementation. The experimental diets had no effect on the performance and meat quality, with the exception of colour.

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P-153 (ID 190)

EFFECT OF TYPE OF CEREAL AND OAT HULLS ADDITION ON GASTROINTESTINAL MORPHOLOGY IN BROILERS ORALLY INFECTED WITH *CAMPYLOBACTER JEJUNI*

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Abstract

An experiment was conducted within the EU-FP7 project CAMPYBRO for evaluating the effect of type of cereal and oat hulls (OH) addition on gastrointestinal (GIT) morphology in broilers. There were five treatments: corn [C], wheat [W] or barley [B], and the inclusion of 5% of OH in C and W diets. A total of 180 one-day-old Ross 308 broilers were divided into cages (3 birds/cage) and experimental treatments (12 cages/treatment). At 14 d, all broilers were orally gavaged with 100 µl of a solution containing 1x10^5 cfu/ml of ST-45 *C. jejuni*. *Campylobacter* effect on GIT morphology could not be analysed since all birds were infected. On days 35 and 42, the performance parameters and the weight of GIT, empty BW (EBW), proventriculus (PRO), gizzard (GIZ), and the pH at PRO and GIZ were taken. Data were analysed by GLM procedure of SPSS (v.19.0). From 0-42d birds fed B ate more feed than birds fed C-OH. Age of animals significantly affected all the GIT parameters studied decreasing their proportion in relation to BW, GIT or EBW. Birds fed B showed the biggest GIT as %BW, and OH increased the GIT weight in C and W diets and the relative importance of the GIZ, but decreased it in the PRO. Dietary treatment did not affect the pH at GIZ, but OH increased the pH at the PRO, especially in W diet. It is concluded that type of diet modifies the GIT morphology.

Keywords: *Campylobacter jejuni*, cereals, oat hulls, broiler, gastrointestinal tract

Introduction

To date, several research have investigated beneficial effects of adding oat hulls (OH), or other types or fibrous fractions into the diet of broiler chickens (HETLAND et al., 2003). Moderate amounts of some fiber sources dilutes the diet but might improve the motility and function of the gastrointestinal tract (GIT) and, eventually benefit digestive physiology without compromising growth in broiler chickens (MATEOS et al., 2012). The objective of this study was to evaluate the effect of different cereal sourced and oat hulls (OH) addition on gastrointestinal morphology in broilers orally infected with *Campylobacter jejuni*.

Material and Methods

A total of 180 Ross 308 broilers (50% male and 50% female) from 1 to 42 days of age were used and allocated to the experimental treatments. There were five treatments applied from 1 to 42 d of age: corn (C), wheat (W) or barley (B), and the inclusion of 5% of OH in C and W diets. The trial was carried out in an Animal Biosafety Level 2 Unit for broilers. Broilers were housed in cages in groups of three. Mash (non-pelleted) feeds were fed *ad libitum* with no added coccidiostat, growth promoter or veterinary antibiotics. At 14 d of age, all broilers were orally gavaged with 100 µl of a solution containing 1 x 10^5 CFU/ml of ST-45 *C. jejuni* strain. Performance was calculated by cage at 21 and 42 days. On days 35 and 42, twelve broilers per treatment were euthanized and the weight of the GIT, empty BW (EBW), proventriculus (PRO), gizzard (GIZ), and the pH at PRO and GIZ were taken. Data were analysed by GLM procedure (SPSS v.19.0). The model included the dietary treatment, the age and its interaction. A Tukey's test was used for mean comparison.

Results

There was no effect of dietary treatment on growth or feed conversion at any of the studied periods. From 0-21d, broilers fed wheat+OH showed higher feed intake that birds fed corn or wheat-based diets, showing the rest of treatments intermediate results. From 21-42d, broilers fed barley ate more feed than birds fed corn+OH and wheat+OH, showing the rest of treatments intermediate values. For the whole trial, broilers fed barley ate more feed than birds fed corn+OH (Table 1).

Table 1. Effect of dietary treatment on average daily gain (ADG, g), average daily feed intake (ADFI, g) and feed conversion ratio (FCR, g/g) from 0-21, 21-42 and 0-42 d of age

Treatment	0-21 d			21-42 d			0-42 d		
	ADG	ADFI	FCR	ADG	ADFI	FCR	ADG	ADFI	FCR
Barley	31.7	64.1^{ab}	2.038	78.1	147.9^{a}	1.921	54.9	106.2^{a}	1.949
Corn	32.4	56.9^{b}	1.815	74.2	137.9^{ab}	1.897	53.8	98.3^{ab}	1.837
Corn + 5% OH	32.2	64.3^{ab}	2.013	69.8	120.6^{c}	1.745	51.1	92.7^{b}	1.817
Wheat	31.5	57.8^{b}	1.849	73.9	135.5^{ab}	1.854	53.3	97.7^{ab}	1.844
Wheat + 5% OH	28.6	69.5^{a}	2.075	76.0	130.6^{bc}	1.731	52.0	100.5^{ab}	1.941
SEM (n=12)	1.192	1.445	0.091	1.920	2.062	0.049	0.988	1.447	0.033
P	0.519	**0.001**	0.509	0.339	**0.001**	0.244	0.408	**0.001**	0.193

SEM = Standard error of mean. Different superscripts in same column are significant (a-c: P ≤ 0.05).

Campylobacter effect on GIT morphology could not be analysed since all birds were infected. Age of animals significantly affected to all the parameters studied showing, in general, bigger size of organs at 42d than at 35d, but lower importance expressed as percentage of BW, GIT, or EBW (Table 2).

Barley-based diets showed the biggest GIT in weight and in %BW, and consequently the lowest EBW, significant compared to corn and wheat diets (Table 3). Unusually, OH inclusion in corn and wheat diets decreased the relative importance of the proventriculus, compared to the cereals alone (4.14^{ab}, 4.65^{a}, 3.96^{b}, 4.56^{a} and 3.91^{b} %BW for barley, corn, corn+OH, wheat and wheat+OH, respectively). As expected, OH inclusion in corn and wheat diets increased the relative importance of the gizzard compared to the cereals alone (2.87^{ab}, 2.67^{bc}, 3.14^{a}, 2.47^{c} and 2.89^{ab} %BW for barley, corn, corn+OH, wheat and wheat+OH, respectively). This was a clear effect of long size particles of fiber, which stimulate the gizzard function and muscle development. Dietary treatment did not affect the pH at gizzard but, unexpectedly, OH increased the pH at the proventriculus, especially in wheat-based diets (3.69^{c}, 3.72^{bc}, 4.16^{ab}, 3.70^{c} and 4.26^{a}, for barley, corn, corn+OH, wheat and wheat+OH, respectively).

Conclusions

Under our experimental conditions, it is concluded that cereal type and quantity of fibre modifies the GIT morphology.

Acknowledgements

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Table 2. Effect of age, type of cereal and oat hulls (OH) inclusion (mean values at 35 and 42 d) on gastrointertinal tract (GIT) morphology and pH

	BW, g	GIT		Empty BW		Proventriculus			
		g	%BW	g	%BW	g	%GIT	%BW	%EBW
Age, d									
35d	1713	166.1	9.69	1547.3	90.31	6.95	4.23	0.41	0.45
42d	2261	208.3	9.20	2052.4	90.80	8.73	4.26	0.39	0.43
SEM (n=60)	27.3	3.82	0.135	24.87	0.135	0.185	0.094	0.007	0.008
Treatment									
Barley	2060	210.7^{a}	10.26^{a}	1849.0	89.74^{b}	8.63^{a}	4.14^{ab}	0.42^{a}	0.47^{a}
Corn	1964	175.3^{b}	8.91^{b}	1788.7	91.09^{a}	8.17^{ab}	4.65^{a}	0.41^{ab}	0.45^{ab}
Corn + 5% OH	1938	182.6^{b}	9.45^{ab}	1755.5	90.55^{ab}	7.13^{b}	3.96^{b}	0.37^{b}	0.41^{b}
Wheat	2017	180.5^{b}	9.00^{b}	1836.8	91.00^{a}	8.04^{ab}	4.56^{a}	0.40^{ab}	0.44^{ab}
Wheat + 5% OH	1956	186.8^{b}	9.60^{ab}	1769.2	90.40^{ab}	7.25^{b}	3.91^{b}	0.37^{b}	0.41^{b}
SEM (n=24)	43.2	6.04	0.213	39.33	0.213	0.293	0.149	0.011	0.013
Probability									
Age	**<0.001**	**<0.001**	**0.012**	**<0.001**	**0.012**	**<0.001**	0.867	**0.051**	**0.038**
Treatment	0.256	**0.001**	**<0.001**	0.359	**<0.001**	**0.001**	**0.001**	**0.002**	**0.002**
Age x Treatment	0.817	0.469	0.692	0.876	0.692	0.481	0.833	0.517	0.502

	Gizzard				pH	
	g	%GIT	%BW	%EBW	Proventriculus	Gizzard
Age, d						
35d	50.45	30.44	2.95	3.27	3.90	3.36
42d	59.92	29.11	2.67	2.94	3.91	3.51
SEM (n=60)	1,203	0.458	0.054	0.062	0.072	0.063
Treatment						
Barley	57.92^{ab}	27.85^{b}	2.87^{ab}	3.20^{ab}	3.69^{c}	3.50
Corn	52.29^{bc}	29.89^{b}	2.67^{bc}	2.93^{bc}	3.72^{bc}	3.23
Corn + 5% OH	60.54^{a}	33.57^{a}	3.14^{a}	3.47^{a}	4.16^{ab}	3.32
Wheat	49.08^{c}	27.39^{b}	2.47^{c}	2.72^{c}	3.70^{c}	3.56
Wheat + 5% OH	56.08^{abc}	30.19^{b}	2.89^{ab}	3.20^{ab}	4.26^{a}	3.55
SEM (n=24)	1.902	0.723	0.085	0.098	0.114	0.100
Probability						
Age	**<0.001**	**0.043**	**<0.001**	**<0.001**	0.952	0.087
Treatment	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	*0.070*
Age x Treatment	0.505	0.066	0.405	0.455	0.373	0.858

SEM = Standard error of mean. Different superscripts in same column are significant (a-c: P ≤ 0.05).

P-157 (ID 142)

EVALUATION OF CRIMPED KERNEL MAIZE SILAGE SUPPLEMENTATION ON BROILER PRODUCTION

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Abstract

A feeding trial was carried out to study the effect of dietary inclusion of crimped kernel maize silage (CKMS) on broiler production and nutrient digestibility. The CKMS had a low pH (4.2) and contained high amounts of lactic acid bacteria (10^8 log cfu/g), and organic acids mainly lactic acid (212 mmol/kg) and acetic acid (96 mmol/kg). Four experimental diets were used: Wheat based feed (WBF), maize based feed (MBF), maize based feed supplemented with 15% CKMS (CKMS-15) and maize based feed supplemented with 30% CKMS (CKMS-30). The body weight at 37 days was highest in broilers fed MBF (2636g) and CKMS-15 (2683g) but lowest when CKMS-30 was fed (2497g). The low body weight in CKMS-30 might be explained by a lower apparent fat digestibility and increased feed particle size as indicated by a higher relative gizzard weight in this group. In general, the apparent digestibility of nutrients was lower when CKMS was fed. The feed dry matter intake was higher in all maize based groups compared to WBF. Further, the intake of water and the ratio of water:dry matter was lower in birds fed the three maize diets, and decreased further following addition of CKMS. Bird mortality decreased and litter quality improved when CKMS was fed, which was reflected by a lower foot pad lesion score in these two groups. With respect to production results and bird welfare, a dietary inclusion level of 15% CKMS showed promising results.

Keywords: poultry, nutrition, broiler, maize silage, fermented feed, production, welfare

Introduction

Maize is one of the most commonly used energy source in broiler feed throughout the world. In Denmark, maize is harvested late in November with an approximate water content of 40%. To provide storage stability and avoid costs related to artificial drying, kernel maize is crimped and ensiled (crimped kernel maize silage, CKMS). Similar to the fermentation of a compound feed (CANIBE AND JENSEN, 2012), CKMS is characterized by a low pH (pH 4) due to the presence of high concentration of lactic acid and acetic acid, and contains large numbers of lactic acid bacteria as a result of the fermentation process. All these factors, alone or in combination have been shown to reduce the colonization of enteropathogens, increase nutritive value of feed, and have positive influence on growth and performance. However, the fermentation of a compound feed has some disadvantages such as loss of synthetic amino acids and poor litter quality in poultry (ENGBERG et al., 2009). Therefore, the supplementation of a compound feed with separately fermented grains might be a better option. The aim of the present study was to investigate the influence of CKMS at inclusion levels of 15% and 30% on broiler performance and nutrient digestibility.

Material and Methods

A production trial was carried out with 736 Ross 308 male broiler chicks from 1-37 days. They were grouped into 4 treatments and were reared in pens (8 replicate pens) with free access of feed and water. The CKMS was prepared by ensiling the crimped kernel maize for approximately 8 weeks with addition of an organic acids (formic, propionic, and benzoic acids). Analysis of DM, pH and microbial counts were carried out on crimped maize before and after ensiling as described by ENGBERG et al. (2009). All groups received the same starter feed (1-7 days) containing 22.5% crude protein (CP) and 11.4 MJ metabolizable energy (ME) per kg. Four different grower diets were used in the period from 8 to 37 days. Group 1 received wheat based feed (WBF) with 59% wheat presenting the usual feeding regime in Denmark. Group 2 received a diet containing 58% maize (maize based feed, MBF). Group 3 and 4 received maize based feed supplemented with 15% CKMS (CKMS-15) and 30% CKMS (CKMS-30), respectively. The CKMS was replaced with maize on DM basis. Additionally, 15-30% and 10% whole wheat was added to WBF and all maize based diets, respectively. Whole wheat and CKMS were mixed with the pelleted compound feed before feeding. Gross energy, crude protein, fat, starch, sugar, ash, total non-starch polysaccharides (NSP), SCFA and lactic acid were determined (ENGBERG et al., 2009).

Body weight, feed-DM intake, feed conversion ratio (FCR), and water consumption of broilers were measured for each pen on day 1 and day 37. The litter DM content in each pen was analysed by freeze drying. Foot pads lesions of broilers (5 birds/pen) were scored on both feet at d 37. A digestibility study was carried out in metabolism cages from d 22-25. The DM, ash, fat, nitrogen and energy content in feed and excreta were analysed as stated above. Four birds from each pen were randomly killed by cervical dislocation on d 36 to measure the gizzard weight. Statistical analyses were performed using the GLM procedure of SAS® applying the following general model: $Y_i = \mu + \alpha_i + \varepsilon_i$, where Y_i was the observed dependent variable, μ was the overall mean, α_i was the effect of treatments and ε_i was the random error. Results are presented as least square means (LSMeans) and pooled standard error (SE). Probability values < 0.05 were considered as significant.

Results

The pH and DM of CKMS was 4.2 and 53.5%, respectively. In CKMS, lactic acid bacteria (LAB) counts increased from 7.5 to 8.2 log cfu/g, while coliform bacteria, lactose negative bacteria, yeast and mould decreased below the detection limit (<3.0 log cfu/g) from 6-7 log cfu/g before ensiling. CKMS contained 212 mmol/kg lactic acid and 96 mmol/kg acetic acid. As a result, higher concentartion of SCFA and lactic acid was determined in diets supplemented with CKMS (Table 1). The body weight of broilers in CKMS-15 was around 50g/bird and 100g/bird higher than birds receiving MBF and WBF, respectively (Table 2).

Table 1 Composition of the starter and grower experimental diets

	WBF	MBF	CKMS-15	CKMS-30
Dry matter (g/kg)	872.4	873.7	821.3	765.9
Per kg DM				
Gross energy (MJ)	19.04	18.65	18.51	18.56
Crude protein (N x 6,25)	221.3	220.6	214.4	217.5
Starch	443.2	475.0	484.9	477.3
Total sugar	49.3	42.7	43.1	42.0
Total NSP	113	108	108	107
Fat	70.4	50.6	48.1	48.4
Ash	53.8	53.6	54.0	53.9
Per kg as fed				
Short chain fatty acids				
Acetic acid (mmol/kg)	6.2	6.4	13.8	18.5
Propionic acid (mmol/kg)	0.0	0.0	3.2	6.8
Benzoic acid (mmol/kg)	0.0	0.0	0.4	1.1
Lactic acid (mmol/kg)	2.1	2.0	26.7	66.3

Feed intake and FCR increased with the supplementation of CKMS. Both water consumption and total water-DM ratio was significantly lower in all maize based diets and further decreased with the supplementation of CKMS (Table 2). Apparent digestibility of fat (P=0.003) was lower in both groups fed CKMS whereas no difference was observed with respect to the digestibility of starch, ME and nitrogen retention. The relative gizzard weight of broilers (g/kg body weight) was higher in CKMS-30 than that of the other groups (P=0.001). Litter moisture (P=0.012) was lower in CKMS-30 (34.6%) and CKMS-15 (38.7%) compared to WBF (45.4%) and MBF (41.2%). Likewise, foot pad score was significantly lower (P<0.001) in CKMS-15 (3.4) and CKMS-30 (3.1) as compared to WBF (44.4) and MBF (28.8). Mortality of broilers was significantly higher (P<0.001) in WBF (7.6%) and MBF (4.9%) than in CKMS-15 (1.1%), whereas no mortality was observed in the CKMS-30.

Table 2 Body weight, feed-DM intake, FCR, water consumption and total water-DM ratio of broilers on day 37

		Diets					
Item	Day	WBF[1]	MBF[2]	CKMS-15[3]	CKMS30[4]	SE	*P value*
Body weight (g)	37	2574[bc]	2636[ab]	2683[a]	2497[c]	36.82	0.008
Feed DM consumption (g)	37	3858[b]	4044[ab]	4349[a]	4296[a]	103.13	0.007
Feed conversion ratio	37	1.50[b]	1.53[b]	1.62[ab]	1.72[a]	0.05	0.005
Water consumption (ml)	37	6118[a]	5632[b]	5693[b]	5291[c]	93.59	<0.001
Total water-DM ratio (ml water/g feed DM) *	37	1.68[a]	1.49[b]	1.47[b]	1.44[b]	0.04	0.001

[a-c]LSMEANS within the same row with different superscripts differ significantly ($P < 0.05$); *Including drinking water and dietary water

Conclusions

The dietary inclusion of 15% CKMS in broilers feed resulted in increased body weight. However, broilers in CKMS-30 showed the lowest body weight which was associated with an increased feed particle size indicated by the higher gizzard weight and reduced fat digestibility in this group (data not shown). Lower intake of water and total water-DM ratio (Table 2) had contributed to an improved litter quality which was reflected by the lower frequency of foot pad lesions in broilers fed CKMS-15 and CKMS-30, indicating a positive attribute from animal welfare perspective. Soluble NSP has been associated with increased water intake (FRANCESCH AND BRUFAU, 2004), and therefore presence of high NSP in WBF compared to maize (Table 1) could be a possible explanation. Furthermore, combination of improved litter condition along with higher acidification of the upper digestive tract related to higher concentration of organic acids in feed (HERES et al., 2004) conataining CKMS-15 and CKMS-30 (Table 1) might have resulted lower mortality in broilers fed CKMS.

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P-158 (ID 126)

EFFECT OF HOP CONE SUPPLEMENTATION ON OXIDATIVE STRESS AND OXIDATIVE STABILITY OF FRESH AND STORED MEAT IN BROILERS

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Abstract

Broilers are frequently exposed to oxidative stress which can lead to reduced health status, performance and stability of meat. Consumer concerns about feed additives led to attempts to find natural bio-efficient antioxidants for animal nutrition. Hops (***Humulus lupulus*** L.), are rich in phenols with antioxidant activity (xanthohumol, isoxanthohumol). The study examined the effects of hop cone supplementation on oxidative stress ***in vivo*** and oxidative stability of meat in broilers. 84 one-day-old male broiler chickens were housed in six floor pens and divided into 3 groups of 28. All groups received a high-PUFA diet unsupplemented or supplemented as follows: no supplementation (C), 0.9 g hops/kg (H1) and 3.6 g hops/kg (H2). Oxidative stress ***in vivo*** was studied by measuring the rate of lymphocyte DNA damage, malondialdehyde (MDA) concentration in plasma and breast muscle and by total plasma antioxidant capacity of lipid- and water-soluble compounds. Oxidative stability of meat was studied by measuring MDA in fresh and stored (refrigerated, frozen) breast meat. Both hop concentrations reduced the rate of blood lymphocyte DNA damage. Higher hop supplementation induced MDA formation and reduced plasma antioxidant activity of lipid-soluble compounds. In H2 group an increased MDA formation was observed also. An effect on other parameters of meat quality was not observed. Further studies are suggested to confirm the results and clarify the possible use of hops in broiler nutrition.

Keywords: hops, antioxidants, PUFA, oxidative stress, broilers

Introduction

In broilers, oxidative stress may occur as a consequence of nutrition such as high amount of dietary polyunsaturated fatty acids (PUFA), high environmental temperatures and also several pathological conditions. Lipid oxidation also has negative effects on meat quality and often determines the shelf life of meat and meat products. The high intake of PUFA is a very important factor that increases the requirement for antioxidants. Recent attempts to find novel bio-efficient antioxidants have focused particularly on natural antioxidants, in order to satisfy consumer concerns over the safety and toxicity of food additives. Hops (*Humulus lupulus* L.) are known as an excellent source of polyphenolic antioxidants including proanthocyanidins, flavanols, phenolic acids, stilbenes, flavonols and prenylated flavonoids. In addition, hop oil and hop acids have been reported as potent antioxidants (Yamaguchi et al., 2009). Much of attention has gone to the polyphenolic content of hops, and specific compounds, such as xanthohumol and 8-prenylnaringenin, have been identified as multipotent bioactive compounds. The objective of this study was to demonstrate the potential effectiveness of hops in terms of animal performance, antioxidant capacity *in vivo* and of oxidative stability of broiler meat under various storage conditions.

Material and Methods

84 one-day-old male Ross 308 broiler chickens were caged in wire-floored cages. The diets were formulated according to Ross 308 broiler nutrition specifications. All groups received a high-PUFA diet containing 7.5 % linseed oil, supplemented with: no supplementation (C), supplemented with 0.9 g of hops/kg (H1) and with 3.6 g of hops/kg (H2). Hops were provided from Slovenian institute for hop research and brewing. After 37 days of treatment, the birds were killed. Blood and breast muscle samples from each bird were collected for analysis. Lymphocytes were isolated from the fresh blood samples. The comet assay was performed according to the standard method with slight modifications as described by Rezar et al. (2003). The concentrations of MDA in blood plasma and meat samples were determined by HPLC. Meat was exposed to different treatments: stored at −70°C till analysis (raw meat), refrigerated for 6 days at 4°C and then frozen for 3 months at −20°C, and refrigerated at −20°C for 3 months. Plasma antioxidative capacity of lipid-soluble substances (ACL) and water-soluble substances (ACW) were determined by photochemiluminescence detection using the Photochem assay (Analytik Jena, Leipzig, Germany). The animal ethics committee of the Veterinary Administration of the Republic of Slovenia approved the study protocol. Data were analysed using the GLM procedure of the SAS/STAT module (SAS 8e, 2000; SAS Institute Inc., Cary, NC). Significance was considered established at $P < 0.05$.

Results

Oxidative stress can occur from providing large amounts of high-PUFA vegetable oils to reach the high energy requirements of poultry or when creating poultry products with improved nutritional quality (products rich in n-3 PUFA). The results from literature show, that bitter acids of hop cones and their analogues have antioxidant activity, potent DPPH radical scavenging activity and lipid peroxidation inhibitory activity (Tahashira at al.. 1995). Plasma malondialdehyde (MDA) was measured as biomarker for oxidative stress *in vivo* (Nielsen et al., 1997). In comparison with C group, the supplement of 3.6 g/kg of hops in the feed significantly increased MDA concentration in plasma, which indicates that the highest level of hops supplementation increased oxidative stress. Oxidative damage to DNA has also been identified as a useful index of oxidative stress. In Table 1, DNA damage in blood lymphocytes is presented as percentage of DNA in the head of the comet. Results of the comet assay indicate that supplementation of 3.6 g hops/kg of feed has protective effect on lymphocyte's DNA due to decreased DNA fragmentation.

Table 1. Concentration of MDA in plasma (nmol/ml), lymphocyte DNA damage (expressed as % DNA in the head of the comet) and antioxidative capacity of water (ACW) and lipid (ACL) soluble antioxidants of chicken's plasma in different experimental groups

Parameter/Group	C	H1	H2	*P- value*
MDA in plasma (nmol/ml)	1.00 [a]	2.06 [a]	13.29 [b]	*< 0.001*
% DNA in head	74.96 [a]	78.12 [ab]	84.12 [b]	*0.0063*
ACW (nmol AA* equiv/ml)	73.81	71.48	69.95	*0.877*
ACL (nmol trolox equiv/ml)	129.42 [a]	125.96 [a]	110.90 [b]	*0.0031*

LS-means (least square means) in the same row without the same superscript ([abc]) differ significantly, $P < 0.05$

*AA – ascorbic acid

A hop is known to have antioxidant properties, therefore we also measured antioxidant capacity of lipid (ACL) and water (ACW) soluble antioxidants in chicken plasma samples to assess oxidative stress. As shown in table 1, concentrations of ACW were similar between the groups, but ACL content were significantly lower in the group supplemented with 3.6 g hops/kg in comparison to the group C, which indicates that lipid soluble antioxidants were consumed as lipid oxidation progressed, which is in concordance with higher concentrations of plasma MDA.

Some previous studies showed that a diet rich in PUFA increased MDA levels in fresh and also in meat, stored under different conditions (Botsoglou et al., 2003). MDA concentration in raw meat was statistically higher in the group H2 in comparison the group C, as was in blood plasma. The MDA levels increased when breast meat was refrigerated for 6 days and then frozen for 3 months at - 20 ^{o}C, but we did not find statistical differences between the groups.

Table 2. Concentration of MDA (nmol/g) in fresh and in different storage samples of breast meat

Treatment/Group	C	H1	H2	*P- value*
Raw meat	17.64 [a]	21.88 [ab]	26.36 [b]	*0.0523*
Frozen for 3 months at -20°C	15.93	13.93	19.37	*0.5908*
Refrigerated for 6 days at 4°C than frozen for 3 months at -20°C	25.46	27.71	34.42	*0.3493*

LS-means (least square means) in the same row without the same superscript ([abc]) differ significantly, $P < 0.05$

Conclusions

The results of the present study demonstrate the inconsistent effects of hop supplementation in two different concentrations on oxidative status of broilers and on the quality of fresh and stored broiler meat. The results of the experiment show, that supplementation of hops in concentration of 3.6 g/kg of feed have a negative effect and increased lipid peroxidation in chickens, measured as MDA concentration in the plasma. At the same time, hops had a protective effect on DNA of lymphocytes, increasing percentage of un-fragmented DNA. The rate of lipid oxidation in fresh meat, measured as formation of secondary product of lipid oxidation (MDA) demonstrates the detrimental effect of hops supplementation. Further studies are needed to confirm the results of the present study.

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P-159 (ID 4)

EFFECT OF REPLACING SOY IN BROILER DIETS ON PERFORMANCE, CARCASS YIELD AND COST OF PRODUCTION

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Abstract

The study aimed to evaluate the effects of replacing soy bean meal (SBM) in broiler diets on production parameters. A randomized trial (36 d) was conducted using 20 pens (20 m^2, 280 Ross PM3 birds). A starter and grower diet (12.6/13.0 MJ ME, 210/200 g CP, 12.8/12.2 g lysine) were fed as crumbs. The diets contained either a high level of SBM (HS; starter/grower: 28.7/26.8% SBM and 2.5/2.5% rape seed meal (RSM)), a medium level of SBM (MS; starter/finisher: 15.0/15.0% SBM, 15.0/15.0% peas, 5.0/5.0% RSM, 5.0/5.0% sun flower meal (SFM), 2.8/2.0% potato protein (PP) or no SBM (NS; starter/grower: 25.0/25.0% peas, 5.0/5.0% RSM, 10.0/10.0% SFM, 5.3/4.5% PP, 4.0/4.0% corn gluten). Treatments were HS/HS, MS/MS, MS/NS, NS/NS for starter/finisher diets. Performance parameters, litter quality, hock burns (HB) and foot pad lesions (FPL) were recorded and analysed by using ANOVA and Tukey-Kramer.

Feed intake for HS/HS, MS/MS, MS/NS, NS/NS was 3438^{a} g, 3481^{ab} g, 3574^{b} g, 3580^{b} g (P, 0.003). Body weight (Ø 2234 g), FCR (Ø 1.57) and mortality (Ø 2.14%) did not differ between treatments. The ratio of water/feed intake was highest in HS/HS. This led to lower litter quality and significantly higher scores for FPL compared to the other treatments. Carcass yield (Ø 69.50%) and composition did not differ between treatments. Feed ingredient costs were 5% higher for NS/NS compared to HS/HS. Overall performance was comparable between treatments, however, SBM led to significantly higher occurrence of FPL.

Keywords: Soy, protein alternatives, broiler, performance

Introduction

Markets and politics demand a more sustainable food production system. In the sustainability discussion soy production and trade is often a controversial issue. Even though 80 - 90 % of soy imported into Switzerland fulfills high standards concerning the sustainability of production, the acceptance for soy imports is missing by part of the consumers. Furthermore, the price for GMO-free soy has risen over the last years.

The objective of the present study was to evaluate the effect of replacing soy bean meal (SBM) in broiler diets on performance, carcass quality and production cost.

Material and Methods

The trial involved four dietary treatments and 5600 as hatched Ross PM3 birds. The birds (280 per pen) were randomly assigned to 20 pens of 20 m^2 each. The trial lasted 36 days. Barn climate was based on the commercial standards for the breed. A starter diet (12.6 MJ ME, 210 g CP, 12.8 g Lysin, 9.0 g Ca und 6.5 g P) and a grower diet (13.0 MJ ME, 200 g CP, 12.2 g w, 7.5 g Ca und 5.5 g P) were fed as crumbs *ad libitum*. The diets contained either a high level of SBM (HS), a medium level of SBM (MS) or no SBM (NS) (Table 1). Treatments were HS/HS, MS/MS, MS/NS, NS/NS for starter/grower diets.

Table 1. Percentage of protein rich ingredients in the different diets (% in starter and grower)

Protein rich ingredients	HS	MS	OS
Soy, %	28.7/26.8	15.0/15.0	0/0
Peas, %	0/0	15.0/15.0	25.0/25.0
Rape cake, %	2.5/2.5	5.0/5.0	5.0/5.0
Sunflower meal, %	0/0	5.0/5.0	10.0/10.0
Potato protein, %	0/0	2.8/2.0	5.3/4.5
Corn gluten, %	0/0	0/0	4.0/4.0

Feed structure and composition were analyzed. Standard performance parameters, water intake, litter quality (% crusted surface), foot pad lesions and hock burns were recorded (0 = no change; 3 = strong change). In addition, carcass grading and composition were evaluated. Data were analyzed by ANOVA and means compared with the test of Tukey-Kramer.

Results

Feed composition was in agreement with calculated values and feed structure was comparable between treatments (data not shown).

Treatments with no SBM in the grower diet led to a significant higher feed intake compared to HS/HS. Body weight, FCR and mortality were not affected by treatments. EBI was between 363.4 and 377.2 (Table 2).

Table 2. Effect of protein rich ingredients on performance of ROSS PM3 birds

Parameters	HS/HS	MS/MS	MS/OS	OS/OS	SEM	P
Feed intake	3438[a]	3481[ab]	3574[b]	3580[b]	26.667	0.003
Body weight, g	2219	2214	2229	2274	33.243	0.571
FCR g/g	1.549	1.572	1.606	1.574	0.015	0.086
Mortality, %	2.07	2.07	2.07	2.36	0.202	0.890
EBI	374.4	368.4	363.4	377.2	8.672	0.683

The ratio of water/feed intake was highest in HS/HS (Table 3). This led to lower litter quality (92% of surface crusted in HS/HS pens) and significantly higher scores for FPL compared to the other treatments. HS/HS also showed the highest average score for HB (Table 3).

Table 3. Effect of protein rich ingredients on water to feed intake, foot pad lesions and hock burns of ROSS PM3 birds

Parameter	HS/HS	MS/MS	MS/OS	NS/OS	SEM	P
g water / g feed	1.935[a]	1.847[ab]	1.693[b]	1.790[ab]	0.049	0.023
Foot pad lesion*	0.418[a]	0.132[b]	0.128[b]	0.094[b]	0.052	0.002
Hock burn*	0.340[a]	0.110[b]	0.080[b]	0.140[ab]	0.052	0.011

*day 35 Score 0 – 3 (0 = no change, 3 = strong change)

All treatments achieved excellent carcass quality with over 97.5% grade 1. Average carcass yield was 69.50% and was not affected by treatment. Dietary protein source did not influence carcass composition (Table 4).

Table 4. Effect of protein rich ingredients on carcass composition of ROSS PM3 birds

Parameter	HS/HS	MS/MS	MS/OS	NS/OS	SEM	P
Breast, %	28.05	27.45	27.95	27.35	0.0032	0.352
Leg, %	30.50	30.15	29.95	30.60	0.0026	0.373
Wing, %	10.30	10.15	10.25	10.30	0.0010	0.878
Rest, %	31.15	32.00	31.90	31.65	0.0029	0.100

Conclusions

Diets with no SBM led to a significantly higher feed intake compared to diets with SBM. However, weight gain and FCR were not affected by diet composition. Overall performance of SBM-free diets was comparable to the standard commercial diet with SBM. Dietary composition did also not influence carcass composition. Carcass composition has been shown to change with suboptimal protein nutrition (SUMMERS ET AL. 1992; FARIA FILHO ET AL. 2005). The lack of difference indicates that all birds received adequate and comparable protein and amino acid supply.

SBM-free diets led to significantly lower water to feed intake. This could partially be explained by the lower potassium concentration in diets without SBM (VIEIRA & LIMA 2005; SMITH ET AL. 2000). The lower water intake led to healthier skin on the legs. Both foot pad lesions and hock burns were lower in diets with no SBM. This is in agreement with TUCKER and WALKER (1999) who also reported a link between litter quality and the occurrence of FPL and HB.

SBM could be replaced with little change in performance and a significant reduction in FPL and hock burns. However, feed ingredient cost were 5% higher for diets without SBM compared to diets with high inclusion of SBM. In the market,

not all alternative protein sources used in the trial (particularly potato protein) are available in sufficient amounts to switch a larger market segment to diets without SBM. Therefore, SBM-free diets could only play a role in niche markets where the extra cost can be passed on to the customer.

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P-161 (ID 257)

GLYCEROL-A GREAT POTENTIAL SOURCE OF ENERGY FOR LAYER

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Abstract

The aim of the study was to investigate the use of purified glycerol from biodiesel production as energy feed-stuff for layer. Purified glycerol is used for drug and cosmetic production. It is also suitable for food and animal feed. Purified glycerol contains 99%wt up for glycerol content, less than 0.01%wt of methanol, 3842-4118 kcal/kg of gross energy and 3,352 kcal/kg ME for poultry. Therefore, the purified glycerol has a high potential as energy feed stuff for domestic animals. The study on productive performance was conducted to determine the use of purified glycerol in layer diet. A total of 480, 24 week of age, Lohmann Brown were divided into 4 treatments including, corn-soy basal diet (CS), CS with 2.5% of glycerol, CS with 5.0% of glycerol and CS with 7.5% of glycerol, respectively. Each treatment consisted of 10 replications with 12 birds each. All diets were isocaloric and isonitrogenous (2,750 kcal/kg, 17.5%CP). Birds were raised in an evaporative system for 4 periods, 28 days per period. There was no significant difference in feed intake, hen house production, hen day production and egg weight in each period. The result revealed that purified glycerol can be used in layer diet at 7.5% without a negative effect on productive performance.

Keywords: Glycerol, gross energy, metabolizable energy, energy source

Introduction

Nowadays, the price of oil is dramatically increasing. Thailand has promoted the alternative energy, biodiesel, to replace using fossil fuel. The raw materials for biodiesel production are mainly palm oil, and palm wax. The other materials used are cooking oil, animal fat and plant oil. Its chemical structure is that of fatty acid alkyl ester (FAAE). Biodiesel as a fuel gives much lower toxic air emissions than fossil fuel. In addition, it gives cleaner burning and has less sulfur content, and thus reducing emission. Because of its origin from renewable resources, it is more likely that it competes with petroleum products in future (HAYYAN *et al*., 2010). Commercially, biodiesel is produced by transesterification of triglycerides which are the main ingredients of biological origin oils in the presence of an alcohol (e.g. methanol, ethanol) and a catalyst (e.g. alkali, acid) with glycerol or glycerine as a major by-product (MA and HANNA, 1999). In recent years, increasing of biodiesel production has been associated with increasing glycerol production. Biodiesel production process produces approximately 10% of crude glycerol (Anonymous, 2007). The crude glycerol can be purified into purified glycerol for 30-45%. This depends on the quality of the raw materials. Purified glycerol is commonly used for drug and cosmetic production. It is also suitable for food and animal feed. The aim of the study was to investigate the use of purified glycerol from biodiesel production as energy feed-stuff for layer.

Materials and Methods

A total of 480, 24 week of age, Lohmann Brown were divided into 4 treatments including, corn-soy basal diet (CS), CS with 2.5, 5.0% and 7.5% of glycerol, respectively. Each treatment consisted of 10 replications with 12 birds each. Each replication contain 4 cages (20 cm × 40 cm × 36 cm per cage) with 3 hens per cage at light at 16 h light per day. Feed in mash form and water were provided *ad libitum*. All diets were isocaloric and isonitrogenous (2,750 kcal/kg ME, 17.5%CP). Birds were raised in an evaporative system for 4 periods with 28 days per period. The ingredients and chemical composition of the diet are presented in Table 1. Laying hens were weighed individually at the beginning and at the end of the experiment. Mortality was recorded as it occurred. Eggs were collected daily and egg production was expressed on a hen-day and hen-house basis. All the eggs laid were collected and weighed to determine the egg weight. Feed intake was weekly recorded then calculated as g per hen per day and expressed in each period.

Table 1. Ingredients and chemical composition of the diets.

	Glycerol Supplementation			
	0	2.5%	5.0%	7.5%
Corn	57.67	54.82	51.97	49.14
Extracted rice bran	6.0	6.0	6.0	6.0
Soybean oil	1.05	0.95	0.85	0.75
SBM 49%	2.46	2.51	2.55	2.59
Glycerol	0	2.5	5.0	7.5
L-lysine	0.09	0.09	0.09	0.09
DL-methionine	0.145	0.145	0.145	0.145
Choline chloride 50%	0.007	0.007	0.007	0.007
Monodical P21	1.466	1.466	1.466	1.466
$CaCO_3$	8.36	8.36	8.36	8.36
Salt	0.36	0.36	0.36	0.36
Px	0.25	0.25	0.25	0.25
Chemical composition (analyzed)				
GE, kcal	4023.79	3976.99	3995.37	3980.73
Crude Protein,%	17.41	17.65	17.48	17.51
Calcium, %	3.15	3.32	3.26	3.17
Phosphorus, %	0.66	0.63	0.61	0.60

Results

During the experimental period % mortality was not affected by treatment. These data are consistent with the study of CERRATE *et al*.(2006). In this study hens fed diet with purified glycerol at 2.5, 5.0 and 7.5% had no significantly effected on feed intake, hen house production, hen day production and egg weight in each period (Table 2). These data are consistent with the study of YALCIN *et al*.(2010) who found that feeding glycerol at 2.5-- 7.5% did not significantly affect body weight, egg production, egg weight and feed efficiency of laying hens. Similarly, CERRATE et al. (2006) found that the inclusion of 2.5 or 5.0% glycerol had no significant adverse effects on feed conversion at any age when compared to control diet (0% glycerol). However, using 10% glycerol in diet caused a reduction the body weight gain.

Conclusion

The inclusion of refined glycerol from biodiesel production up to 7.5% in diets had no adverse effects on feed intake and egg production. Therefore, purified glycerol can be effectively used as an energy source in layer diet.

Acknowledgement

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Table 2. The effects of dietary supplementation of glycerol on feed intake and egg production.

	Glycerol Supplementation (%)				*P-value*	*Pooled SE*
	0	2.5	5.0	7.5		
Period 1						
FI	108.95	105.82	106.05	109.36	0.327	0.3681
Hen day production (%)	92.76	91.91	93.19	93.39	0.9001	0.2139
Hen house production (%)	92.56	91.91	93.19	93.39	0.8910	0.2853
Egg weight (g)	61.72	60.94	61.38	61.15	0.1287	0.1364
Period 2						
FI	112.72	113.63	112.19	114.98	0.6255	0.3545
Hen day production (%)	86.37	85.83	88.11	88.01	0.7234	0.2606
Hen house production (%)	83.45	84.23	83.16	86.76	0.7139	0.3378
Egg weight (g)	61.20	62.73	61.15	62.24	0.0813	0.4279
Period 3						
FI	115.32	115.48	117.08	118.60	0.6326	0.4001
Hen day production (%)	86.97	89.97	91.02	89.09	0.5221	0.3265
Hen house production (%)	83.60	87.11	86.58	87.74	0.8015	0.2150
Egg weight (g)	61.08	64.97	63.64	63.94	0.2303	0.2644
Period 4						
FI	104.03	104.61	103.07	103.73	0.9782	0.4520
Hen day production (%)	84.27	85.59	85.07	83.51	0.9175	0.3279
Hen house production (%)	83.96	81.34	77.47	83.17	0.4608	0.2554
Egg weight (g)	62.62	63.98	63.41	60.78	0.3585	0.2833

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P-162 (ID 201)

EFFECT OF DIFFERENT NSP-DEGRADING ENZYME PRODUCTS IN TWO DIET TYPES ON GUT PARAMETERS AND BROILER PERFORMANCE

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Abstract

The effects of five non-starch polysaccharide (NSP)-degrading enzyme products (differing in claimed activities but all containing xylanase as the main activity) against a control (no enzyme) were studied in two different diet types, one wheat/soya based diet (simple) and one diet with a more varied ingredient mix (complex), also including peas, distillers dried grains with solubles, sunflower meal and rapeseed. 2112 male Ross 308 day old broiler chicks were weighed and assigned to one of 8 replicates for each of the 12 treatments (22 per floor pen of 2.2 m^2). Diets were iso-nutrient, contained phytase and coccidiostats as standard but no growth promoters. As enzyme addition may affect caecal fermentation, at 25 days of age the weight and length of the small intestine and ceaca were measured. The weight of the full intestine relative to the weight of the full caeca was reduced in the presence of the single xylanase enzymes (P=0.03), suggesting improved caecal fermentation. Growth rate was not affected by enzyme addition. FCR (0-42 days) showed a significant (P<0.001) diet*enzyme interaction, with only one of the five enzyme products showing an improved FCR in both diet types. There appeared to be no benefit from using a multi-activity product compared to the use of a single xylanase product in either diet, demonstrating that the benefits of NSP-enzyme addition can be achieved from xylanase alone.

Keywords: xylanase, broiler, alternative ingredients

Introduction

NSP-degrading enzymes are commonly used in a variety of broiler diets across the world. Most of the commercially available products contain xylanase as a major activity, but it is often argued that a complex diet would require an enzyme complex to improve performance rather than just a xylanase. Understanding the mode of action by which NSP enzymes improve nutrient digestibility and performance may assist the debate on whether a single xylanase product works across diet types or whether a wide spectrum of activities is needed to achieve this. Reduction of intestinal viscosity has long been considered a main target for NSP enzymes, and this is relevant in high viscous diets. Xylanase also might increase digesta retention time through the breakdown products from arabinoxylans effecting changes in the caecal microbiome and subsequently inducing a hormonal effect on gastric retention time (Singh et al., 2012). This mechanism would not require an enzyme complex to achieve improved performance across a range of diets, but suggests a single xylanase could have the same effect. The current study was set up to compare five commercial NSP enzyme products (differing in claimed activities but all containing xylanase as the main activity) against a control (no enzyme) in two different diet types, one wheat/soya based diet (simple) and one diet with a more varied ingredient mix (complex), also including peas, distillers dried grains with solubles, sunflower meal and rapeseed.

Materials and Methods

2112 male Ross 308 day old broiler chicks were weighed and assigned to one of 8 replicates for each of the 12 treatments (22 per floor pen of 2.2 m^2). The birds were fed a starter diet from day 0-14, a grower diet from day 14-28 and a finisher diet from day 28-42. The two diet types were iso-nutrient, contained phytase and coccidiostats (Nicarbazin+Narasin in starter and Narasin in grower and finisher) as standard but no growth promoters. Basal diet composition is shown in table 1. The enzyme treatments were based on commercial products available in the European market, defined as follows: Control (no NSP enzyme); Xyl E (single xylanase product); Multi R (multi-enzyme product), Xyl H (single xylanase product), Xyl + ß-Glu A (blend of xylanase and ß-glucanase), Xyl W (single xylanase product). Each product was included at recommended levels for wheat based diets.

Birds and feed were weighed at each feed transition, on day 35 and at the end of the experiment at day 42. At 27 days of age two birds per pen were euthanized; weight and length of the small intestine and ceaca were measured and viscosity of jejunal digesta was measured.

Table 1: Basal diet composition (%)

	Starter (0-14 days)		Grower (14-28 days)		Finisher (28-42 days)	
	Simple	Complex	Simple	Complex	Simple	Complex
Wheat	59.2	48.1	64.0	50.8	67.6	54.5
Soybean meal	30.3	23.2	27.8	17.2	23.9	12.8
Maize DDGS	-	5.0	-	5.0	-	5.0
Peas	-	5.0	-	5.0	-	5.0
Fish meal	2.5	2.5	-	-	-	-
Sunflower meal	-	4.0	-	7.5	-	7.5
Rape seed	-	5.0	-	7.5	-	8
Soybean oil	1.1	1.0	1.0	1.0	1.8	1.0
Animal Fat	2.1	1.6	3.3	2.3	2.3	1.9
Palm oil	-	-	-	-	1.0	1.0
Monocalcium phosphate	0.59	0.48	0.39	0.26	0.22	0.08
Salt	0.19	0.15	0.22	0.15	0.19	0.09
Limestone	1.49	1.49	1.25	1.23	1.09	1.06
Sodium bicarbonate	0.03	-	-	-	0.06	0.11
Premix complex[1]	2.43	2.47	1.95	2.06	1.80	1.94
Calculated nutrients						
Crude Protein (g/kg)	241	243	217	219	202	202
AME broiler (Kcal/kg)	2800	2800	2900	2900	3000	3000
Digestible Lysine (g/kg)	12.5	12.5	11.2	11.2	10.0	10.0
Digestible M+C (g/kg)	9.3	9.3	8.5	8.5	7.8	7.8
Digestible Thr (g/kg)	8.1	8.1	7.1	7.1	6.4	6.4
Calcium (g/kg)	8.4	8.4	6.4	6.4	5.4	5.4
Ret P[2] (g/kg)	4.0	4.0	3.2	3.2	2.8	2.8

[1)]Premix complex comprises vitamins, trace minerals, amino acids, coccidiostats and phytase.

[2)]Calculated retainable P includes contribution from phytase

Results and Conclusion

Nutrient and enzyme levels in the diets were within acceptable limits of variation. Intestinal viscosity was not affected by diet or enzyme (table 2), which means enzyme benefits can not be explained by a reduction in digesta viscosity.

At 42 days of age (table 2) there was a significant ($P<0.001$) interaction between diet type and enzyme product for FCR. One single xylanase product gave a significantly improved FCR in both diet types (simple: 1.600 cf 1.642; complex: 1.618 cf 1.643) whilst in the simple diet three of the products showed no significant improvement and in the complex diet one of the other products showed no significant improvement.

The weight of the full small intestine relative to the weight of the full caecum was significantly reduced by the three single xylanase enzyme products as compared to the control diet. A reduction in this ratio can indicate a more active caecal fermentation, which could be the result of the supply of arabinoxylo-oligosaccharides, as discussed by Singh et al (2012). This finding thus supports the hyphothesis that a single xylanase product can work across a variety of diet types.

Table 2: Results		Gain 0-42 d (g)	FCR 0-42 d (g:g)	Digesta Viscosity (cPs)	Intestine Wt relative to caecum
Interaction Effect					
Diet	**Enzyme**				
Simple	Control	3379	1.642[cd]	2.84	9.0
Simple	Xyl E	3413	1.600[a]	2.60	7.1
Simple	Multi R	3387	1.602[a]	2.63	8.1
Simple	Xyl H	3387	1.640[bcd]	3.11	8.8
Simple	Xyl + ß-Glu A	3440	1.619[abc]	2.71	8.5
Simple	Xyl R	3379	1.646[d]	2.94	7.4
Complex	Control	3366	1.643[d]	2.60	9.6
Complex	Xyl E	3322	1.618[ab]	2.83	8.1
Complex	Multi R	3319	1.648[d]	3.15	9.2
Complex	Xyl H	3364	1.619[ab]	2.76	7.5
Complex	Xyl + ß-Glu A	3334	1.619[ab]	2.79	9.0
Complex	Xyl R	3298	1.612[a]	2.83	7.1
LSD		74.8	0.023	0.765	1.59
P		0.421	<0.001	0.620	0.255
Diet Effect					
Simple		3397[b]	1.625	2.80	8.2
Complex		3334[a]	1.626	2.83	8.4
LSD		30.5	0.0094	0.765	0.65
P		<0.001	0.725	0.620	0.407
Enzyme effect					
Control		3372	1.642	2.72	9.3 [d]
Xyl E		3368	1.609	2.71	7.6 [ab]
Multi R		3353	1.625	2.89	8.6[bcd]
Xyl H		3375	1.629	2.94	8.2[abc]
Xyl + ß-Glu A		3387	1.619	2.75	8.7 [cd]
Xyl R		3338	1.629	2.88	7.2 [a]
LSD		52.9	0.0163	0.541	1.12
P		0.511	0.004	0.931	0.003

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Mineral nutrition

P-166 (ID 309)

DIETARY SELENIUM SUPPLEMENTATION OF BREEDERS INFLUENCES GROWTH AND SELENIUM STATUS OF PROGENY

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Abstract

The impact of selenium (Se) sources fed to breeders on muscle Se status and progeny growth was studied. Breeders were divided into 3 groups differing by the dietary Se source as followed: sodium selenite (SS), selenized-yeast (SY) and hydroxy-selenomethionine or HMSeBA (SO). Each breeder group generated 290 day-old chick. At d0, 30 chicks per group were selected to measure muscle Se content and the 260 remaining chicks were divided into 13 replicate pens of 20 chicks for 21 days. Progeny of the 3 groups all received the same standard broilers diet containing only SS. On D12 and 21, growth performances were measured and one chick per pen was selected to measure breast Se content. Breeder Se source had no effect on the body weight at hatch, body weight gain or feed intake during the entire experimental period ($P > 0.05$). However, feed conversion ratio (FCR) of progeny issued from SO fed breeders was significantly improved between D0 to 12 and D0 to 21 comparatively to SS ($P < 0.05$). Muscle Se content of day-old broiler chicks was higher for SO group compared to other treatments ($P < 0.05$). In addition, muscle Se content of day-old broiler appeared to be significantly related to FCR for the periods: D0 to 12 and D0 to 21 ($P < 0.01$). These results showed the higher ability of HMSeBA compared to other Se source in breeder diet to improve chick Se status at hatch which is related to FCR improvement during 0-21 day period of growth.

Keywords: selenium, hydroxy-selenomethionine, broiler breeder, progeny

Introduction

Several studies showed the benefits of dietary organic selenium (Se) supplementation in breeders on egg quality and fertility compared to inorganic Se (Pappas et al. 2005a). However, few studies observed the effect of breeder nutrition on the performance of the offspring. Indeed, young broiler chicks are facing strong oxidative stress during their first days of life due to important physiological changes, such as, adaptation to aerobic life, active development of physiological systems such as digestive tract, hormonal and neuronal systems (Moran 2007). Surai (2000) had shown an increase of the Se content of day-old-chick at hatch and higher glutathione level and glutathione peroxidase (GPx) activity in liver of their chicks at least 5 days after hatch when breeders were supplemented with selenized yeasts. Moreover, Pappas et al. (2005b) had observed a positive effect of the breeders Se intake on the GPx activity in tissues of their offspring throughout the 4 weeks following hatch. Recently, Jlali et al. (2013) demonstrated that egg selenium content can be tremendously increased with different organic selenium sources when compared to inorganic selenium sources, resulting in selenium deposition higher with hydroxy-selenomethionine (HMSeBA) compared to selenized yeast. The present work aimed evaluating the effect of breeder selenium sources on selenium status and growth performance of their progeny.

Materials and Methods

A total of 870 day-old chicks from 3 different broiler breeders groups (3 x 290) were used in this study. The 3 breeder groups differing in dietary Se sources and level as followed: sodium selenite at 0.3 mg Se/kg (SS); selenized yeast at 0.2 mg Se/kg (SY); hydroxy-selenomethionine or HMSeBA at 0.2 mg Se/kg (SO). On the 290 day-old chicks per group, 30 were randomly selected and euthanized on the arrival of birds to measure their birth Se status. The *pectoralis major* were collected and pooled by 5 chicks in order obtaining 6 pools of muscle per breeder groups. The 260 remaining chicks were allocated in 13 pen replicates of 20 chicks for 21 days. Whatever the origin of the chicks, they received the same starter and grower commercial broiler diets containing only sodium selenite as Se supplementation at 0.2 mg Se/kg of feed. The body weight, feed intake and FCR of birds were measured on day 12 and 21 and muscle samples have been collected on 1 bird per pen at D12 and D21 to follow Se status of birds. The total selenium concentration in

diets and muscles was determined by inductively coupled mass spectrometry (ICP-MS) according the method of Bierla et al. (2008). All data were analyzed using GLM procedure of SAS 9.1.3 (SAS Inst. Inc., Cary, NC).

Results

The measurement of Se level of the breeder diets confirmed targeted levels (data not shown). The progeny starter and grower broiler diets naturally contained 0.08 and 0.11 mg Se/kg, respectively and resulted to 0.26 and 0.35 mg Se/kg, respectively after SS supplementation.

Table 3: Broiler growth performance in relation with selenium sources distributed to breeders

Breeders diet	SS	SY	SO	SEM	P-value
D0-12					
BW D0 (g)	40.31	40.23	40.31	0.19	0.876
BW D12 (g)	327.4	328.3	331.0	43.7	0.366
BWG (g)	287.1	288.1	290.7	45.0	0.381
FI (g)	357.4	354.0	355.9	60.6	0.544
FCR (g/g)	1.245[a]	1.229[ab]	1.225[b]	0.0003	0.025
D12-21					
BW D21 (g)	710.6	711.6	717.5	180.7	0.389
BWG (g)	383.2	383.3	386.5	75.9	0.566
FI (g)	613.6	610.4	612.8	124.7	0.743
FCR (g/g)	1.602	1.593	1.586	0.0003	0.096
D0-21					
BWG (g)	670.3	671.4	677.2	182.3	0.395
FI (g)	971.0	964.4	968.7	277.0	0.591
FCR (g)	1.449[a]	1.436[ab]	1.431[b]	0.0002	0.007

a, b: Means with different superscripts are statically different ($P < 0.05$)

The growth performances of day-old chicks are presented in Table 1. The breeder Se sources had no effect on hatch weight of the offspring, feed intake (FI), body weight gain (BWG). However, broilers from breeders fed with SO had a better FCR between D0 to D12 and D0 to D21 ($P < 0.05$) compared to the broilers from breeders fed with SS, while chicks issued from SY treatment presented intermediary FCR. Chick muscle Se content at the different sampling days are presented in Figure 1A. At hatch, broilers from breeders fed with organic Se source (SO and SY) had a higher muscle Se content ($P < 0.001$) compared to SS maternal treatment, with an additional concentration using SO compared to SY treatment ($P <0.001$). These results are consistent with those of Jlali et al. (2013) who reported egg Se content in the same hierarchy with those sources. The link between egg selenium content and day old chick selenium status had been also reported by Surai (2000). However, after 12 days, muscle Se content was no longer discriminating maternal treatments. The significant decrease from D0 to D12 could be explained, on one hand by the low efficiency of inorganic Se source supplied during growth to maintain muscle Se content, and, on the other hand by the selenium turnover to build selenoprotein, such as GPx, for antioxidant defenses (Surai, 2002). Our results corroborates a study of Wang et al. (2011) who fed sodium selenite or selenomethionine in breeder and studied the offspring performances. They also reported significantly higher antioxidant status of chicks from organic selenium group with higher GPx and SOD activities in breast muscle compared to inorganic group.

A B

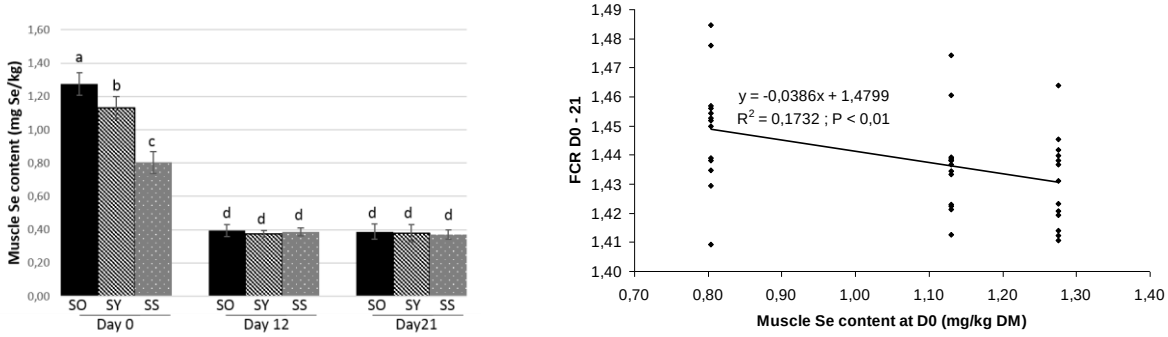

Figure 3: A) Evolution of the broilers muscle Se content in relation with dietary selenium source of breeders.
B) Effect of muscle selenium content at hatch on 0-21 days FCR in broilers

SO significantly improved FCR during the 0-21 day period compared to SS. The three different selenium sources supplied to breeder were related to progeny breast muscle selenium concentration and FCR (Figure 1B). Day old chick Se content had a significant effect on the FCR of broilers between D0 to D12 and between D0 to D21 ($P < 0.01$). The improvement of broilers FCR with the use of organic selenium forms in breeder feeds could be explained by higher muscle Se reserves that allowed birds to manage selenium demand during the early growth phase and ensure better selenoprotein production to maintain their antioxidant system (Pappas et al. 2005b, Wang et al 2011). These results point-out the importance of day old chick selenium status on their subsequent performances. This resulted in an average of FCR improvement of 1.25 % during the 0-21 day period for broilers from SO treatment compared to broilers from SS treatment.

Conclusions

This study showed the higher ability of HMSeBA to supply selenium through maternal diet into eggs and chick progeny comparatively to seleno-yeasts and sodium selenite. Day old chick selenium status was correlated to 0-21 day FCR, highlighting the importance of selenium status for optimal performances and certainly maintain antioxidant defenses during this critical phase of chick early life.

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P-167 (ID 308)

TOWARD AN EASY ASSESSING METHOD TO MEASURE SELENIUM BIOAVAILABILITY IN BIRDS

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Abstract

The aim of this study was to develop an easy assessment method to determine the selenium (Se) bioavailability of different selenium sources in birds. The first step was to measure the kinetic of selenium deposition in muscle and feathers of broilers fed different selenium sources. A total of 576 chicks were divided into 4 treatments with 8 replicates of 18 birds per pen for 21 days. The diets used in the experiment were supplemented with different Se sources as followed: negative control (NC) not supplemented in Se; sodium selenite (SS); selenized yeast (SY) and hydroxy-selenomethionine or HMSeBA (SO) at 0.2 mg Se/kg of feed. Total selenium content in breast muscle and feathers was assessed on day 0, 7, 14 and 21. Only SO increased muscle Se content at D7 compared to D0 ($P < 0.05$), while all other treatments exhibited decrease of Se muscle content ($P < 0.05$) from D0 to D7. At D21, the muscle and feather Se content were highly correlated ($R^2 = 0.927$; $P < 0.0001$). This study shows that breast muscle selenium measured as early as at D7 allows discriminating selenium sources as well as at D21. Moreover, the feather Se content measured at 21 days appeared a good and convenient indicator of selenium status in birds that can be used at farm level. These results also underline the benefit of a pure organic selenium source such as hydroxy-selenomethionine to rapidly increase selenium status in early growth phase.

Keywords: selenium, hydroxy-selenomethionine, feather, poultry

Introduction

The tissues of chick embryos and newly hatched chicks contain a high proportion of polyunsaturated fatty acids. During their first days of life, birds face a rather stressful period, and are highly susceptible to oxidative stress (Surai and Fisinin, 2014). In addition, their hepatic concentration of vitamin E and carotenoids decrease by 20-fold during the 10 first days of life (Surai 2002). However, the glutathione peroxidase (GPx) liver activity increased in the same time. In practice, signs of selenium (Se) deficiency are generally observed around 7 days post-hatch, when vitamin E level is low and GPx activity not yet sufficient (Surai, 2002). Traditionally, to ensure adequate antioxidant status of young broilers, Se supplementation is provided in the diets, using sodium selenite (SS) or organic forms like seleno-yeasts (SY) containing mainly SeMet in variable amount according to the strain and growing yeast conditions (Surai and Fisinin, 2014; Simon et al. 2013). The Se status of wild birds is currently evaluated with the measurement of Se content of their feathers (Burger, 1996). The purpose of this study was to evaluate the possibility to use muscle or feather Se contents to compare the bioavailability of different selenium sources.

Material and Methods

A total of 576 day-old chicks from a commercial hatchery were allocated to 4 treatments with 8 replicates of 18 birds per pen. The birds received a starter diet from D0 to D11 and a grower diet from D12 to D21 and each treatment was supplemented with different Se sources as follow: NC, basal diet not supplemented with Se; SS, SY and SO, basal diet supplemented with 0.2 mg Se/kg from sodium selenite, selenized yeast and HMSeBA respectively. At the end of each phase: body weight (BW) and pen feed intake (FI) were recorded and used for calculation of feed conversion ratio (FCR). Moreover, on D0, 7, 14 and 21, one bird per pen (8 per treatment) was randomly selected and euthanized. The feathers of the wings and the two *Pectoralis major* muscle were collected and pooled for two birds before being frozen at -20°C for subsequent analysis. The total Se concentration of diets, feathers and muscles were determined by inductively coupled mass spectrometry (ICP-MS) according to Bierla et al. (2008). All data were analyzed using GLM procedure of SAS 9.1.3 (SAS Inst. Inc., Cary, NC).

Results

The feed analyses indicated an expected native Se content of 0.13 and 0.10 mg Se/kg in starter and grower diet, respectively (data not shown). The different treatments had no effect on growth performance during the 21 days of experiment (average BW = 918 g and average FCR = 1.39 g/g). These results are consistent with other works that did not show effects on performance in relation with dietary selenium (Se) supplementation in broilers under standard conditions (Briens et al. 2013).

The evolution of muscle Se content through the experiment depending on the Se source is presented in Figure 1. Selenium sources significantly affected the Se deposition in *pectoralis major* muscle (P < 0.001) at D7 and the same effects remained until 21 days old. At D7, chicks fed with the diet supplemented with SO treatment showed a significant increase of muscle Se content compared to hatch level (+ 41%) whereas other treatments showed significant decrease in muscle selenium content (-15, -37 and -43 % for SY, SS and NC respectively). SY supplementation restored muscle selenium content after 14 days at a similar level than D0. SS treatment appeared to keep muscle selenium content stable from D7 to D21, whereas for NC treatment muscle selenium content decreased until D14. These results are consistent with previous works studying muscle Se concentration with different Se sources supplemented for 21 day (Briens et al. 2013, 2014). From D7 to D21, the muscle Se content of broilers fed SS diet remains stable, whereas the muscle Se content of broilers fed NC continued to decrease. This finding showed that even a relatively high feed Se basal level (0.13 to 0.10 mg Se/kg in NC) cannot maintain Se status of broilers. The slight decrease of muscle Se content of chicks fed with SY during the first days of life and the subsequent recovery between D7 and D14 could be related to the Se form in yeasts, SeMet incorporated in proteins, that may be not optimally digested during the first week because of suboptimal gastrointestinal functions of the young chicks whereas a free seleno amino acid like SO may be directly absorbed.

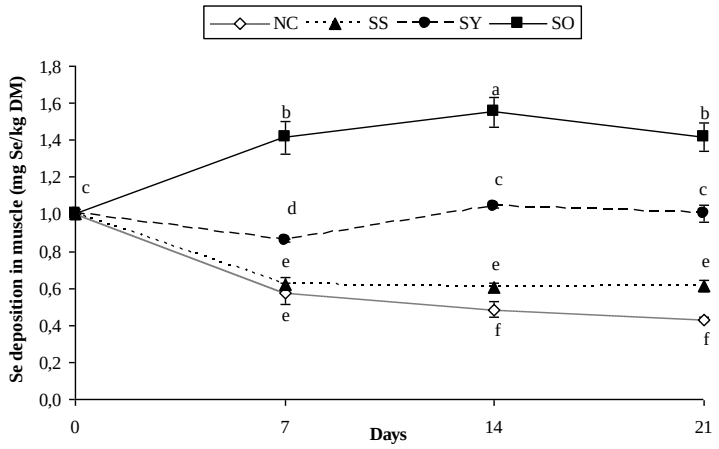

Figure 4: Kinetic of muscle Se content in *Pectoralis major* muscle in function of the dietary Se source. Results are expressed as mean ± SD and different letters indicate statistical differences at P < 0.05.

Se concentration in feathers collected on day 7 and 21 on birds fed NC, SY and SO diet was summarized in Figure 2A. Whatever the dietary treatment, the Se concentration in feathers decreased (P < 0.001) with age of the birds. At both sampling time (7 or 21 days) feather Se content appeared significantly affected (p<0.05) by dietary Se content and source as follow: NC < SY < SO. The present results are in agreement with the study of Sevcikova et al. (2006). The correlations between muscle and feather Se content at D7 and D21 were highly significant (P < 0.001) with a better accuracy at D21 (R^2 = 0.927) than at D7 (R^2= 0.542) (Figure 2B). This could be explained because at latter age the second generation of feathers starts to grow and push out the down feather (Leeson and Walsh, 2004). These results allow to propose to measure feather Se content at 21 days of age as a simple and accurate indicator to follow the muscle Se status of birds and to compare bioavailability of selenium sources.

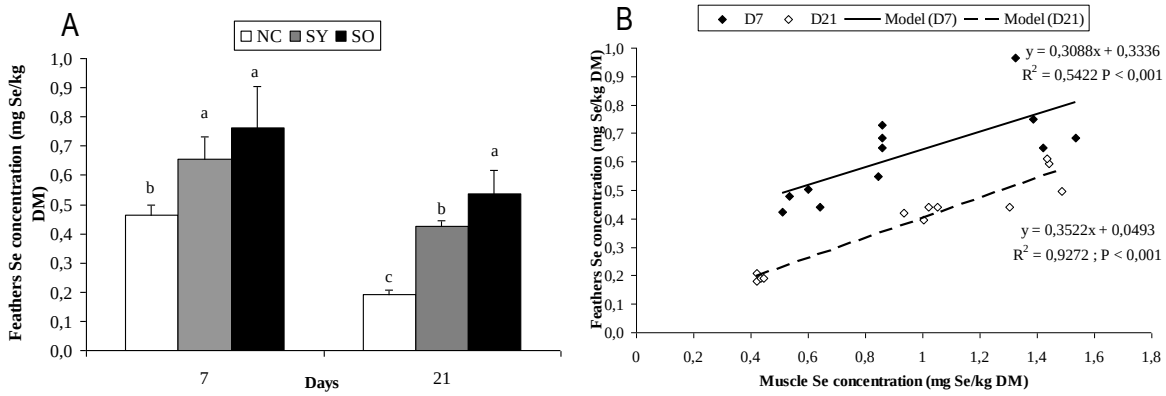

Figure 5: A: Se deposition in feathers in function of dietary selenium source at day 7 and 21. Results are expressed as mean ± SD and different letters indicate statistical differences at P < 0.05; B: Correlation between Se deposited in feathers and in *Pectoralis major* muscle.

Conclusions

The selenium status and the efficacy of different selenium sources can be evaluated through a non-invasive procedure: the measure of feather Se content. Muscle and feather Se content are highly correlated at D21, and transfer Se efficiency is dependent of the Se source. In those conditions HMSeBA showed the best ability to increase bird Se deposition, an indicator of their Se status.

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P-171 (ID 79)

BROILER PERFORMANCE AND PREVALENCE OF FOOTPAD LESIONS IN RESPONSE TO PHYTASE, CALCIUM AND PHOSPHORUS CONCENTRATIONS

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Abstract

Taking into account the concerns about the environment, nutrition and farm profits, animals have to be fed precisely to meet their phosphorus (P) requirements. The objective of the current trial was to provide efficacy data of a 6-phytase for use in broilers at different calcium (Ca) levels and Ca/P ratios. Phytase was introduced at different dosage levels (100g/tonne and 200g/tonne) into diets i) with a standard Ca and reduced P level (imbalanced diet); ii) with a reduced Ca and P level or iii) with a normal Ca and P level (balanced diets). The performance of the balanced treatments without phytase supplementation did not differ significantly. The balanced diets (with and without phytase supplementation) had a significantly lower WAFC1500 (FC to obtain a body weight of 1500g) compared to the imbalanced diets. Adding phytase to the low Ca-low P diet did further increased the performance and the obtained results were even better (not significantly) than those fed the standard Ca and P diet. A higher dosage of phytase combined with an imbalanced diet had a significant effect as the higher supplementation level could compensate for the shortcomings of the imbalanced diet. The highest prevalence of footpad lesions was seen at broilers fed the imbalanced diets. The overall results emphasise that dietary Ca and P concentrations can be reduced but only in a balanced way. If done in combination with phytase even higher performance results were obtained than when using standard dietary Ca and P levels.

Introduction

Due to the environmental pressure, but also from a nutritional and economical point of view, the phosphorus (P)-content of a diet is an increasingly important issue and animals must be fed near their P requirements. However, it is very difficult to know the exact P requirements of broilers as there are interactions between eg. the calcium (Ca) level, P level and phytase (L´ETOURNEAU-MONTMINY et al., 2010). Therefore, the objective of this trial was to provide efficacy data of a 6-phytase for use in broiler diets at different Ca levels and Ca/P ratio's.

Materials and Methods

One day old, male broiler chicks (Ross 308) were used in this trial (Origin: Belgabroed Hatchery, Merksplas-Belgium). For the trial, 36 pens were used and male birds were housed with 35 per pen. The floor was covered with fresh wood shavings and no additional filling or cleaning of the floor was executed during the trial. Central water heating and infrared bulbs (1 per pen) provide optimal house temperature. The experiment consisted of 6 dietary treatments with each 6 replicates of 35 birds or 1260 birds in total. The first dietary treatment consisted of a corn-soybean diet which met all dietary recommendations for chickens from 1 to 39 days of age. For the second, fifth and sixth treatment Ca and P level were reduced during the entire period. For the third and fourth treatment only the P level was reduced during the entire period. All treatments were formulated to be isocaloric and isonitrogenous. For treatment 3 and 5 and for treatment 4 and 6 Optiphos 2500 Granular was supplemented at 250 (100 g/tonne) and 500 OTU (200g/tonne), respectively. A 3-phase feeding scheme was applied with periods of 13 days each.

Average pen weight was recorded at day old, 13, 21, 26 and 39 days of age. Feed intake was recorded for 1-13, 13-21, 21-26 and 26-39 days. Feed conversion, daily growth rate, bird-days and daily feed intake per bird will be calculated for 1-13, 13-21, 21-26, 26-39 days and also for 1-26 and 1-39 days. Dead animals were recorded during the entire period. Foot pad dermatitis of 8 birds per pen was determined. Daily mortality and cullings were recorded for each pen. Corrections for mortality calculating zootechnical performances were done using the number of 'animal days' (number of animals x days alive).

All zootechnical parameters were analysed by a linear mixed model with treatment as a fixed factor and block as a random factor (Statistica 11.0 program). Data that differ significantly have a P-value below 0.05. The scoring data were analysed using a proportional odds logistic regression model for ordinal responses (*polr* function in *MASS* package in R 3.0.1).

Results

Main performance results are presented in table 1. Lowest body weight was obtained if broilers were given the imbalanced diet with low phytase supplementation (Tr3). Until d21 of age, body weight of broilers fed Tr3 was significantly lower compared to broilers fed Tr4 to Tr6. From d26 onwards, the difference in body weight of the broilers fed the imbalanced diets (Tr3 and Tr4) was no longer present. But body weight of Tr3 fed broilers remained significantly lower compared to the supplemented NC groups (Tr5 and Tr6). No significant differences between the balanced diets without phytase supplementation (Tr1 and Tr2) were observed until slaughter age. If broilers were given the normal Ca, low P diet (Tr2), body weight at slaughter age decreased with 32.9 g compared to their normal Ca, normal P fed counterparts (Tr1). Supplementing phytase at the NC groups (Tr4 and 5) significantly increased the body weight at d26 and d39. The higher supplementation level of phytase did not result in a further significant increase of body weight. However, a higher supplementation level for the unbalanced diet (Tr4 vs Tr3) significantly increased body weight until d21. This increase was no longer significant at the 5% level after d21 of age.

Table 1: Effect of dietary treatments on body weight (BW) (g), feed intake (g/day) and feed conversion during the starter (d0-d13), grower (d13-d26) and finisher (d26-d39) period.

	Tr1	Tr2	Tr3	Tr4	Tr5	Tr6	P-value
BW (g)							
d13 (g)	303.8^{bc}	305.7^{abc}	294.6^{c}	318.3^{ab}	326.9^{a}	315.5^{abc}	0.002
d26 (g)	1100.8^{abc}	1083.1^{bc}	1027.3^{c}	1108.9^{ac}	1129.2^{ab}	1160.7^{a}	0.0004
d39 (g)	2290.4^{abc}	2257.5^{bc}	2214.8^{c}	2297.4^{abc}	2363.1^{a}	2351.8^{ab}	0.0008
FI (g/day)							
d0-d13	27.7^{b}	28.4^{ab}	28.4^{ab}	28.9^{ab}	30.2^{a}	28.9^{ab}	0.02
d13-d26	89.6^{ab}	88.0^{ab}	85.0^{b}	91.4^{a}	91.6^{a}	93.1^{a}	0.006
d26-d39	155.8	154.8	153.9	154.5	158.4	158.0	NS
FC							
d0-d13	1.389^{a}	1.413^{ab}	1.480^{b}	1.371^{a}	1.394^{a}	1.388^{a}	0.009
d13-d26	1.462	1.473	1.510	1.503	1.487	1.432	NS
d26-d39	1.704	1.714	1.685	1.691	1.671	1.726	NS
$WAFC_{1500}$	1.524^{a}	1.539^{ab}	1.608^{b}	1.535^{ab}	1.522^{a}	1.493^{a}	0.002

Tr = treatment; BW = body weight; FI = feed intake; FC = feed conversion, $WAFC_{1500}$ = weight adjusted feed conversion = FC to btain a body weight of 1500g, Tr 1 = normal calcium (Ca) and normal phosphorus (P) level, Tr 2 = low Ca and low P level, Tr 3 = normal Ca and low P level + normal phytase level, Tr 4 = normal Ca and low P level + high phytase level, Tr 5 = low Ca and low P level + normal phytase level, Tr 6 = low Ca and Low P level + high phytase level

$^{a-c}$ means with the same letter are not significantly different from each other at $P \leq .05$.

Daily feed intake was significantly lower for the PC (Tr1) group during the starter phase, however during the grower phase the NL dietary treatment group (Tr3) had the significantly lowest feed intake. Broilers fed the low Ca, low P (NC) diet had a feed intake comparable with the other dietary treatments. Adding both levels of phytase to the diets increased the feed intake of the broilers but not significant with exception of the imbalanced diet during d21 to d26. Dietary treatments did not significantly influence feed intake during the finisher phase. For the entire period, broilers fed Tr3 and Tr5 had the lowest and highest feed intake, respectively.

During the starter period there was a significant effect of diets on feed conversion, however during grower and finisher this effect was no longer observed. During the starter period, the most favourable feed conversion was obtained if broilers were fed the imbalanced diet with a high dosage of phytase (Tr4) or the low Ca low P diet supplemented with phytase (Tr5 and Tr6). Due to a higher feed intake, feed conversion of Tr5 was comparable with Tr4. Feed conversion was significantly lower for the Tr3 fed broilers due to a low body weight gain. Supplementing a higher phytase level in combination with the imbalanced diet significantly improved feed conversion.

Broilers fed the negative control group without phytase supplementation (Tr2) had a significantly lower tibia ash percentage compared to the birds of the other dietary treatments and the highest prevalence of footpad lesions was seen at broilers fed the imbalanced diets.

Conclusions

It can be concluded that a reduction of the minerals should be done in a balanced way to obtain maximal performance results. However, this study indicates that a higher dosage of phytase can compensate for these shortcomings. Furthermore, reducing the Ca and P level in a balanced way resulted in comparable results as the positive control group, however the significantly lower tibia ash % can be an indication of too low levels of both minerals in this study. Supplementing phytase did increase performance and the obtained results were even better than those of the PC group, but not at the 5% significance level. The higher dosage of phytase combined with the low Ca and P diet did not further improve the zootechnical results of the broilers.

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P-172 (ID 291)

EFFECTS OF DIETARY COARSENESS AND WET FEEDING ON P-UTILISATION OF LOW AVAILABLE P DIETS IN YOUNG (PRE-)LAYER HENS

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Abstract

An experiment was conducted to study phosphorus utilization in 624 White Leghorn hens (15 to 28 wks) as affected by diets differing in particle size distribution ('feed structure'), conformation ('wet vs dry feed') and available phosphorus content in a 2x2x2 factorial design. Eight treatments were tested in 48 pens (6 replicate pens per treatment): a fine or a coarse mash diet, that was either fed in wet (1kg feed : 1L water) or dry form, and contained either a low (1.6g/kg) or standard (2.9g/kg) level of available phosphorus (aP). Experimental diets were maize based and contained no phytase supplements. The hypothesis was that a coarse and/or wet diet improves gastrointestinal tract development and alleviates the detrimental effects of a P-deficient diet by increasing the degradation of phytate. The hens were fed *ad libitum* provided in one meal per day. Feed intake (g/b/d) was increased when hens were fed the wet diet as compared to the dry diet in wk 19-22 (101.7 g vs. 98.7 g, P<0.001) and in wk 23 to 27 (116.0 g vs. 113.5 g, P<0.001). Feed structure affected intake in wk 19 to 22 (101.0 g fine diet vs. 99.4 g coarse diet; P=0.011) and in wk 23 to 27 (115.5 g fine diet vs. 114.1 g coarse diet; P=0.012). Rate of lay was highest for hens fed the combination of the wet and fine diet (93.01% vs. 90.17%; P=0.016). The coarse diet reduced egg mass production (49.69 g/d vs. 50.92 g/d, P=0.009). Tibia ash and mineral content and phytase activity in the GI-tract will be measured in due time and presented at the conference.

Keywords: young layer, diet structure, wet feed, phosphorus utilisation, performance

Introduction

A large majority of the phosphorus (P) found in plant ingredients of poultry feeds is bound to phytate, rendering this P less available to the bird, unless broken down by the enzyme phytase. Despite improvements with the addition of microbial phytase, certain amounts of inorganic phosphates are still required in the diet. Hence the need to investigate possible feeding strategies to improve P utilisation in poultry is evident. Providing birds with coarsely ground diets (larger particle sizes) and feeding it in wet form have shown to be beneficial to poultry. It has been demonstrated that feeding broilers a coarse corn mash diet increased bone ash and P and Ca retention (Kasim and Edwards, 2000) compared to those fed a fine corn mash diet, indicating an improved utilisation of P possibly due to an increased degradation of phytate P. Wet feeding improves feed intake and digestibility (Yasar and Forbes, 1999). If and to what extend wet feeding would improve the activity of phytase in the gut is unknown. It has, however, been shown that wetting feed reduces phytate by fermentation, hence increasing the level of available P (aP) in the diet (e.g.:Blaabjerg et al. (2014)). This study was aimed to investigate the effects of a coarse mash diet vs. a fine mash diet in combination with feeding the diet soaked or dry on egg production, bone health and gastro-intestinal development in young layers fed a normal or deficient level of calculated aP. It is hypothesized that coarse and wet feeding would counteract the negative effects of a deficient aP level.

Material and Methods

A total of 624 110-day of White Novogen pullets were assigned to 8 treatment groups, in a 2x2x2 factorial design. Each treatment group consisted of 6 replicate pens, with 13 birds per pen. Dietary treatments consisted of a low P (1.6 g aP/kg diet) versus high P level (2.9 g aP/kg diet), dry versus wet feeding method and fine versus coarse mash diet. Diets were maize based, no phytase supplements were used and had a similar ingredient composition. Anorganic phosphate (MCP) was used to upgrade the low aP diets to the high aP diets. Diets were ground using a roller mill, with a roller distance of 2.3 mm for the fine diets and a distance of 3.4 mm for the coarse diets. The wet diets were prepared fresh daily prior to feeding by mixing the feed with tap water in a 1:1 ratio. The hens were fed *ad libitum* provided in one meal per day. Feed intake and egg production were recorded weekly. At 22 and 28 wks of age two hens per replicate were culled for GI-tract measurements and tibia collection. Chyme was collected from the crop, proventriculus and gizzard and small intestine, used for pH measurements and stored for later analysis of phytase activity. Empty organs

were weighed. For the measurement of tibia breaking force a three point bending method was used, with the Instron 5564 texture analyser (compression speed was 2mm/min). Results are presented as mean ± SEM.

Results

Feed intake (of the air-dry feed) was increased by wet feeding compared to dry feeding in the pre-layer period (wk 16-17; 87.3 g vs. 79.5 g, P<0.001) and in the transition period (wk 19- 22; 101.7 g vs. 98.7 g, P<0.001). Feed intake in the transition period was also increased by the high P level compared to the low P level (101.0 g vs. 99.5 g, P=0.010) and the fine diet compared to the coarse diet (101.1 g vs. 99.4 g, P=0.011). FCR was unaffected by treatments. Rate of lay in the transition period was 68.43% and did not differ between treatments. Egg mass production was increased by the high P level compared to the low P level (33.07 g/d vs. 31.76 g/d, P=0.025) and by wet feeding compared to dry feeding (32.91 g/d vs. 31.73 g/d, P=0.035).

Table 1: Production performance measurements of hens fed diets differing in P level (P), conformation (C) and structure (S).

			Production performance				
P	**C**	**S**	Age 23 - 27 wk				
			Feed intake g/d	**FCR**[1]	**Rate of lay** %	**Egg mass** g/hen/d	**Egg weight** g/egg
High P	Dry	Fine	112.7 ± 0.7	2.22 ± 0.02	91.08 ± 0.91	50.69 ± 0.44	55.97 ± 0.29
		Coarse	113.4 ± 0.7	2.26 ± 0.03	91.99 ± 0.96	50.14 ± 0.53	54.97 ± 0.30
	Wet	Fine	120.3 ± 0.6	2.29 ± 0.03	93.72 ± 0.89	52.58 ± 0.59	56.31 ± 0.26
		Coarse	113.1 ± 0.7	2.33 ± 0.03	87.35 ± 1.20	48.36 ± 0.71	55.76 ± 0.31
Low P	Dry	Fine	112.9 ± 0.9	2.29 ± 0.04	89.61 ± 1.27	49.37 ± 0.86	55.40 ± 0.31
		Coarse	115.1 ± 1.3	2.34 ± 0.03	89.45 ± 1.36	49.22 ± 0.74	55.19 ± 0.24
	Wet	Fine	115.9 ± 0.7	2.27 ± 0.03	92.29 ± 1.13	51.05 ± 0.61	55.49 ± 0.25
		Coarse	114.8 ± 0.7	2.26 ± 0.04	91.51 ± 1.36	51.02 ± 0.44	55.80 ± 0.21
Significance							
P level (P)			0.715	0.396	0.695	0.540	**0.039**
Conformation (C)			**<0.001**	0.547	0.402	0.066	**0.003**
Structure (S)			**0.012**	0.095	0.051	**0.009**	**0.010**
P * C			**0.035**	**0.005**	**0.040**	0.077	0.441
P * S			**<0.001**	0.612	0.167	**0.018**	**0.006**
C * S			**<0.001**	0.545	**0.016**	0.056	0.143
P * C * S			**0.028**	0.436	**0.042**	**0.046**	0.900

[1] FCR= Feed conversion ratio (based on egg mass).

Results from the early production period, wk 23-27, are shown in Table 1. There were no main treatment effects on rate of lay, but there were interaction effects. Hens fed the combination of the wet and fine diet had a higher rate of lay than hens fed the wet and coarse diet (93.01% vs. 89.43%, P=0.012), while no difference in rate of lay was found between the fine and coarse diets within the dry fed groups (90.35 % and 90.72 %). The hens fed the high P, wet and fine diet had a higher rate of lay than hens fed the high P, wet and coarse diet, but both groups did not differ from the other six groups. Egg mass production was reduced in hens fed the coarse diet compared to those fed the fine diet (49.69 g/d vs. 50.92 g/d, P=0.009). The hens fed the high P, wet and fine diet had a higher egg mass production than the hens fed the high P, wet and coarse diet and the hens fed the low P and dry diets, but did not differ from all other groups.

The average tibia breaking force was 132.33 N at wk 22 and 132.10 N at wk 28 and did not differ between groups. Relative empty gizzard weight was increased by the coarse diet compared to the fine diet at 22 wks of age (15.72 g/kg BW vs. 13.77 g/kg BW, P<0.001) and at 28 wks of age (14.32 g/kg BW vs. 13.51 g/kg BW, P=0.034). Empty gizzard weight was also increased by the low P diet compared to the high P diet at 22 wks of age (15.22 g/kg BW vs. 14.26 g/kg BW, P=0.010), but this effect was no longer significant at 28 wks of age. However, hens fed the low P and coarse diet had higher relative gizzard weights than hens fed the high P and coarse diet (15.08 g/kg BW vs. 13.56 g/kg BW, P=0.029), the low P and fine diet (13.34 g/kg BW, P=0.010) or the high P and fine diet (13.68 g/kg BW, P=0.049). Results for tibia ash and mineral content and phytase activity in the GI-tract are still pending.

Conclusions

Wet and coarse feeding did not counteract negative effects of the low P diet, as this diet had barely affected performance and had no effect on tibia strength, despite containing a level of only 1.6 g/kg aP. This could indicate that an aP level of less than 2.9 g/kg is sufficient for maintaining egg production in young layers. Wet feeding increased feed intake throughout the whole trial, similar to other findings (Yasar and Forbes, 1999). The coarse diet had negatively affected feed intake, a finding which is in contrast to Safaa et al. (2009). Hens fed the combination of the wet and fine diet showed the highest feed intake and egg performance. P level interaction effects for performance appear to be caused by the contrast between the group fed the high P, wet and fine diet, which performed well, and the group fed the high P, wet and coarse diet, which performed rather poorly. The coarse diet increased empty gizzard weight, similar to a number of other studies (Svihus, 2014). The positive effect of the low P diet on gizzard weight has, to the knowledge of the authors, not been demonstrated before and could indicate an adaptive response.

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P-173 (ID 65)

THE EFFECT OF LIMESTONE PARTICLE SIZE AND PHYTASE SUPPLEMENTATION ON PERFORMANCE, EGG-SHELL QUALITY AND BACTERIAL SPECIES IN ILEUM

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Abstract

The aim of the study was to examine the influence of limestone particle size and phytase (F) on performance of hens, physical parameters of eggs, digestibility of minerals and microbial colonization of the digestive tract. One hundred and sixty 60-week-old Lohmann Brown hens were housed in enriched cages for 12 months. The 2 x 2 factorial experiment included two levels of 3-phytase NATUPHOS® (0 and 350 FTU/kg) and two types of grain size of limestone (coarse limestone (CL) and mix of CL and fine limestone (FL) (65:35)) in mixed feed. All of the diets contained 4.4 g/kg of total phosphorus and 34 g/kg of calcium. Coarse limestone with F addition significantly increased hen-day egg production (P=0.018). The highest daily feed intake (P=0.001) and feed conversion (P=0.005) were recorded in the case of mix of CL and FL and F addition. The phytase supplement at 350 FTU/kg significantly increased shell percentage (P=0.007), shell index (P=0.003), shell strength (P=0.017) and shell thickness measured at blunt end (P=0.015), at the middle (P=0.009), at sharp end (P=0.015) and the average value of shell thickness (P=0.006). Albumen percentage and yolk percentage were influenced neither by limestone particle size nor by F supplementation. Albumen quality evaluated on the basis of Haugh units was higher (P=0.039) when using the mix of limestone. The phytase addition into the mixed feed nonsignificantly increased calcium and phosphorus digestibility in ileum by 6.2 and 9.1%, respectively. Bacteria belonging to genera *Turicibacter* and *Lactobacillus* occurred with higher frequency in ileum of hens fed with mixed feed enriched with the phytase.

Keywords: coarse limestone, 3-phytase, eggshell, microbial colonization, hen.

Introduction

It is estimated that between 6 and 8% of the total eggs laid worldwide, are either cracked or broken before they reached the final consumer. Both in terms of economy and in relation to the health risks associated with a cracked shell, the eggshell quality still remains one of the main interests of the poultry industry. Coarsely ground limestone can increase the utilization of calcium (Ca) for shell formation and also for percentage a thickness of eggshell. Toward the end of the laying period, the utilization of Ca generally decreases. The utilization of Ca is 80% at the start of the laying cycle and 50% at the end of the laying cycle. It is assumed that coarsely ground limestone remains longer in the small intestine and Ca will be released during the night, when the shell is formed. Also phytase has positive effect on the shell quality. The addition of phytase completely overcomes the adverse effects associated with low intake of inorganic phosphorus (P) by animals and improves the bioavailability of Ca and eggshell quality at the marginal level of Ca 3.4%. In addition, phytase supplementation decrease P excretion in manure and reduce the potential environmental problems (Jalal and Scheideler, 2001). Exogenous phytases are mainly active in acidic pH which is in the crop, proventriculus and gizzard. There are several commercial phytase products such as 3-phytase NATUPHOS® produced by strain *Aspergillus niger*.

Materials and Methods

One hundred and sixty 60-week-old Lohmann Brown hens were randomly assigned to 4 treatments with 4 replicate cages at 10 hens per cage. The hens were housed in three-floor enriched cages. The 2 x 2 factorial experiment was employed: 2 levels of phytase (F; 0 and 350 phytase units (FTU)/kg) and 2 types of grain size of limestone (coarse limestone (CL; 1 – 2 mm) and mix of CL and fine limestone (FL; < 0.5 mm) (65:35)) in mixed feed. NATUPHOS® (BASF, Ludwigshafen, Germany), a preparation of 3-phytase (EC 3.1.3.8) produced by *Aspergillus niger*, was chosen as the source of the F. The experiment lasted 12 months. All of the diets contained (per kg): approximately 11.5 MJ of apparent metabolizable energy (AMEN), 170 g of crude protein, 34 g of Ca and 4.4 g of total phosphorus. The number of eggs and hens and their health status were monitored daily. The hen-day egg production and feed intake were calculated weekly on a per-cage basis. Egg weights were determined once per week. For the physical parameter determination, eggs were collected in the 65th, 68th, and 70th week of the hens' age, and a total of 360 eggs were analyzed. The

shell breaking strength was determined on the vertical axis using an Instron 3360 apparatus (Instron, Canton, MA, USA). Haugh units and shell index were calculated according to the formulas. The shell thickness (values measured at the sharp and blunt ends and the equator, and the three obtained values were averaged) was measured using a micrometer after removing the shell membranes. Analyses of the P and Ca content of the eggshells were conducted once during the experiment in the 66th week of age of the hens (4 eggs per sample; n = 8). Dry samples of the eggshells were ashed at 550°C. The total P in the dried eggshells was assayed using a vanadate-molybdate reagent (AOAC, 2005; method 965.17). The calcium content was determined by atomic absorption spectrometry performed using a Solar M6 instrument (TJA Solutions, Cambridge, UK). Two groups of hens were used for the molecular analysis of microbial colonization (PCR-DGGE; n = 10) and digestibility determination (n = 10) in ileum: CL without and with F Natuphos® addition (350 FTU/kg). Digestibility determination using titanium dioxide as a marker was performed according to the modified methodology of Short et al. (1996). The resulting values were analyzed using Procedure of SAS (Statistical Analysis System, Version 9.2, 2003).

Results

As is evident from Table 1, CL with F addition at 350 FTU/kg significantly (P=0.018) increased hen-day egg production (88.6%) contrary to alone CL (82.5%) or mix of CL and FL with F supplement (82.7%). Significantly lower daily feed intake (P=0.001) and feed conversion (P=0.005) were recorded in the hens fed with the mix of CL and FL without F addition and in the diet with CL together with F. The egg weight was not influenced.

Table 1. The effect of phytase and limestone particle size on performance

Phytase (F; FTU/kg)	0		350		SEM	Probability		
Limestone (L)	FL+CL	CL	FL+CL	CL		F	L	F x L
Hen-day egg production (%)	84.9^{ab}	82.5^{b}	82.7^{b}	88.6^{a}	0.88	NS	NS	0.018
Egg weight (g)	66.7	66.5	66.4	65.9	0.19	NS	NS	NS
Feed intake (g/day/hen)	116.2^{b}	116.9^{b}	122.4^{a}	116.3^{b}	0.57	0.009	0.012	0.001
Feed conversion ratio (g/g)	2.08^{b}	2.15^{ab}	2.30^{a}	2.03^{b}	0.030	NS	NS	0.005

FTU = phytase units; FL = fine limestone; CL = coarse limestone; NS = non-significant

$^{a–b}$means in the same row with different superscripts differ significantly

As regards the physical parameters of egg quality (Table 2), F addition increased eggshell quality. The phytase supplement at 350 FTU/kg significantly increased shell percentage (P=0.007), shell index (P=0.003), shell strength (P=0.017) and shell thickness measured at blunt end (P=0.015), at the middle (P=0.009), at sharp end (P=0.015) and the average value of shell thickness (P=0.006). Limestone particle size influenced only Haugh units.

Table 2. The effect of phytase and limestone particle size on egg quality

Phytase (F; FTU/kg)	0		350		SEM	Probability		
Limestone (L)	FL+CL	CL	FL+CL	CL		F	L	F x L
Haugh units	79.8	79.9	82.7	77.4	0.69	NS	0.039	NS
Albumen percentage (%)	64.4	64.5	63.9	64.4	0.18	NS	NS	NS
Yolk percentage (%)	25.8	25.4	25.9	25.2	0.16	NS	NS	NS
Shell thickness								
Blunt end (µm)	347	359	364	370	3.0	0.015	NS	NS
Equator (µm)	356	363	371	380	3.0	0.009	NS	NS
Sharp end (µm)	359	364	374	380	3.2	0.015	NS	NS
Average (µm)	354	362	370	377	2.8	0.006	NS	NS
Shell breaking strength (g/cm^2)	3,893	3,826	4,165	4,320	78.4	0.017	NS	NS
Shell index (g/100 cm^2)	8.6	8.7	8.9	9.1	0.06	0.003	NS	NS
Shell percentage (%)	9.8	10.0	10.2	10.4	0.07	0.007	NS	NS

FTU = phytase units; FL = fine limestone; CL = coarse limestone; NS = non-significant

Nonsignificant increase of Ca and P digestibility in ileum was ascertained after F addition into the mixed feed (Table 3). In addition, F supplement had an impact on microbial colonization of the digestive tract of hens. Bacteria belonging to genera *Turicibacter* and *Lactobacillus* occurred in ileum with higher frequency.

Table 3. The effect of phytase and limestone particle size on calcium and phosphorus digestibility in ileum

Phytase (FTU/kg)	0	350	SEM	Probability
Limestone	CL	CL		
Calcium (%)	49.9	53.0	4.44	NS
Phosphorus (%)	42.9	46.8	2.32	NS

FTU = phytase units; CL = coarse limestone; NS = non-significant

Conclusions

Feeding the hens a diet with coarse limestone with grain size of 1 – 2 mm, as a source of calcium, enriched with the 3-phytase at 350 FTU/kg leads to increase of performance. The addition of phytase alone improves eggshell quality, increases calcium and phosphorus digestibility and changes bacterial colonization of ileum.

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P-175 (ID 180)

THE EFFECT OF CALCIUM AND HOUSING SYSTEM ON PERFORMANCE AND EGG COMPOSITION OF TWO CONTRAST GENOTYPES

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Abstract

The objective of this study was to compare performance and egg composition of Lohmann White and Czech Hen housed in cages and on litter system in accordance to different calcium levels (3.0 vs. 3.5 %). Higher hen-day egg production (P≤0.001) and feed intake (P≤0.001) were observed in Lohmann White. Feed intake was significantly (P≤0.001) higher on litter (159.49 g/day) in comparison with cages (89.30 g/day). Egg weight was significantly (P≤0.001) affected by the interactions of all evaluated factors. The heaviest eggs (61.8 g) were detected in Lohmann White housed on litter with 3.5 % Ca and the lightest (47.3 g) were in Czech Hen housed in cage system with 3.0 % Ca. Significantly (P≤0.001) higher albumen percentage was observed in Lohmann White (61.1 %) than Czech Hen (57.6 %). Czech Hen produced eggs with significantly (P≤0.001) higher yolk percentage (26.3 %) than Lohmann White (22.7 %). Significantly (P≤0.001) higher eggshell percentage (12.2 %) was obtained in eggs from Czech Hen in comparison with Lohmann White (11.4 %). The performance and egg composition were affected by genotype and housing system; however, egg weight by interactions of all evaluated factors. Growing Ca content increased egg weight.

Keywords: Laying hen; genotype; housing; Ca; performance

Introduction

The hen performance and egg quality parameters are affected by many factors including calcium content of feed, housing system and hen genotype. Ca requirements by laying hens are considered an ongoing challenge for nutritionists and poultry producers. SAFAA et al. (2008) indicated that, increasing Ca levels significantly improved egg production, egg weight and eggshell weight. The effect of housing system on hens performance were described by ENGL-MAIEROVÁ et al. (2014) who found lower feed consumption and higher egg production in conventional cages compared to litter system. Egg weight also varies according to different housing systems. SINGH et al. (2009) detected heavier eggs on litter, whereas, TŮMOVÁ et al. (2011) in cages. Depending on laying hen genotype variances, there are differences in hen performance. Higher egg production was found in commercial genotype compared to the non-commercial ones by SINGH et al. (2009). Egg quality characteristics including egg weight, yolk weight, albumen weight and albumen/yolk ratio differed between variable brown genotypes (TŮMOVÁ et al., 2011).

This study aimed on evaluating of hen performance and egg quality characteristics in Lohmann White and Czech Hen housed in cages and on litter with different feed calcium content.

Material and Methods

Laying hens performance and egg quality parameters were evaluated in an experiment with 132 laying hens of Lohmann White and pure breed Czech Hen. The hens were housed in conventional cages Eurovent (72 hens, 550 cm^2 / hen) and on six littered pens (60 hens, 7 hens /m^2, 10 hens /pen). In both housing systems laying hens were fed feed mixtures differed in Ca content, K (control group) 3.48 % and P (experimental group) 3.0 %. The feed mixture between 20 to 40 weeks of age consisted of 16.66% crude protein (CP), 11.4 MJ of ME; and 15.37% CP, 11.48 MJ ME from 40 to 60 weeks of age.

Eggs for physical quality determination were collected in four week interval two days in row. Eggs were weighted after collection. Albumen and yolk weights were individually measured. Eggshell weight was determined after drying. The albumen, yolk, shell percentage and yolk/albumen ratio were calculated. Hen performance and egg quality data were evaluated by three-way analysis of variance using the GLM procedure (housing, genotype, calcium interactions) of SAS (SAS Institute Inc., Cary, Nc, 2003).

Results

The hen performance parameters (Table 1) were significantly (P≤0.001) affected by genotype. Higher hen-day egg production and daily feed intake were revealed to Lohmann White in comparison with Czech Hen. Similar results were reported by SINGH et al. (2009) who found higher egg production from commercial genotype in comparison with the non-commercial ones. Significant effect of Ca level (P≤0.05) was observed in egg weight with heavier eggs at 3.5% than 3.0% Ca content, which corresponds with the results of NARVAEZ-SOLARTE et al. (2006) who found an increasing linear effect of Ca level on egg weight. The effect of housing system was detected in daily feed intake with higher daily consumption on litter than cages. Similarly, ENGLMAIEROVÁ et al. (2014) detected higher feed consumption on litter compared to conventional cages.

Table 1. Main effects of genotype, housing system, and Ca content on hen performance.

Factor	Treatment	Ca	Hen-day egg production (%)	Feed intake (g)	Egg weight (g)
Czech Hen	Cage	3.00	19.18	65.12	47.34^{e}
		3.50	13.23	65.38	51.63^{cd}
	Litter	3.00	21.30	150.70	50.91^{d}
		3.50	19.15	143.70	48.20^{cd}
Lohmann White	Cage	3.00	88.20	116.06	60.86^{ab}
		3.50	81.03	110.62	60.45^{b}
	Litter	3.00	86.90	169.00	58.44^{c}
		3.50	89.55	174.55	61.83^{a}
	RMSE		11.17	10.31	6.04
Genotype			0.001	0.001	0.001
Housing			0.927	0.001	0.001
Calcium			0.959	0.560	0.033
Genotype x Housing x Ca			0.784	0.365	0.001

$^{a, b, c, d, e}$ statistically significant differences (P≤0.01) within columns are indicated by different superscripts.

Significant interaction between genotype, housing system and Ca levels was detected in egg weight. The heaviest eggs (61.83 g) were found in Lohmann White housed on litter with 3.5% Ca, while, the lightest eggs (47.34 g) were in Czech Hen housed in cages with 3.0 % Ca. Egg quality parameters were affected mainly by genotype (Table 2). Significantly (P≤0.001) higher albumen percentage and shell percentage was found in eggs from Lohmann White in comparison with Czech Hen. On the other hand, eggs from Czech Hen showed higher values of yolk percentage and albumen/yolk ratio than Lohmann White genotype. These results in agreement with the founding of (TŮMOVÁ et al., 2011) who reported significant differences in yolk weight, albumen weight and albumen/yolk ratio between variable brown genotypes. Increasing Ca levels significantly (P≤0.009) increased eggshell percentage which correspond

Table 2. Main effects of genotype, housing system, and Ca content on egg quality parameters.

Factor	Treatment	ca	Albumen percentage (%)	Yolk percentage (%)	Shell percentage (%)	Albumen / yolk ratio
Czech Hen	Cage	3.0	57.59	28.46	11.62	53.77
		3.5	57.21	26.58	10.87	56.25
	Litter	3.0	57.16	24.15	11.84	54.64
		3.5	58.40	26.11	11.39	51.99
Lohmann White	Cage	3.0	61.46	22.94	12.17	43.19
		3.5	61.32	22.72	12.45	42.97
	Litter	3.0	60.44	22.34	12.05	45.81
		3.5	61.30	22.68	12.28	43.35
	RMSE		2.62	6.74	0.890	6.41
Genotye			0.001	0.001	0.001	0.001
Housing			0.072	0.092	0.314	0.055
Calcium			0.015	0.878	0.009	0.002
Genotye x Housing x Ca			0.425	0.099	0.189	0.126

with SAFAA et al. (2008) who reported an increasing in eggshell percentage with increasing Ca levels.

Conclusions

Results of the study show that only egg weight was affected by interaction of all evaluated factors. Better performance, higher egg weight and albumen proportion in Lohmann hen is the result of the long term selection. There was a minor effect of housing system on proportion of egg components but Ca had a higher impact on egg composition.

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P-176 (ID 341)

EFFECT OF FORM OF SELENIUM USED BROILER BREEDERS' DIET ON CHICKS' GROWTH PERFORMANCE

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Abstract

The present study was conducted to evaluate performance of broilers chicks obtained from breeders receiving two different sources (Se-hydroxy-4-methylselenobutanoic acid; HMSeBA *vs.* selenium yeast) of organic selenium. Two hundred and twenty, 54 weeks old Ross-308 male (20) and female (200) breeders were fed standard breeder (female) diets containing organic selenium sources; HMSeBA or Selenium yeast from 54 to 62 weeks of age. For hatchings process, eggs were collected, selected and stored in a cool room until owing 700 eggs, 350 from each group (35 x 10 subgroup) between 58-59 weeks of age for the first hatched and 61-62 weeks of age for the second hatched, respectively. Eggs were then incubated in a single stage incubator using 35 eggs per replication in order to obtained chicks to assess effect of selenium sources in maternal diets on growth performance of progeny. Following hatchings, chicks were weighed than accommodated in 20 pens, each treatment group had 10 pens, each sized 2x3m and equipped with a tube feeder and water drinkers on litter; wood shaving litter 7-8 cm height. Chicks were fed ad libitum with standard starter, grower, finisher and withdrawal diets containing no organic selenium source but inorganic selenium for a period of 35 days under a 23:1 light: dark photoperiod. Chick's performance was assesed by measuring weigh, weigh gain, feed intake and feed conversion efficiency. At 35 days of age birds were weighed and transferred to slaughterhouse. The data obtained in the experiment were analysed using t-test.

The results obtained during the first performance tests showed that chicks from maternal receiving HMSeBA had higher body weight gain at 7 and 14 days of age ($P<0.05$). At 35 days of age, the chicks obtained from the breeder receiving HMSeBA had numerically higher ($P>0.05$) body weight, feed intake and better feed efficiency ($P>0.05$) than those of maternal receiving selenium yeast. The results of the second performance test revealed only numerically difference ($P>0.05$) in the favour of chicks obtained from the breeders receiving HMSeBA. However, the performance values obtained from the both groups were found to be greatly higher than breeder's expectation in the both trials. All these results may suggest that providing HMSeBA to breeder diets could be of value increasing progeny performance in terms of growth performance with a higher efficacy.

Keywords: Organic Selenium, Breeder, Egg, Hatching, Broiler, Performance

Introduction

It is well documented that selenium with the conjunction of vitamin E is the vital nutrient source to maintain health, growth and product quality in mammals and birds (1). Vitamin E is a fat-soluble nutrient found in body fat depots, plasma lipoproteins, and cell membrane phospholipids, where it serves as an important antioxidant (2). Selenium (Se) fulfils an antioxidant role as a component of glutathione peroxidises (GSHPx). Se is widely distributed in the body, but the most labile reservoir is in the liver (2). It is found in body tissue principally as selenomethionine (SeMet) or as selenocysteine (SeCys), the latter found in GSHPx. Both nutrients are should be examine together because of their related functions and similar deficiency signs. However, Se and vitamin E each have unique metabolic roles, and the factors that alter the oxidative state of an animal may differentially affect their dietary needs. It is now well known that Se plays an important role in maintaining semen quality (3). An optimal Se status in male birds is considered to be an important factor in ensuring the fertility of breeding stocks, while an optimal Se status of the eggs of females needs for antioxidant system to maintain embryo development and also hatchability (4).

In poultry nutrition, selenium requirements meet through trace mineral premixes. Recent literature showed that besides the rational source of supplemental selenium in mineral forms mainly sodium selenite in animal diets, organic selenium sources have also been developed through selenize yeast with Se in the form of selenomethionine and also 2-hydoxy-4-methylselenobutanoic acid (HMSeBA) in recent years (5). Comparative studies showed that organic Se source provides some advantages with higher bioavailability and lower inclusion rate in the diet (5). However no comparative studies have been undertaken to evaluate different sources of organic selenium in female broiler breeders in terms of reproduction performance, egg quality, hatchability and chicks' performance.

The present project was aimed to evaluate organic sources of Se-hydoxy-4-methylselenobutanoic acid (HMSeBA of Adisseo; Selisseo) in contrast to another organic selenium source (selenium yeast) used in broiler breeders' diet on chicks' growth performance.

Materials and Methods

The present study was carried out in the Broiler Breeder Unit of the Experimental Farm of the Department of Animal Science, Faculty of Agriculture, University of Çukurova, Adana-Turkey. Two hundred and twenty, 54 weeks of age Ross-308 male (20) and female (200) breeders were used in the experiment lasted 9 weeks. Throughout the experimental study standard breeder (female) diet based on corn and soya was used supplemented with selenium in the form of SE-yeast or HMSeBA at 200 ppb.

During the experiment, hatching performance of the eggs obtained from the birds receiving HMSeBA or Se-yeast was performed by 21 day-incubation twice. For hatchings process, egg selected and stored in a cool room until owing 700 eggs, 350 from each group (35x10 subgroups) between 58-59 weeks of age for the first hatched and 61-62 weeks of age for the second hatched, respectively. Eggs were incubated in a single stage incubator using 35 eggs per replication. Temperature was controlled according to McQuoid (6). Hatching was completed on the 21st day. The chicks were carefully removed from the pouches and their weights were determined by using an electronic scale with a sensitivity of 0.01 g. Chicks were then accommodated in 20 pens, each treatment groups has 10 pens sized 2x3m and equipped with a tube feeder and water drinkers on litter; wood shaving litter 7-8 cm height. Chicks were fed 35 days ad libitum under a 23:1 light: dark photoperiod. The chicks obtained from the eggs of a group/subgroup were housed by name of the maternal group/subgroup number to fellow on the same axis to maternal to its chicks. Chicks were received four stages (starter; CP:24%, ME:3050 Kcal/kg, grower; CP:22%, ME:3150 Kcal/kg, finisher; CP:21%, ME:3250 Kcal/kg, and withdrawal; CP:20%, ME:3250 Kcal/kg, feeds) feeding programme with the diets containing conventional vitamins and trace mineral premixes, with 150 ppb selenium as sodium selenite. During the experiment, live weight, feed intake and feed efficiency were recorded weekly in subgroup (10 replicates in each group) level. At 35 days of age, birds were attained the weight given for 42 days of age by the Breeder Handbook (7), therefore feeding was ceased and birds were taken slaughterhouse one week earlier. The data obtained in the study were analysed using t-test procedure of SAS; the Statistical Analysis System (8). Result obtained in this study will be presented as means per bird with standard errors of the difference between means (SED) and CV.

Results and Discussion

At the end of the first and the second incubations, all the chicks obtained at hatching were subjected to growth performance test by feeding starter, grower, finisher and withdrawal feeds containing no organic selenium source but inorganic selenium (sodium selenite) for a period of 35 days during which all the animals were allocated in a group named by its maternal group. When the project was proposed in the first place, broiler performance tests at the end of the both incubation were planned to carry out 42 days, however, in order to control leg problems due to enormously fast growing during the testing period did not let trials to be run 42 days. At 35 days of age in the first and the second trials broilers had to be taken slaughterhouse. The results are given here were obtained at 35 days of age in the first and the second performance test.

The results obtained during the first performance test were given in Table 1. The chicks of the both groups grew very fast. Especially chicks from maternal receiving HMSeBA had mean slaughter weight of 2670 grams at 35 days of age, being a 7-days earlier and 0.09 better feed conversion rate (1.52) than breeder's expectation (1.61) at 35 days of age. Chicks from maternal receiving Se-Yeast had mean slaughter weight of 2606 grams at 35 days of age, being a 5-days earlier and 0.10 better feed conversion rate (1.51) than breeder's expectation (1.61) at 35 days of age. The results also showed that at the end of the feeding period, the chicks, whose maternal received HMSeBA attained body mass almost 6% higher than those of the birds of Se-Yeast group. The both groups had similar liveability, feed intake and also feed conversion rate (Tables 1 and 2) throughout the feeding period.

The results obtained during the second performance test were given in Table 3. The chicks of the both groups grew very fast similar to the first performance test. Especially chicks from maternal receiving HMSeBA had mean slaughter weight of 2531 grams at 35 days of age, being a 6-days earlier and 0.11 better feed conversion rate (1.49) than breeder's expectation (1.61) at 35 days of age. Chicks from maternal receiving Se-Yeast had mean slaughter weight of 2481 grams at 35 days of age, being a 4-days earlier and 0.06 better feed conversion rate (1.55) than breeder's expectation (1.61) at 35 days of age. The results also showed that at the end of the feeding period, the

chicks, whose maternal received HMSeBA attained body mass almost 6% higher than the birds of Se-Yeast group. The both groups had similar liveability and feed intake (Tables 3 and 4) but HMSeBA group attained 0.06 better feed conversion rate (Table 4) than Se-yeast group.

Table 1. Body weight gain of chicks obtained at the first incubation (g/chick).

Parameters	**HMSeBA** (n=10)			**Se-Yeast** (n=10)			P=
	Mean	Stderr	CV	Mean	Stderr	CV	
Number of eggs placed hatchery	**35**	0.00	0.00	35	0.00	0.00	0.0000
No of total chicks at hatching	**22.40**	1.56	22.09	21.22	1.31	18.52	0.5762
Body weight at day 0	**49.25**	1.28	0.28	50.78	0.78	3.43	0.1021
B.W. gain at 7 days old	**134.66**	2.00	3.32	119.81	4.90	9.15	**0.0230**
B.W. gain at 14 d old	**445.89**	5.92	2.97	419.80	9.27	4.94	**0.0451**
B.W. gain at 21 days old	**893.38**	12.80	3.20	855.43	1.94	3.12	0.0620
B.W. gain at 28 days old	**1756.64**	21.87	2.78	1725.51	18.06	2.34	0.3042
B.W. gain at 35 days old	**2620.76**	34.77	2.97	**2555.55**	40.84	3.57	0.2587
B.W. at 35 days old	**2670.01**	21.99	2.72	**2606.32**	40.11	3.44	0.3201
Expected body weight at 35 days old	**2021**						**Difference**
No of birds at 35 days old	**209** (107+102)			**202** (103+99)			7 (3.5%)
Body Mass (kg/group)	**558** (2670x209)			**526** (2606x202)			32 (6%)
Liveability (%)	**93%** (209/224)			**92%** (202/220)			1%

Table 2. Cumulative feed intake and feed conversion efficiency of chicks obtained at the first incubation.

	Feed Intake (g/chick)					Feed Conversion Rate (g feed/g gain).				
Weeks of age	**HMSeBA**		**Se-Yeast**		P=	**HMSeBA**		**Se-Yeast**		**P=**
1	**Mean**	sderr	**Mean**	Sderr		**Mean**	Sderr	**Mean**	Sderr	
2	**159.68**	7.34	**161.28**	8.01	0.8863	**1.19**	0.05	**1.36**	0.10	0.1624
3	**605.79**	19.95	**618.85**	31.97	0.7379	**1.36**	0.03	**1.48**	0.09	0.2229
4	**1483.92**	42.05	**1503.51**	71.12	0.8185	**1.46**	0.04	**1.46**	0.09	0.3654
5	**2553.23**	68.30	**2530.78**	97.68	0.3343	**1.45**	0.04	**1.47**	0.05	0.5004
6	**3973.32**	89.20	**3859.62**	108.3	0.1663	**1.52**	0.04	**1.51**	0.04	0.4107
Expected at 6 weeks of age	**3248**					**1.61**				

Table 3. Growth performance (g/chicks) of chicks obtained at the second incubation.

Parameters	**HMSeBA** (n=10)			**Se-Yeast** (n=10)			P=
	Mean	Stderr	CV	Mean	Stderr	CV	
Number of eggs placed hatchery	**35.00**	0	0	**33.70**	1.3	12.2	0.3306
No of total chicks at hatching	**22.80**	1.5	20.76	**21.80**	1.39	20.15	0.6302
Body weight (B.W.) at day 0	**44.67**	0.35	1.77	**44.09**	0.26	1.29	0.2230
B.W. gain at 7 d old	**90.70**	2.83	6.98	**89.42**	2.51	6.27	0.7429
B.W. gain at 14 d old	**349.52**	8.06	5.16	**341.86**	8.56	5.60	0.5332
B.W. gain at 21 d old	**955.95**	17.24	4.03	**924.98**	13.30	3.21	0.1927
B.W. gain at 28 d old	**1708.89**	37.84	4.95	**1658.12**	24.09	3.25	0.2906
B.W. gain at 35 d old	**2486.39**	42.62	3.83	**2480.79**	50.31	4.53	0.4724
B.W. at 35 d old	**2531.06**	42.46	3.75	**2480.79**	50.31	4.53	0.4671
Expected body weight at 35 days old	**2021**						**Difference**
No of birds at 35 days old (male+female)	**210 (99+111)**			**202 (94+108)**			8 (3.5%)
Body Mass (kg/group)	**531** (2531x210)			**501** (2480x202)			30 (6%)
Liveability (%)	**92% (210/228)**			**92% (202/218)**			-

Table 4. Cumulative feed intake and feed conversion efficiency of chicks obtained at the second incubation.

	Feed Intake (g/chick)					Feed Conversion Rate (g feed/g gain).				
Weeks of age	**HMSeBA**		**Se-Yeast**		P=	**HMSeBA**		**Se-Yeast**		**P=**
1	**Mean**	sderr	**Mean**	Sderr		**Mean**	Sderr	**Mean**	Sderr	
2	**114.65**	5.78	**111.06**	7.79	0.5276	**1.25**	0.04	**1.24**	0.08	0.3143
3	**482.59**	21.94	**489.95**	15.22	0.8743	**1.38**	0.06	**1.43**	0.06	0.5344
4	**1295.50**	40.34	**1278.83**	27.25	0.7558	**1.35**	0.04	**1.38**	0.03	0.6059
5	**2336.88**	42.47	**2323.26**	31.90	0.8042	**1.37**	0.03	**1.40**	0.02	0.4355
6	**3702.46**	59.95	**3765.21**	46.41	0.4318	**1.49**	0.01	**1.55**	0.04	0.2324
Expected at 6 weeks of age	**3248**					**1.61**				

Conclusion

The results obtained in the experiment showed that chicks from the maternal receiving HMSeBA had numerically higher (P>0.05) slaughter weight and better (P>0.05) feed conversion rate than chicks of Se-Yeast group.

The performance values of broilers obtained from the both groups were found to be greatly higher than breeder's target in both trials.

The results of the present study reported here suggest that providing selenium in organic form, especillay HMSeBA could be of value increasing progeny performance in terms of growth performance.

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P-177 (ID 342)

EFFECT OF SELENIUM SOURCE ON EGG PRODUCTION AND EGG QUALITY OF BROILER BREEDERS

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Abstract

The present study was conducted to evaluate two different sources (Se-hydoxy-4-methylselenobutanoic acid; HMSeBA vs. selenium yeast) of organic selenium in female broiler breeders in terms of egg production, fertility, egg quality, hatchability and chick growth performance. Two hundred and twenty, 54 weeks old Ross-308 male (20) and female (200) breeders were used in the experiment. Standard breeder (female) diet based on corn and soya were used. Birds were placed in a complete randomized design with 2 dietary treatments (HMSeBA vs. Selenium yeast) and 10 replications of 10 females and 1 males per pen sized 2x1.5m. Each pen had five nests with a female tubular feeder and one individual male feeder, and nipple drinkers. The experiment occurred while the broiler breeders were from 54 to 62 weeks of age and the lighting and feeds were supplied according to the Breeders' recommendations. Eggs were collected everyday and measured for exterior and interior quality measurements once a week. Throughout the experiment, feed intake, feed conversion efficiency, egg production, egg yield, hen-day egg production were recorded. The data obtained in the study were analysed statistically by t-test.

The results showed that birds attained almost 240 grams of weigh in both groups without any significant difference (P>0.05). Feeding the birds according to the breeder's recommendation maintained animal performance as breeders' target in terms of body weight, fertility and also hatchability and hatching yield. The results showed that providing selenium in the form of HMSeBA or Se-yeast did not affect egg production significantly (P>0.05), but the group receiving HMSeBA gave numerically higher number of eggs and attained higher egg weigh and egg mass with better feed conversion rate. The results with respect to egg quality showed that at the last week of the trial eggs obtained from the group receiving HMSeBA exhibited better values in terms of egg shell strength (P<0.07), shell thickness (P<0.09), yolk height (P<0.06) and yolk width (P<0.01). The results obtained in the trial suggest that providing dietary organic selenium in the form of HMSeBA could be of value in improving egg quality of broiler breeder hens.

Keywords: Organic Selenium, Breeder, Egg, Fertility, Hatching, Broiler, Performance

Introduction

It is well documented that selenium with the conjunction of vitamin E is the vital nutrient source to maintain health, growth and product quality in mammals and birds (1). Vitamin E is a fat-soluble nutrient found in body fat depots, plasma lipoproteins, and cell membrane phospholipids, where it serves as an important antioxidant (2). Selenium (Se) fulfils an antioxidant role as a component of glutathione peroxidises (GSHPx). Se is widely distributed in the body, but the most labile reservoir is in the liver (2). It is found in body tissue principally as selenomethionine (SeMet) or as selenocysteine (SeCys), the latter found in GSHPx. Both nutrients are should be examine together because of their related functions and similar deficiency signs. However, Se and vitamin E each have unique metabolic roles, and the factors that alter the oxidative state of an animal may differentially affect their dietary needs. It is now well known that Se plays an important role in maintaining semen quality (3). An optimal Se status in male birds is considered to be an important factor in ensuring the fertility of breeding stocks, while an optimal Se status of the eggs of females needs for antioxidant system to maintain embryo development and also hatchability (4).

In poultry nutrition, selenium requirements meet through trace mineral premixes. Recent literature showed that besides the rational source of supplemental selenium in mineral forms mainly sodium selenite in animal diets, organic selenium sources have also been developed through selenize yeast with Se in the form of selenomethionine and also 2-hydoxy-4-methylselenobutanoic acid (HMSeBA) in recent years (5). Comparative studies showed that organic Se source provides some advantages with higher bioavailability and lower inclusion rate in the diet (5). However no comparative studies have been undertaken to evaluate different sources of organic selenium in female broiler breeders in terms of laying performance and egg quality.

The present project was aimed to evaluate effect(s) of organic sources of Se-hydoxy-4-methylselenobutanoic acid (HMSeBA of Adisseo; Selisseo) in contrast to the other organic selenium source as selenium yeast on laying performance and egg quality of broiler breeders.

Materials and Methods

The present study was carried out in the Broiler Breeder Unit of the Experimental Farm of the Department of Animal Science, Faculty of Agriculture, University of Çukurova, Adana-Turkey. Two hundred and twenty, 54 weeks of age Ross-308 male (20) and female (200) breeders were used in the experiment lasted 9 weeks. Experimental animals were weighed at the first day and last day of the trial to assess weigh changes throughout the experiment, in which standard breeder (female) diet based on corn and soya was used.

Two hundred female Ross-308 and 20 Ross-308 male broiler breeders were placed in the unit at 52 weeks of age. After 2 weeks pre-feeding period they were allocated according to body weight and also egg production in a completely randomized design into 2 treatment (0.2 ppm Se-Yeast or 0.2.ppm HMSeBA) groups with 10 replicate pens, 10 females and 1 males per pen sized 2.0 x 1.5 m in the breeder unit, where 20 subgroup pens were available for the trial. Extra 5 males were raised separately to replace sexually inactive or dead males.

Each pen of the breeder unit had five nests bedded wood shaving, sizeing 25x43x35 cm each, a female tubular feeder and one individual male feeder, and nipple drinkers. Animals were placed on wood shaving litter 7-8 cm height. The experiment lasted while the broiler breeders were from 54 to 62 weeks of age and the lighting and feeds (female: 156 gram/day, male: 130 gram/day) were supplied according to Ross Breeders recommendations (6) without drinking water restriction. The environmental temperature was maintained within the animal comfort zone using foggers and tunnel ventilation. Male performance was watched and the male with low mating performance was replaced with a spare one. Eggs were collected every day and measured for exterior quality (size, weigh, and crack incidents) measurements. Weekly egg production, hatching eggs, and eggs with defects were obtained through daily collections and were expressed in percentage. Defective eggs were considered those with physical deformities and those bearing cracks. Double yolk and floor eggs will be added up to total production, but were not incubated. In the experiment feed intake, feed conversion efficiency, egg production, egg yield, hen-day egg production, mortality rate were recorded. In the third week of the trial and thereafter (57-62th weeks of age) eggs obtained on the third day of every week analysed for interior quality (albumen, yolk measurements, Haugh Unit etc.) and also exterior quality measurements such as egg shape index, shell thickness, shell weigh, shell strength etc. The data obtained in the study were analysed using t-test procedure of SAS; the Statistical Analysis System (7). Result obtained in this study will be presented as means per bird with its standard error.

Results and Discussion

In order to observe body weigh changes of breeders throughout the experiment, at the beginning of the experiment when the birds 54th week of age, and at the end of the experiment when the birds 62nd week of age, all the birds were weighed individually. The results with respect to body weight changes of the hen showed that birds attained almost 240 grams of weigh in both groups without significant difference (Table 1). Throughout the experiment, the birds were given feed according to Breeder company recommendation. Foods were given to female birds 156 gram/day, male birds 130 gram/day. Feeding the birds according to the breeder's recommendation keep animal performance as breeders' target in terms of body weight, fertility and also hatchability and hatching yield. The results with respect to body weigh changes and feed intake are given in Table 1.

Table 1. Live weigh changes and feed intake of birds throughout the experiment.

Parameters	**HMSeBA (n=10)**		**Se-Yeast (n=10)**		**P=**
	Mean	Std Error	**Mean**	Std Error	
Live weigh at the first day of the trial (g/hen)	**4696.7**	27.25	**4737.9**	29.83	0.3213
Live weigh at the last day of the trial (g/hen)	**4940.0**	35.75	**4976.2**	36.02	0.4862
Weigh changes (g/hen)	**243.3**	39.84	**238.33**	22.28	0.9172
Feed Intake (g/hen/day)	**156**		**156**		
Feed Intake (g/cock/day)	**130**		**130**		

After 2 weeks pre-feeding period from 52-53 weeks of age, animals were grouped according to body weight and also egg production. At 54 weeks of age and thereafter laying performance parameters were analysed weekly. The results with respect to laying performance at 56 and 62 (two weeks after and eight weeks after the initiation of the experiment) weeks of age are given in Table 2. The results showed that providing selenium in the form of HMSeBA or Se-yeast did not affect egg production significantly (P>0.05). However, mean production values at 62nd week of age seemed to be favour of HMSeBA groups (Table 2). Addtionally, the performance values obtained at 56-62 weeks of age in the present trial were higher than the values set by the Breeder Company (6).

Table 2. Egg Production of the breeders at the second week and last week of the trial

	56th weeks of age					62nd weeks of age				
Parameters	**HMSeBA** (n=10)		**Se-Yeast** (n=10)		P=	**HMSeBA** (n=10)		**Se-Yeast** (n=10)		**P=**
	Mean	Stderr	**Mean**	Stderr		**Mean**	Stderr	**Mean**	Stderr	
Egg production (number/week)	**4.78**	0.34	**4.65**	0.25	0.293	**4.13**	0.22	**3.86**	0.20	0.368
Egg weight (g/day)	**46.79**	3.03	**45.59**	2.22	0.215	**41.79**	2.23	**38.85**	2.02	0.342
Egg weight (g/egg)	**68.67**	0.25	**68.76**	0.38	0.850	**70.76**	0.22	**70.52**	0.50	0.657
Egg mass (g/week)	**327.6**	23.47	**319.37**	17.22	0.215	**292.54**	15.38	**271.95**	14.17	0.342
Feed Conversion Rate (g feed/g egg)	**3.19**	0.23	**3.52**	0.24	0.332	**3.74**	0.22	**4.01**	0.23	0.402

The results with respect to quality of eggs at the beginning (56th week of age) and at the last weeks (62nd week of age) of the experiment are given in Tables 3. The results showed at the beginning of the experiment all the quality measurements of the treatment groups were similar to each other. However at the last weeks of the trial eggs obtained from the group receiving HMSeBA exhibited better values in terms of egg shell strength (P<0.07), shell thickness (P<0.1), yolk height (P<0.06) and yolk width (P<0.01). The improvement in egg quality could be attributed to selenium and selenomethionine role in synthesis of egg shell matrix, mineralization and also its contribution to antioxidant capacity and membrane integrity.

Table 3. Quality measurements of eggs obtained at the beginning and end of the trial.

	56th weeks of age					62nd weeks of age				
Parameters	**HMSeBA** (n=10)		**Se-Yeast** (n=10)		P=	**HMSeBA** (n=10)		**Se-Yeast** (n=10)		**P=**
	Mean	Stderr	**Mean**	Stderr		**Mean**	Stderr	**Mean**	Stderr	
Egg weight (g)	**69.25**	0.774	**68.40**	0.772	0.436	**72.66**	0.88	**71.68**	0.65	0.3688
Albumen weight (g)	**40.09**	0.532	**39.68**	0.624	0.615	**41.35**	0.79	**40.79**	0.57	0.5676
Yolk weight (g)	**22.54**	0.241	**21.95**	0.308	0.134	**24.02**	0.43	**23.52**	0.31	0.1363
Egg shell weight (g)	**6.63**	0.099	**6.72**	0.090	0.485	**7.43**	0.14	**7.53**	0.14	0.6279
Shell strength (kg/cm^2)	**3542**	181.0	**3564**	155.1	0.858	**3793**	173.0	**3599**	152.86	**0.0664**
Egg width (mm)	**45.54**	0.18	**45.72**	0.332	0.992	**45.54**	0.17	**46.06**	0.16	0.3908
Egg length (mm)	**59.93**	0.36	**60.07**	0.399	0.676	**60.17**	0.49	**61.26**	0.36	0.4717
Egg Shape index (%)	**76.08**	0.50	**76.23**	0.61	0.779	**75.85**	0.61	**75.28**	0.53	0.8537
Yolk height (mm)	**18.96**	0.134	**19.03**	0.182	0.788	**19.94**	0.26	**19.37**	0.14	**0.0521**
Albumen height (mm)	**5.89**	0.221	**5.91**	0.189	0.159	**6.42**	0.29	**5.63**	0.21	0.1669
Yolk width (mm)	**47.16**	0.637	**46.69**	0.512	0.626	**46.17**	0.50	**50.27**	1.08	**0.0012**
Albumen width (mm)	**86.59**	2.942	**85.24**	2.013	0.706	**88.40**	3.62	**87.17**	1.81	0.7586
Albumen length (mm)	**106.48**	4.014	**100.9**	1.861	0.214	**112.37**	3.79	**110.20**	1.75	0.59780
Shell thickness (µm)	**324.72**	3.703	**333.1**	4.316	0.142	**358.47**	5.65	**339.78**	5.11	**0.0981**
Haugh Unit	**71.60**	1.27	**71.66**	1.60	0.977	**74.33**	1.79	**70.86**	1.37	0.2123

It is well documented that selenium is an essential nutrient. Its role in metabolism is mainly related to the synthesis of Se-amino acid and Se-protein complexes that act as potent antioxidants. In addition to its antioxidant function, selenium has potential to affect egg quality. Wakebe (8) and Papas et al. (9) showed that Se addition to layer diets can

mitigate the reduction of Haugh units in stored eggs. Our results on increased shell strength are confirmed by previous studies (10), they reported that shell strength could be related to higher Se concentration in the shell and shell membrane. These two last factors are particularly increased when diet contains organic Se sources, suggesting that the high Se concentration could be the main factor for increased shell strength through its role in formation of organic matrix. It is well-known that eggshell membrane is mainly consisted of fibrous protein or collagen-like proteins (11), which derives organic matrix. It has been shown that fortifying poultry feeds with selenium, especially organic source, have a great potential to increase egg selenium contents (12). Our results suggest that selenium in the egg have a powerful agent with its antioxidant properties and also being a significant factor in synthesis of organic matrix of egg shell. It is also reported that the natural selenium-containing antioxidant enzymes like glutathione peroxidise and thioredoxing reductase have the potential to reduce the effect of free radicals on the aging process (13). Disulfide bonds (bonds between sulphur atoms) can cross-link proteins, decreasing enzyme function and increasing the sinew associated with aging collagen. Protein oxidation is known to be related to selenium deficiency (14). The present knowledge with respect to selenium and its protective effect on structural protein could be suggested that selenium is a key factor for the formation of organic matrix derived by shell membrane by maintaining integrity of fibrous protein or collagen-like proteins, leading to increase in shell strength.

Conclusion

The results with respect to body weight changes of the hen during the trial showed that birds attained almost 240 grams of weigh in both groups without a significant difference ($P<0.5$).

Providing selenium in the form of HMSeBA or Se-yeast did not affect egg production significantly ($P>0.05$).

The performance values of breeders obtained from the both groups in the present study were higher than the values set by Breeder Company; Ross.

The breeder receiving dietary HMSeBA exhibited better values in terms of egg shell strength ($P<0.07$), shell thickness ($P<0.1$), yolk height ($P<0.06$) and yolk width ($P<0.01$).

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P-180 (ID 306)

PHOSPHORUS AND CALCIUM DIGESTIBILITY IS IMPROVED WITH THE ADDITION OF A CARBOHYDRASE COMPLEX IN BROILER CHICKENS FED WHEAT-SOYBEAN-BASED DIETS

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Abstract

This study aimed to evaluate the efficacy of a carbohydrase complex (CC) in improving the digestibility of P and Ca in broilers' wheat-soybean-based diets, compared to that observed for a single 6-phytase. Four diets were formulated with different available phosphorus levels (2.0, 2.7, 3.4, and 4.1 g kg^{-1}), with a fixed Ca:P ratio. To each diet, either CC (Rovabio® ADVANCE L) at 200 mL ton^{-1} or a 6-phytase (Phyzyme® XP 5000L) at four different inclusion levels (0, 80, 160, and 240 FTU kg^{-1}) was added. For each diet, six birds were reared between d 10-28 in individual cages suitable for digestibility trials. Excreta collections were performed at d 15-18 and d 25-28 for the determination of the apparent digestibility of P and Ca. We estimated the potential of the CC in comparison with the phytase effect using the factorial regression of dietary P levels and the phytase inclusion. No significant interaction was observed between P levels and phytase inclusion. P levels promoted a linear reduction ($P<0.001$) of the digestibility of both minerals in the two periods of evaluation. The phytase inclusion promoted a linear improvement ($P<0.05$) of P and Ca digestibility. The addition of the CC increased ($P<0.05$) the P and Ca digestibility. For P apparent digestibility, the improvement rate was 7.9% and 3.8% respectively at d 18-21 and d 25-28; for Ca, it was 5.0% and 5.8%. These improvements were observed regardless the level of P in the diets.

Keywords: NSP-enzymes, phytase, calcium, phosphorus, digestibility.

Introduction

The model the action of the carbohydrases in improving the global digestibility of feeds is mediated by effects on digesta viscosity, on the physical accessibility to nutrients and on modifications in the intestinal microflora. All these effects are associated to the partial or complete hydrolysis of both soluble and insoluble non-starch polysaccharides (NSP) chains from plant raw material (Adeola and Cowieson 2010). Since these NSP, as main constituents of the cell wall, act to 'envelope' nutrients in the cell cytoplasm; this effect of NSP is frequently called "cage effect" (Smeets et al. 2014). Thus, by adding NSP-carbohydrases into feeds, not only nutrients directly released by the hydrolysis (such as monomeric sugars from fibres, for example) are made available to the normal digestion process of animal, but all other nutrients of interest present inside intact vegetable cells and other caged compartments can be acceded (Smeets et al 2013). Several studies have reported the effect of NSP-carbohydrases in promoting accessibility and indirectly improvement of digestibility of amino acids, starch and fat (Slominsky 2011). However, little is known about the effect on other compounds with nutritional and economic interest This study aimed to evaluate the efficacy of a carbohydrase complex (CC) in improving the digestibility of P and Ca in broilers' wheat-soybean-based diets, compared to that observed for a single 6-phytase.

Material and Methods

This study was carried out in the Centre of Expertise and Research in Nutrition (CERN), Adisseo France SAS, in Malicorne, France (46°18'14" N, 2°44'07" E), between October and November 2014. The 120 male Ross PM3 broilers were divided in 20 treatments, with six repetitions (individual cages suitable for digestibility trials) of 1 birds each between 1 and 28 days of age, in 2 feeding phases: initial (d1-7), grower (d8-28). Four diets were formulated with different available phosphorus levels (2.0, 2.7, 3.4, and 4.1 g kg^{-1}), with a fixed Ca:P ratio of 1.40. To each diet, either CC (Rovabio® ADVANCE L at 200 mL ton^{-1}) or a 6-phytase (Phyzyme® XP 5000L) at four different inclusion levels (0, 80, 160, and 240 FTU kg^{-1}) was added. The CC and the 6-phytase were not evaluated in combination. Excreta collections were performed at d15-18 and d25-28 for the determination of the apparent digestibility of P and Ca. By considering the factorial regression of dietetic P level and phytase inclusion rate, were estimated the digestibility improvement potentials on P and Ca of the CC in comparison with the phytase effect, in FTU kg^{-1}.

Results

No significant interaction was observed between dietary available P levels and phytase inclusion. P levels promoted a linear reduction (P<0.001) of the digestibility of both minerals in the two periods of evaluation. The phytase inclusion promoted a linear improvement (P<0.05) of P and Ca digestibility. The prediction equations obtained from the factorial regression of dietary available P levels and phytase levels on P and Ca apparent digestibility had R^2 between 0.35 and 0.60 (Table 1).

Table 1. Prediction equations obtained from the factorial regression of dietary available P levels (x, in %) and phytase levels (y, in FTU kg^{-1}) on apparent digestibility P and Ca at d18 and d28.

Parameter	Prediction equation	R^2 (Prob.)
Dig. P (d18)	$69.193 + 53.308x + 0.102y – 210.713x^2 – 0.148xy – 6.063E10^{-5}y^2$	0.349 (0.032)
Dig. P (d28)	$72.763 + 57.365x + 0.063y – 220.908x^2 – 0.091xy – 5.373E10^{-5}y^2$	0.506 (0.004)
Dig. Ca (d18)	$69.357 + 102.603x + 0.082y – 262.085x^2 – 0.140xy – 1.416E10^{-6}y^2$	0.436 (0.027)
Dig. Ca (d28)	$67.847 + 22.160x + 0.006y – 195.413x^2 – 0.064xy – 1.182E10^{-5}y^2$	0.591 (0.009)

The addition of the CC significantly increased (P<0.05) the P and Ca digestibility at d18 and d28 (Table 2). For P apparent digestibility, the improvement rate was 7.9% and 3.8% respectively at d 18-21 and d 25-28; for Ca, it was 5.0% and 5.8% (Table 3).

Table 2. Digestibility of P and Ca in different diets varying the available P levels with or without the addition of a carbohydrase complex (CC) at d18 and d28.

Dietary P_{av} levels (%)	Dig. P (d18, %)		Dig. Ca (d18, %)		Dig. P (d28, %)		Dig. Ca (d28, %)	
CC Addition	without	with	without	with	without	with	without	with
0.20	71.40	73.60	72.31[b]	75.51[a]	75.67[b]	81.2[a]	65.14	66.05
0.27	71.16	73.70	74.25	77.24	72.15	72.55	60.66	62.32
0.34	60.57[b]	69.54[a]	67.31[b]	72.75[a]	67.00	68.47	50.23[b]	55.16[a]
0.41	55.43[b]	60.93[a]	60.68	62.72	58.97[b]	61.97[a]	44.65[b]	48.79[a]
Average (all P levels)	64.64[b]	69.44[a]	68.64[b]	72.06[a]	68.45[b]	71.05[a]	55.17[b]	58.08[a]

[a] Different letters in same line means significant difference (P<0.05)of digestibility between “without” and “with” addition of the CC by the Mann-Whitney non-parametric test.

Table 3. Improvement rate of digestibility of P and Ca in different diets varying the available P levels promoted by the addition of a carbohydrase complex (CC) at d18 and d28.

Dietary P_{av} levels (%)	Dig. P (d18)	Dig. Ca (d18)	Dig. P (d28)	Dig. Ca (d28)
0.20	3.08%	4.42%*	7.32%*	1.40%
0.27	3.56%	4.03%	0.56%	2.74%
0.34	14.82%*	8.08%*	2.19%	9.82%*
0.41	9.93%*	3.37%	5.08%*	9.27%*
Average (all P levels)	7.85%*	4.98%*	3.79%*	5.81%*

* Significant (P<0.05) improvement rate of digestibility by the Mann-Whitney non-parametric test.

By using the prediction equations with the improvements observed with the addition of CC, it was found that this complex had a potential compared to 99.6 FTU kg^{-1} for P digestibility and 108.9 for Ca digestibility (Table 4).

Table 4. Improvement rate of digestibility of P and Ca in different diets varying the available P levels promoted by the addition of a carbohydrase complex (CC) at d18 and d28, compared with that observed for the effect of inclusion of a 6-phytase (presented in FTU kg^{-1}).

Dietary P_{av} levels (%)	Dig. P (d18)	Dig. Ca (d18)	Dig. P (d28)	Dig. Ca (d28)
0.20	56.30	58.24*	193.44*	143.45
0.27	53.14	119.31	24.28	131.17
0.34	158.45*	140.82*	107.01	89.98*
0.41	131.77*	58.76	72.87*	127.90*
Average (all P levels)	99.92*	94.28*	99.40*	123.11*

* Significant (P<0.05) improvement rate of digestibility by the Mann-Whitney non-parametric test.

These results confirms the hypothesis that NSP-carbohydrases are capable to release internal nutrients present caged by cell wall in plant compartments, even if those nutrients are not directly linked to enzyme substrates: the NSP. Once released, these internal nutrients become accessible for normal digestion process. Even if most part of phosphorus inside plant tissues are presented as phytate – normally indigestible for monogastrics – endogenous alkaline phosphatase can hydrolase the phosphate radicals from myo-inositol mono, di and triphosphate (Abudabos 2012). Besides, all sources of phosphorus (plant or mineral) in the experimental feed were took into account in the measured digestibility. The expected reduction of digest viscosity by the CC can also played an important role in the indirect improvement of mineral digestibility, as observed in several studies with other nutrients (Smulikowska and Mieczkowska 1996; Langhout et al. 1997).

Conclusions

The study demonstrated the efficacy of the evaluated carbohydrase complex in improving the digestibility of phosphorus and calcium, giving a strong scientific support to the concept of global digestibility improvement by this enzymatic complex.

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P-185 (ID 287)

BONE ASH AS A CRITERION FOR ASSESSING MINERALISATION IN BROILERS

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Abstract

Two common divergences in the methodology for determining the degree of bone mineralisation were examined. The first objective was to compare the effect of varying fat extraction time on poultry tibia bones. The second objective was to evaluate the effect of autoclaving bones prior to fat extraction on bone ash. 264 day old Ross 308 male broilers were fed standard commercial diets and were allocated to 33 replicate pens; 8 birds per replicate. At 42d, 1 bird per replicate pen was culled. For objective 1, all adhering tissues were removed from the right tibia bones. The bones, including their cartilage caps, were then dried at 105°C for 24h prior to fat extraction by Soxhlet extraction for 1h using petroleum ether, and then dried at 105°C for 24h and dry bone weight recorded. This process was cumulatively repeated 8 times in total. For objective 2, the left tibia bones were autoclaved and then dried at 105°C for 24h prior to fat extraction in petroleum ether for 8h. All bones from right and left tibias were then ashed at 650°C for 24h. There were significant increases in fat extracted for each additional hour from 1-5h, $P<0.05$. After 5 hours of extraction no significant differences were observed in percentage fat extracted. Autoclaving bones prior to fat extraction did not have any significant effect on bone ash.

Keywords: poultry, bone mineralisation, bone ash, methodology

Introduction

Proper bone mineralisation is important considering the fast rate of growth of modern broilers and the amount of weight put on relatively immature bones. Fast growing broiler chickens are known to be susceptible to bone abnormalities, causing major problems for broiler producers (Shim *et al.,* 2008); the skeleton remains a weak link in physically supporting heavy carcasses in young birds (Applegate and Lilburn, 2002).

Bone status is commonly used as an indicator of mineral adequacy in poultry diets (Onyango *et al.,* 2003), and various bone measurements have already been identified as indicators of bone mineralisation (Hall *et al*., 2003, Shim *et al*., 2012). Tibia ash remains the most common bone used in evaluating bone mineralisation in poultry research (Shaw *et al.,* 2010).

The Association of Official Analytical Chemists recommended method of determining tibia ash (AOAC, 2000) is widely cited in literature. The process involves determining ash on a fat-free, dry weight basis. However different divergences to this methodology have been cited in literature (Yan *et. al.,* 2005) and it is thought that the variations in methodology may have an effect on the result obtained.

This study was conducted to evaluate the effect of two common divergences in the bone ash methodology: the effect of varying time on fat extraction using the Soxhlet method, and the effect of autoclaving prior to fat extraction on bone ash.

Material and Methods

264 day- old Ross 308 male broilers fed standard commercial diets were allocated to 33 replicate pens; 8 birds per replicate. Diets and water were provided ad libitum to all birds during the experimental procedure. At 42d, 1 bird per replicate pen was culled by cervical dislocation and tibia bones were manually removed and frozen at -20°C until further analysis.

For objective 1, all adhering tissues were removed from the right tibia bones. The right tibia bones, including their cartilage caps, were dried at 105°C for 24h prior to fat extraction by Soxhlet extraction for 1h using petroleum ether. They were then dried at 105°C for 24h and dry bone weight recorded. This process was cumulatively repeated 8 times in total. For objective 2, the left tibia bones were autoclaved and dried at 105°C for 24h prior to fat extraction in petroleum ether for 8h. All bones from right and left tibias were then ashed at 650°C for 24h.

Data was analysed using the IBM SPSS statistical software, version 22. For objective 1, data was analysed as a one-way repeated measure ANOVA and post hoc test was determined using the Bonferroni procedure. For objective 2, one-way ANOVA was performed on data using the univariate model procedure. Post hoc test was determined using the Turkey's Honestly Significant Difference procedure.

Results

Objective 1: Effect of varying time on fat extraction

The total % mean fat extracted increased significantly for every additional hour of fat extraction upto 5 hours, and for every additional hour of fat extraction, there was a reduction in the additional % mean fat extracted.

Table 1. The effect of varying time on fat extraction

Mean % additional fat extracted per hour		
Fat extraction time (h)	**% additional mean fat extracted**	**SE***
1	5.724[a]	0.405
2	3.893[b]	0.232
3	2.406[c]	0.153
4	1.504[d]	0.145
5	0.720[e]	0.101
6	0.446[f]	0.064
7	0.354[f]	0.052
8	0.349[f]	0.053

* Standard error

Means with different letters differ significantly ($P < 0.05$).

Objective 2: Effect of autoclaving prior to fat extraction

Autoclaving bones prior to fat extraction did not have any significant effect on bone ash weight or percentage ash $P>0.05$

Table 2

Processing method	**Ash weight (g)**	**SE***	**Ash %**	**SE***
Fat extracted bones[1]	2.3282	0.093	42.12[a]	0.355
Autoclaved bones[1]	2.3048	0.091	42.95[a]	0.284

* Standard error

1 represents the mean of 33 observations per treatment

Conclusions

The result from this study shows a minimum of 6 hours is required when using the Soxhlet extraction method to extract fat prior to ash determination. Autoclaving prior to fat extraction did not have any effect on bone ash parameters (ash weight and ash percentage). It is of paramount importance a common methodology is adopted by researchers for measuring ash content when evaluating tibia bone mineralisation in poultry.

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P-188 (ID 119)

THE IMPACT OF SODIUM AND CHLORIDE LEVELS AND RATIOS ON THE PERFORMANCE OF BROILERS FED DIETS CONTAINING LASALOCID AT TWO DIETARY LEVELS

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Abstract

The objective of the study was to evaluate the performance parameters of broilers fed with two different levels of a divalent ionophore (lasalocid) in combination with 12 different sodium:chloride (Na:Cl) ratios at moderate to low field coccidiosis infection pressure. A total of 4224 Cobb 500 male broilers were divided among 192 pens across 24 treatments. Results indicated that there was an interaction between electrolytes (Na & Cl), thus the ratios of these key nutrients can be as important as the actual levels. Best performance was achieved when the Cl level in the diet was 20% higher than the Na level. Water consumption was not statistically affected by the lasalocid dose, but it was in respect to dietary Na treatments. Also, broilers receiving a moderate (90 ppm) level of lasalocid during the latter stages of the growing cycle had higher body weights and lower feed conversions than those receiving a high (125 ppm) level. However, since there is a clear dose – anticoccidial efficacy response in ionophores, it could be that the situation would look differently under high coccidiosis infection pressure: there, the merits of a higher inclusion rate in preventing coccidiosis might become more important than a possible drawback. For this reason infection pressure should be taken into account in determining the actual anticoccidial dose in feed, when dose range is available.

Introduction

In feed anticoccidials are largely used as a prevention tool in control of coccidiosis in poultry under modern production practices. In its commission report 233/2008, the European Commission recognizes their use as “essential”. Ionophores are the most commonly used anticoccidial drugs in the modern poultry production. They are lipid-soluble molecules that bind to and transport ions - mainly mono- and divalent cations, across the bipolar lipid layer of biological membranes, which is normally non permeable for such ions. This leads to disruption of transmembrane ion concentration gradients of microorganisms which suppresses their proliferation. Due to this ion binding and transporting mode of action of ionophores, it has been speculated that different levels of electrolytes may affect performance of broilers fed with ionophores (Frigg & Broz, 1983; Cervantes and all., 1982; Christmas & Harms, 1988). It is known that levels of sodium (Na) and chloride (Cl) vary among commercial broiler diets, giving great variability to dietary electrolyte balance (DEB) levels. The study design evaluated the performance parameters of broilers fed with two different levels of a divalent ionophore (lasalocid) in combination with 12 different sodium:chloride (Na:Cl) ratios, under moderate to low field coccidiosis infection pressure.

Materials and Methods

Animals. 4224 Cobb 500 males were divided into 192 pens at 1d of age. Birds were housed in an environmentally controlled building in 0.9m x 1.5m concrete floor pens at the stocking density of 16 birds per m^2. Birds were vaccinated for Marek’s disease *in ovo* and for Newcastle and infectious bronchitis by spray application at day of hatch.

Experimental design. Table 1 describes the group distribution for the study. The study had 24 different groups divided into two different levels of a divalent ionophore (lasalocid) in combination with 12 different Na:Cl ratios. No phytase has been used in order not to deviate the available levels from the analytical ones.

For each Na level, there were three relative percentage levels of Cl (approximately 80%, 100% and 120% of the Na levels, respectively). Each treatment had eight replicates distributed in a randomized complete block design, with the pen as the experimental unit. Birds were kept for 42 d with feed *ad libitum*. Feed schedule consisted of 3 different diets: Starter (0-18 d), Grower (19-28 d) and Finisher (29-42 d).

Data collection. At the beginning of each feed phase (d 0, 18, and 28), water consumption was measured for a period of four days. Feed consumption and body weight gain was measured at 18, 28, 35 and 42 d. Mortality was observed twice a day and all mortality was necropsied and weighted for feed conversion adjustment. On Study Day 42 all birds in every pen were scored for foot pad lesions. Each bird was scored according to the following: 0 = Normal (no burn, scab or lesion); 1 = Pad Burn (dermis only); 2 = Pad Scab (healing) on one or both feet; 3 = Pad Lesion (open sore) on one or both feet.

Treatment	Lasalocid (ppm)	Starter (0-18 days)		Grower (19-28 days)		Finisher (29-42 days)	
		%Na	%Cl	%Na	%Cl	%Na	%Cl
1	90	0.16	0.12	0.16	0.12	0.16	0.12
2			0.16		0.16		0.16
3			0.2		0.2		0.2
4	90	0.19	0.15	0.19	0.15	0.19	0.15
5			0.19		0.19		0.19
6			0.23		0.23		0.23
7	90	0.22	0.18	0.22	0.18	0.22	0.18
8			0.22		0.22		0.22
9			0.26		0.26		0.26
10	90	0.18	0.14	0.2	0.16	0.22	0.18
11			0.18		0.2		0.22
12			0.22		0.24		0.26
13	125	0.16	0.12	0.16	0.12	0.16	0.12
14			0.16		0.16		0.16
15			0.2		0.2		0.2
16	125	0.19	0.15	0.19	0.15	0.19	0.15
17			0.19		0.19		0.19
18			0.23		0.23		0.23
19	125	0.22	0.18	0.22	0.18	0.22	0.18
20			0.22		0.22		0.22
21			0.26		0.26		0.26
22	125	0.18	0.14	0.2	0.16	0.22	0.18
23			0.18		0.2		0.22
24			0.22		0.24		0.26

Statistical analysis. The model for this study was a randomized complete block with the pen as the experimental unit and repeated measures. All hypothesis tests were conducted at the 0.05 level of significance using two-sided tests. Primary variables were weight gain per bird, calculated by dividing pen weight by the number of animals and subtracting D0 pen weight divided by the number of animals at D0, and adjusted feed conversion, calculated on study d 18, 28, 35 and 42 using the total feed consumption for the pen divided by the total weight of surviving birds with D0 weight subtracted.

Results

A number of interactions were identified as related to sodium and chloride levels. Evaluation of the effect of chloride at the different levels of sodium found that at 18 d of age, for birds receiving high levels of dietary sodium (0.22%), the high chloride (0.26%) treatments produced significantly lower feed conversions than did the medium chloride levels. Also, at d 28, 35, and 42, for those birds receiving high dietary sodium levels (0.22%), the high chloride (0.26%) treatments produced feed conversions that were lower than either the medium (0.22%) or low (0.18%) chloride levels.

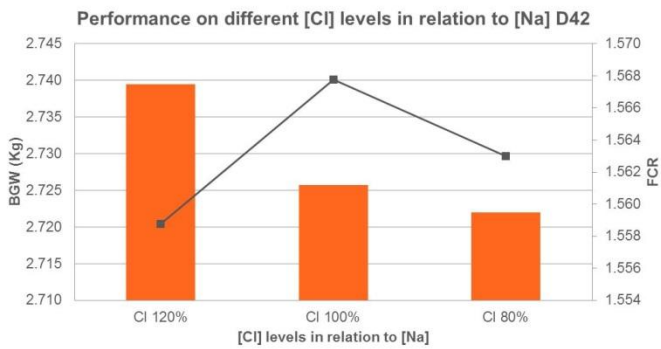

Fig 1. Male broiler performance at different Cl level in relation to Na (high - Cl 20% more than Na; moderate - Cl equal to Na; and low - Cl 20% less than Na) at 42 days

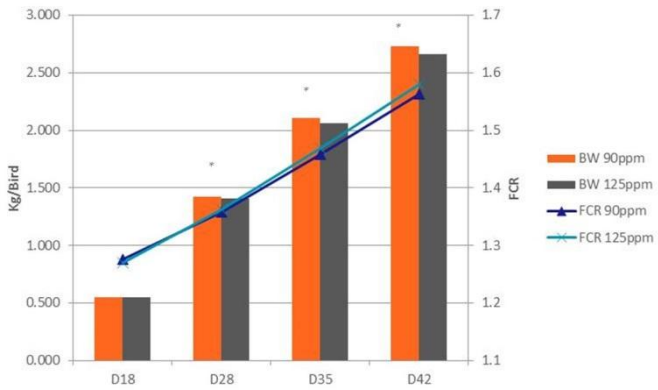

Fig 2. Male broiler performance at 18, 28, 35 and 42 days with 90 or 125ppm of Avatec independently of the Na:Cl ratios

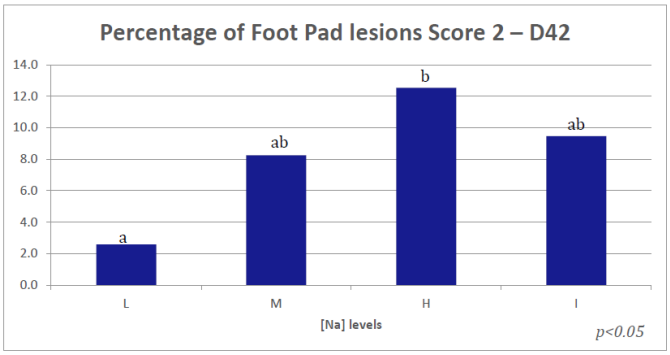

Fig.3 Percentage of Footpad lesions score 2 at 42 days.

Moreover, for birds receiving low dietary levels of sodium (0.16%), those treatments that received low chloride (0.12%) levels produced feed conversion ratios at d 28, 35, and 42 that were lower than those receiving high (0.20%) chloride levels. Evaluation of the effect of sodium at different levels of chloride found that for birds receiving high levels of dietary chloride (T3/15, T6/18, T9/21 and T12/24), those receiving high (0.22%), medium (0.19%), or progressive (0.18 to 0.22%) sodium levels produced significantly lower feed conversions at 28, 35, and 42 d than birds receiving low (0.16%) levels of dietary sodium. Additionally, for birds receiving medium levels (100%) of dietary chloride, those that received medium sodium levels (0.19%) exhibited lower feed conversions at 28, 35, and 42 d than did those birds receiving high sodium levels (0.22%).

For the overall compound effect of the different Cl levels (120% of Na, 100% of Na and 80% of Na), no statistical significance was obtained as there were interactions between the different Na levels. Anyway numerical trend is clear that best performance (both BW and FCR) is obtained when Cl level exceeds Na level (fig 1).

Performance data for birds receiving 90 and 125 ppm of lasalocid are shown in Figure 2. BW and FCR were not significantly different between 90 and 125ppm treatments at d 18. Cumulative body weight gains for birds receiving 90 ppm of lasalocid (T1-T12) were superior to those receiving 125 ppm (T13-T24) at d 42. FCR followed the same pattern, with birds receiving diets with 90 ppm of lasalocid (T1-T12) exhibiting significantly lower feed conversions than did those receiving 125 ppm (T13-T24) at d 42.

Water consumption was not statistically affected by the lasalocid dose, but it was in respect to dietary Na treatments in the period between D28-D31, where birds with the high sodium level (0.22%) consumed significantly more water than the low levels (0.16%) of sodium. When foot pad lesion scores were analyzed, it was noticed that foot pad scores as measured by the % of those scoring #2, tracked with sodium levels. This is tracking with water consumption. Note that no treatments produced litter conditions that would be described as “wet”

Discussion and Conclusions

The trial data indicated that there was interaction between electrolytes (Na & Cl), thus the ratios of these key nutrients can be as important as the actual levels. For broilers receiving medium (0.19%) or progressive (0.18% / 0.20% / 0.22%) dietary sodium levels, there was a trend for increased weight gains and reduced feed conversions when dietary chloride levels were 120% of respective sodium levels. Body weight gain and feed consumption were also affected by different levels of ionophores inclusion in the diets. In the conditions present in this study, where coccidial challenge was at moderate to low field infection pressure, broilers receiving a high (125 ppm) level of lasalocid during the latter stages of the growing cycle had lower body weights and higher feed conversions than those receiving a moderate (90 ppm) level. This appears to be due, at least in part, to a reduction in feed intake. This is in agreement with findings of Frigg and Broz (1983), who described that high levels of monensin reduced feed intake in older birds. However, since there is a clear dose – anticoccidial efficacy response in ionophores, it could be that the situation would look differently under high coccidiosis infection pressure: there, the merits of a higher inclusion rate in preventing coccidiosis might become more important than a possible drawback. For this reason infection pressure should be taken into account in determining the actual anticoccidial dose in feed, when dose range is available. Water intake and FPD lesions increased significantly with the increase of Na level, but there was no interaction with lasalocid level

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P-189 (ID 123)

THE EFFECT OF HOUSING SYSTEM AND CALCIUM ON EGGSHELL QUALITY

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The aim of study was to evaluate the effect of housing system and feed calcium content on quality of the eggshell. The laying hens (ISA Brown) were housed in enriched cages (750 cm^2 per hen), on litter (7 birds per m^2) and in aviary (15 birds per m^2). There were two groups differing in calcium content (3 % and 3.5 %) in the diet. The significant interaction between housing system and calcium content was found in egg weight ($P \leq 0.002$). The heaviest eggs (62.2 g) were detected in aviary with 3% calcium, whereas the lightest eggs (60.4 g) were laid in cage with 3% calcium in diet. Housing system had significant effect on egg weight ($P \leq 0.002$), when heavier eggs were produced on litter with compared to other housing systems. The significant interaction of housing and Ca was also found in eggshell strength ($P < 0.001$), the highest values were in cage with 3.5% calcium and the lowest on litter with 3.5% calcium. This parameter was affected by housing system with higher values in enriched cage. Calcium content not significantly influenced eggshell thickness, however, eggshell strength was significantly higher in 3.5% Ca. These results regarding the quality of the shell probably related to the different needs of calcium in different systems of housing.

characteristics	Ca (%)	Housing system			significance		
		cage	litter	aviary	Housing system	Ca	Housing x Ca
Egg weight (g)	3,0	60.4^{d}	61.1^{bcd}	62.2^{ab}	0.002	NS	0.002
	3,5	60.7^{cd}	62.1^{a}	60.6^{cd}			
Eggshell weight (g)	3,0	6.0^{b}	6.2^{a}	6.2^{a}	NS	NS	0.002
	3,5	6.2^{a}	6.1^{ab}	6.2^{a}			
Eggshell thickness (mm)	3,0	0.431	0.363	0.365	NS	NS	NS
	3,5	0.370	0.359	0.367			
Eggshell strength (g/cm^2)	3,0	4651.2^{b}	4637.6^{b}	4605.8^{b}	< 0.001	0.001	< 0.001
	3,5	4893.1^{a}	4563.1^{b}	4798.5^{a}			
Eggshell ratio (%)	3,0	12.2^{ab}	12.1^{b}	11.6^{d}	< 0.001	0.022	< 0.001
	3,5	12.3^{a}	11.8^{c}	12.2^{ab}			
Eggshell colour	3,0	31.3^{a}	30.5^{b}	30.9^{ab}	0.006	< 0.001	< 0.001
	3,5	28.9^{c}	30.3^{b}	31.1^{ab}			

P-190 (ID 260)

DETERMINATION OF PHOSPHORUS DIGESTIBILITY OF MONO-DICALCIUM PHOSPHATE AND MONOCALCIUM PHOSPHATE IN BROILER CHICKENS

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Abstract

This study was conducted to determine the preceacal digestibility of phosphorus of mono-dicalcium phosphate (MDCP) and monocalcium phosphate (MCP) in broiler chickens using the regression method. Prior to formulating experimental diets, Ca and P concentation of MDCP and MCP were analyzed and found to be 16.1 and 21.3%, 14.5 and 22.4%, respectively. Basal diet was formulated to contain 0.24% total P and each of test ingredients was supplemented into the basal diet to produce 3 experimental diets. The MDCP and MCP test diets containing 0.31, 0.38, 0.45 and 0.30, 0.36, 0.42% total P, respectively. The total Ca to total P ratio in all diets was maintained at 1.2:1 to 1.4:1. Titanium dioxide at the level of 0.3% was used as indigestible marker. One-day-old male broiler chicks were fed with a commercial starter diet from 1 to 20 day of ages. At 21 day of ages, a total of 252 birds were assigned into 7 experimental diets with 6 replicate cages (6 birds per cage). Birds were fed with experimental diets for 8 days. At 29 day of ages, all birds were sacrificed and ileal digesta were collected from the posterior half of ileum. Coefficients of P digestibility of MDCP and MCP were calculated by linear regression analysis. The analyzed concentrations of P increased with raising inclusion levels of MDCP and MCP in the diets and were in agreed with the calculated values. Coefficients of prececal digestibility of P in MDCP and MCP were 0.67 and 0.63, respectively. The present study indicated that the availability of P in MDCP and MCP are lower from the values currently used.

Keywords: phosphorus, digestibility, feed ingredient, broiler

Introduction

Phosphorus is one of major minerals for poultry. The database for accurate phosphorus availability of feed ingredients is considered a useful tool for improving the accuracy of feed formulation. However, there are several approaches used to determine P availability (Shastak and Rodehutscord, 2013). Prececal phosphorus digestibility is the measurement of phosphorus disappearance at the end of ileum. Prececal digestibility have been adopted for estimate the digestibility of several feedstuffs due to this method based on quantitative measurements. Therefore, the value will be unaffected by post-ileal microbial activity and the phosphorus excretion in the urine can be excluded.

MDCP and MCP are P sources that are commonly used in poultry diets. However, the data of digestible P of MDCP and MCP are limited. Therefore, this study was conducted to determine the preceacal digestibility of P from MDCP and MCP in broiler chickens using the regression method.

Material and Methods

Animals

One-day-old male broiler chicks of Ross 308 were raised in evaporative cooling system house with rice husk as litter material and fed with a commercial starter diet from 1 to 20 day of ages. At 21 day of ages, birds were individually weighed. A total of 252 birds that had body weight in the range of 10% from average body weight were introduced to the experiment, such that the average weight across experimental treatments was similar. Birds were assigned into 7 experimental diets with 6 replicate cages (6 birds per cage) and fed with experimental diets for ad libitum intake for 8 days.

Experimental diets

Prior to formulating experimental diets, MDCP and MCP were analyzed for Ca and P content. Basal diet was formulated to contain 0.24% total P. Each of test ingredients was supplemented into the basal diet at the expense of a P-free ingredient to produce 3 experimental diets. The MDCP and MCP test diets contained 0.31, 0.38, 0.45 and 0.30, 0.36, 0.42% total P, respectively. The total Ca to total P ratio in all diets was maintained at 1.2:1 to 1.4:1 by the addition of

ground limestone. Titanium dioxide at the level of 0.3% was used as indigestible marker. Experimental diets were offered in mash form. Ingredient and nutrient composition of experimental diets are shown in Table 1.

Sample Collection and Chemical Analysis

At 29 day of ages, all birds were sacrificed and ileal digesta were collected from the posterior half of ileum. The ileum defined as the section between Meckel's diverticulum and 2 cm prior to the ileo-caeco-colonic-junction. The content was flushed out with distilled water, pooled for all birds from one cage, immediately frozen and later freeze-dried and ground.

Diets and ileal digesta samples were analyzed for DM, Ca, total P, and Ti. Dry matter was determined by method as described by AOAC (2005). Ca and P concentrations were determined by the inductively coupled plasma atomic emission spectroscopy method (ICP-OES). Titanium dioxide was determined by the colorimetric method as described by Short et al. (1996).

Calculations

The precaecal digestibility of P (pcdP) from the supplemented sources were calculated according to the procedure outlined by Rodehutscord (2013).

The prececal digestible P was calculated for each diet and replicated pen using the following equation:

pcdP (%) = 100 – [100 × (MDiet × PDigesta) / (MDigesta × PDiet)] [equation 1]

where

MDiet and MDigesta = analysed marker concentration in the diet or digesta (g/kg DM);

PDiet and PDigesta = analysed P concentration in the diet or digesta (g/kg DM).

The content of pcdP in the diet was calculated as:

pcdP (g/kg of diet) = pcdP (%) × PDiet /100 [equation 2]

The pcdP (g/kg of diet) was plotted against total P concentration (g/kg of diet) in a linear regression analysis. The slope of the regression line gives the coefficient of precaecal digestibility of P from the supplemented source.

Results

The concentration of Ca and P in MDCP and MCP samples was 16.1 and 21.3%, 14.5 and 22.4%, respectively. The analyzed concentrations of P increased with an increase inclusion levels of MDCP and MCP in the diets and the values were in agreed with the calculated values (Table 1). Strong linear relationships ($P<0.001$) were observed between dietary total P concentration and content of pcdP in the diet for both MDCP and MCP. The digestibility coefficients of P in MDCP and MCP were 0.67 and 0.63, respectively (Table 2).

Conclusions

Inorganic phosphate is assumed to be highly availability P sources. Under the condition of the present study, the primary conclusion could be made as the digestibility of P in MDCP and MCP are lower from the available values currently used to formaulted diet (INRA, 2002). The similar coefficient of P digestibility between of MDCP and MCP were observed.

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Table 1. Ingredient and nutrient composition (%) of experimental diets

Ingredient composition	Basal diet	MDCP			MCP		
		Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
Test ingredient	-	0.33	0.64	0.97	0.26	0.51	0.78
Corn	51.40	51.40	51.40	51.40	51.40	51.40	51.40
Soybean meal, 45% CP	23.60	23.60	23.60	23.60	23.60	23.60	23.60
Dried egg albumen	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Dextrose	13.65	13.26	12.90	12.52	13.28	12.94	12.60
Soybean oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Limestone	0.39	0.45	0.50	0.55	0.50	0.59	0.66
Salt	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Sodium bicarbonate	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Trace mineral-vitamin pre-mix[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Choline chloride	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Titanium dioxide	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Calculated analysis							
ME, kcal/kg	3,148	3,134	3,122	3,109	3,135	3,123	3,112
Crude protein	21.0	21.0	21.0	21.0	21.0	21.0	21.0
Calcium	0.33	0.41	0.48	0.55	0.41	0.48	0.55
Total phosphorus	0.24	0.31	0.38	0.45	0.30	0.36	0.42
Ca:total P ratio	1.4	1.3	1.3	1.2	1.4	1.3	1.3
Analyzed composition							
Calcium	0.34	0.40	0.48	0.53	0.42	0.48	0.52
Total phosphorus	0.26	0.32	0.41	0.44	0.31	0.35	0.41

[1]Supplied per kilogram of diet: vitamin A, 12,000 IU; vitamin D3, 3,000 IU; vitamin E, 15 IU; vitamin K3, 1.5 mg; thiamine, 1.5 mg; riboflavin, 5.5 mg; pyridoxine, 2 mg; vitamin B12, 0.01 mg; nicotinic acid, 25 mg; pantothenic acid, 12 mg; folic acid, 0.5 mg; biotin, 0.12 mg, Mn, 80 mg; Zn, 60 mg; Fe, 40 mg; Cu, 8 mg; I, 0.5 mg; Co, 0.1 mg; Se, 0.1 mg

Table 2. Linear relationship between pcdP (g/kg of diet) and total P concentration (g/kg of diet) of MDCP and MCP

Item	Regression equation	SE of the slope	SE of the intercept	r^2	Digestibility coef-ficient
MDCP	Y = 0.6747X - 0.1723	0.0372	0.1481	0.94	0.67
MCP	Y = 0.6316X - 0.0212	0.0469	0.1758	0.91	0.63

P-191 (ID 163)

INHIBITORY ACTION OF ANALYTICAL GRADE ZINC OXIDE AND OF A NEW POTENTIATED ZINC OXIDE ON THE IN VITRO GROWTH OF ESCHERICHIA COLI AND OF *SALMONELLA ENTERICA* STRAINS

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Abstract

Zinc oxide (ZnO) is a feed additive well known for its antimicrobial activity. A new source of potentiated ZnO and a regular ZnO product were compared; their inhibitory action on the *in vitro* growth of enteric bacterial strains was studied in liquid media.

Two pathogenic *E. coli* strains, *Salmonella enterica* biovar Enteritidis (DSM9898) and *S. enterica* biovar Typhimurium were inoculated in 96 well microtiter plates with Zn concentrations ranging from 0.00125 to 0.32 µg Zn/mL, for each Zn source (regular ZnO and potentiated ZnO HiZox®), using Brain-Heart-Infusion (BHI) medium at pH 4.6 and 6.5. Measurements were taken every 2 min for 8h for media at pH 6.5 and every 4 min for 16h at pH 4.6. Coefficients for lag time and maximum growth in the stationary phase were calculated.

Lag phase, exponential growth and stationary phase could be distinguished at sub-inhibitory concentrations. Compared to regular ZnO, lag times were significantly ($p<0.05$) higher in potentiated ZnO for all the strains except for *S.* Typhimurium, at all Zn concentrations and at both pH, but only below 0.01 µL/mL for *E. coli* PS7 at pH 6.5; results were higher for *S.* Typhimurium at pH 6.5 and similar at pH 4.6. All strains showed globally higher maximum growth for standard ZnO compared to potentiated ZnO at all Zn dosages at pH 4.6. Maximum growth tended to be lower for both *S. enterica* in potentiated ZnO at pH 6.5.

In conclusion, compared to the regular ZnO, the new source of potentiated ZnO improved growth inhibition against two pathogenic *E. coli* and two *S. enterica* strains.

Keywords: zinc, inhibitory action, enteric bacteria

Introduction

The inhibitory action of a potentiated ZnO (HiZox®) and analytical grade ZnO on the *in vitro* growth of pathogenic enteric bacterial strains was studied in liquid media. Not only the typical minimal inhibitory concentration (MIC), which determines complete inhibition at certain concentrations, but also growth below these concentrations were investigated in order to assess the growth repressing effect of ZnO at different concentrations.

Material and Methods

The following strains were compared:

- *Escherichia coli* PS 79 (O147:K89:K88)
- *Escherichia coli* PS 7 (O138:K81)
- *Salmonella enterica* subsp. *enterica* biovar Enteritidis DSM 9898
- *S. enterica* subsp. *enterica* biovar Typhimurium DSM 5569

Saturated suspensions of Zn-containing media were produced by adding 10g of ZnO (analytical grade ZnO or HiZox®) to a total of 100 mL BHI media at pH 4,6 and pH 6.5. After incubation for 1h, the suspension was autoclaved. After centrifugation the supernatants were analysed for total Zn concentrations. All media were diluted with BHI for final concentrations of 0.00125 to 0.32 µg Zn/mL medium.

Cells of each strains were inoculated in 96 well microtiter plates. A microplate reader with 650 nm filter was used to record turbidity as measure for bacterial growth. Measurements were taken every 2 min over a period of 8h for media at pH 6.5 and every 4 min for 16h for media at pH 4.6.

A nonlinear regression analysis (3-sigmoidal curve fit) was employed to calculate coefficients for lag time [min] and maximum growth [OD]. Three-dimensional plots of incubation time vs. ZnO concentration vs. growth were used to visualize the effects of ZnO concentrations below MIC values. Curve fits were deemed satisfactory, when r^2 values of >0.999 were achieved from the growth data, which were compared at each Zn-concentration using the t-test. The level of significance was set to up ≤0,05.

Results

Compared to the analytical grade ZnO, potentiated ZnO showed increased solubility at both pH values: +30% at pH 4.6 and +410% at pH 6.5 in BHI medium.

Growth of all tested strains at pH 6.5 below the inhibitory Zn-concentration of 0.32 µg/ml yielded typical three phase, sigmoid growth curves only at 0.04 µg/ml and lower concentrations. Here, lag phase, exponential growth and stationary phase could be distinguished. Therefore, a 3-parameter sigmoid curve fit was applied to these growth curves to describe the influence of different Zn-sources on bacterial growth. Zn-concentrations of 0.08 and 0.16 µg/ml yielded growth curves that showed starting inhibition of growth.

The lag phase describes the time period of bacterial growth before the onset of exponential growth and is a measure of the "fitness" of a bacterial strain in the medium. At pH 6.5, compared to analytical grade ZnO, *E. coli* PS79 tended to show higher lag times for potentiated ZnO at all Zn-concentrations, while *E. coli* PS7 showed higher lag times only at 0.00125 – 0.005 µg/ml Zn. Both *Salmonella* strains displayed higher lag times for potentiated ZnO at all subinhibitory Zn concentrations than for analytical grade ZnO. The lag time for all strains was markedly higher at pH 4.6 than at pH 6.5. Compared to analytical grade ZnO, *E. coli* strains showed increased lag times at all subinhibitory Zn concentrations in potentiated ZnO supplemented medium. The growth of *S. enterica* strains was severely reduced at pH 4.6, even without Zn in the medium. As a consequence, sigmoidal growth curves were only observed for low Zn-concentrations (0 – 0.02 µg/ml for *S.* Enteritidis; 0 – 0.01 µg/µml for *S.* Typhimurium). The identical lag times for *S.* Typhimurium must therefore be attributed to extremely low growth at pH 4.6 for this strain.

The maximum growth coefficient describes the final cell density in the stationary phase. At pH 6.5 and at subinhibitory concentrations, maximum growth was influenced by Zn-source. *Salmonella* strains displayed higher growth in media supplemented with analytical grade ZnO. Growth at pH 4.6 for 8h was not sufficient to generate typical sigmoid curves that are usually seen in bacterial in vitro growth. Instead, 16 h incubation had to be employed to describe growth behaviour with a sigmoid curve fit. All tested strains showed higher maximum growth for ZnO than for potentiated ZnO. However, growth was severely reduced for *S. enterica* strains in acidic pH and thus, results should be interpreted with caution.

Although identical total Zn-concentrations were employed, one has to keep in mind that there may be a difference between total Zn and the "bioavailability" of the different Zn-sources at low Zn-concentrations. As potentiated ZnO displayed a 5-fold increase of solubility, the actual available Zn-concentration may be higher due to aggregation of analytical grade ZnO to components of the growth medium.

As the total Zn-concentrations were identical in tested media, the increased bactericidal effect of potentiated ZnO must be attributed to the "bioavailability" of Zn^{2+}. Potentiated ZnO may show less aggregation to components of the medium and thus, the amount of available Zn^{2+} may be higher. This would be especially effective at lower pH, as ZnO exhibits increasing solubility as pH decreases.

Conclusions

Compared to analytical grade ZnO, potentiated ZnO HiZox® led to increased growth reduction against two pathogenic *E. coli* and two *S. enterica* strains. Minor strain specific differences regarding the growth reducing efficiency were observed. The reduction of growth, particularly at low pH, may be attributed to the higher solubility of HiZox®, which may originate from its higher surface area.

P-192 (ID 255)

NON-PHYTATE PHOSPHORUS LEVELS AND PARTICLE SIZE OF CALCIUM SOURCE IN THE DIET OF COMMERCIAL LAYERS AT THE END OF THE FIRST PRODUCTION CYCLE

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Abstract

This study evaluated the effects of particle size distribution of calcium source in different levels non-phytate phosphorus diets on first-cycle layer performance and egg quality. A completely randomized experimental design with 5 treatments include control: 100% medium limestone particle size distributions and 100 % Non-phytate phosphorus Level requirement, treatment 2: 50% fine and 50% coarse and 70 % Non-phytate phosphorus Level requirement, treatment 3: 50% fine and 50% coarse and 50 % Non-phytate phosphorus Level requirement, treatment 4: 30% fine and 70% coarse and 70 % Non-phytate phosphorus Level requirement, treatment 5 30% fine and 70% coarse and 50 % Non-phytate phosphorus Level requirement was applied, with four replicates of 12 birds each. The treatments did not influence the most of evaluated performance and internal and external egg quality parameters. However, limestone particle size distribution quadratically affected with percentage of defective eggs, with the lowest percentage obtained with the distribution 50 % fine and 50% coarse limestone and 50 % Non-phytate phosphorus Level requirement. Increasing dietary non-phytate phosphorus significantly increased eggshell weight per surface area. It was concluded that the combination of the dietary 70 % Non-phytate phosphorus Level with 50% replacement of fine-particle limestone by coarse limestone results in better eggshell and increases the number of marketable eggs.

Keywords: Commercial Layers, Non-phytate phosphorus Levels, Particle Size of calcium source, first-cycle layer performance, egg quality

Introduction

Calcium is one of the essential minerals in poultry nutrition. In addition to its vital functions as the main component of bone structure and participation in acid-base balance and enzymatic system, calcium is the also the main component of the eggshell. It is estimated that each egg contains 2.2g of calcium, present mainly in the eggshell. When limestone particle size is reduced, its solubility increases. Calcium has several functions in bird metabolism and it is the main component of the eggshell. Dietary calcium levels, particle size and solubility, according to source, influence eggshell quality. Therefore, the knowledge on the quality of feedstuffs used for bird feeding is essential to promote better performance. The present study aimed at determining the effects of particle size distribution of calcium source in different levels non-phytate phosphorus diets on first-cycle layer performance and egg shell quality.

Material and Methods

The experiment was carried out at the experimental facilities of the Poultry Sector of the Islamic Azad University, Kashmar branch, Kashmar. Iran. In this study, 240, 22-week-old Hy- line layers in their first production cycle were used. Birds were housed in a layer house equipped with 60 metal cages (48cm long x 42cm deep x 45cm high). A photoperiod of 15 hours of light per day was applied. Composition of basal diet shown in Table 1.

A completely randomized experimental design with 5 treatments include 100% medium limestone particle size distributions and 100 % Non-phytate phosphorus Level requirement, 50% fine and 50% coarse and 70 % Non-phytate phosphorus Level requirement, 50% fine and 50% coarse and 50 % Non-phytate phosphorus Level requirement, 30% fine and 70% coarse and 70 % Non-phytate phosphorus Level requirement, 30% fine and 70% coarse and 50 % Non-phytate phosphorus Level requirement was applied, with four replicates of 12 birds each. The following performance parameters were evaluated. Egg production data were collected daily, and egg weight and feed intake were determined weekly. Eggshell quality and internal egg quality were evaluated every 28 days during a total period of 56 days. The obtained results were recorded and presented as mean values for the 56-day period. Birds were offered feed and water ad libitum during the entire experimental period (22 to 30 weeks).

Table 1. Composition of experimental diets (as feed basis)

Ingredients	%		
Yellow Corn	60.70	Calculated value:	
Soybean meal, 445 g crude protein / kg	16.10	Crude protein (g/kg)	14.70
Flaxseed	0.0	ME (Mcal/kg)	2700
Wheat bran	3.40	Calcuim (g/kg)	3.20
Barley	10.00	AvaiableP ppppPPphosphorous (g/kg)	0.28
Dicalcium Phosphate	0.69		
Oyster shells	7.90		
NaCl	0.26		
Vitamin premix[a]	0.50		
Mineral premix[b]	0.40		
DL-Methionine	0.05		
Total	100		

[a]Vitamin premix supplied the following per kilogram of diet: Vitamin A, 440.000 IU; Vitamin D3, 80.000 IU; Vitamin E, 96. mg; Vitamin K3, 2000 mg; Vitamin B1, 6120 mg; Vitamin B2, 3000 mg; Vitamin B6, 612 mg; Calcium Pantothenate, 8800 mg; Niacin, 50 mg; Biotin, 2 gr; Folic acid, 1.25 mg; Vitamin B12,640 mg (Telavang co., Tehran, Iran).

[b]Mineral permix supplied the following per kilogram of diet: Cu ($CuSO_4 \cdot 5H_2O$, 25.45% Cu) 8 gr; Fe ($FeSO_4 \cdot 7H_2O$, 20.29% Fe) 100 gr; Mn ($MnSO_4 \cdot H_2O$,32.49% Mn) 64.52 gr; Zn (ZnO, 80.35% Zn) 33.8 gr; Se ($NaSeO_3$, 45.56% Se) 8 mg (Telavang co., Tehran, Iran).

Results

The treatments did not influence the most of evaluated performance and internal and external egg quality parameters. However, limestone particle size distribution quadratically affected with percentage of defective eggs, with the lowest percentage obtained with the distribution 50 % fine and 50% coarse limestone and 70 % Non-phytate phosphorus Level requirement. Increasing dietary non-phytate phosphorus significantly increased eggshell weight per surface area. Egg loss (defective and broken eggs) results are consistent with those obtained by Oliveira et al. (2002), who worked with 2.8 to 4.4% Ca levels.

There was a significant effect ($p<0.05$) of limestone particle size distribution on the percentage of defective eggs (Table 2), with the highest percentage (0.71%) when 30% fine limestone and 70% coarse limestone were fed, and the lowest percentage with 50% fine and 50% coarse limestone. The calcium derived from dietary limestone is influenced by limestone particle size. When particle size distribution contained more than 50% coarse limestone, calcium release decreased due to the lower solubility of Ca in coarser particles, explaining the higher percentage of broken and defective eggs caused by limestone particle size distribution of 30% fine and 70% coarse particles

Table 2. Effect of limestone particle size distribution on the percentage of defective eggs.

Variables	Treatments					SEM	P-values
	Control	T2	T3	T4	T5		
Broken eggs (%)	2.09[a]	2.01[a]	2.43[b]	2.61[C]	2.73[C]	0.17	0.042
Defective eggs(%)	0.39[a]	0.37[a]	0.41[b]	0.59[b]	0.65[b]	0.04	0.039
Egg shell(%)	9.88[b]	9.67[a]	9.61[a]	9.91[b]	9.86[b]	0.08	0.7761

[a, b] Means within a row lacking a common superscript differ ($P < 0.05$).

Conclusions

It was concluded that the combination of the dietary 70 % Non-phytate phosphorus Level with 50% replacement of fine-particle limestone by coarse limestone results in better eggshell and increases the number of marketable eggs.

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Protein and amino acids nutrition

P-197 (ID 271)

FACTORIAL MODEL TO ESTIMATE DIGESTIBLE VALINE, ISOLEUCINE AND TRYPTOPHAN REQUIREMENTS OF BROILER BREEDER HENS

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Abstract

The objective of this study was to determine amino acid utilization efficiency (*k*) and develop a factorial model to estimate valine (Val), isoleucine (Ile) and tryptophan (Trp) intakes. One hundred ninety-two hens were used in each amino acid (AA) assay. The experimental design consisted of eight treatments, eight replicates and one hen per cage. The diets were formulated by dilution technique using a summit diet and a nitrogen free diet, resulting in AA levels that ranged from 1.97 to 9.85g/kg for Val and Ile, and 5 to 2.5 g/kg for Trp. Each assay lasted nine weeks. Data obtained were AA intake (AAI), body weight (BW) and egg output (EO). Broken line with one inclination was used to evaluate the responses and with two inclinations to estimate *k*. The factorial model was $AAI=[AA_m\times(BW\times0.196)^{0.73}]+[(N_{egg}\times EO\times AA_{egg})/k]$, where AA_m is AA for maintenance (247, 134 or 37 mg/$BP_m^{0.73}$ for Val, Ile and Trp respectively) determined in previous studies at Unesp; $BP_m^{0.73}$ is mature body protein; *k* is 0.66 for Val, 0.60 for Ile or 0.56 for Trp determined in this study; N_{egg} is the N content in the egg (1.89 g/100g); AA_{egg} is the AA content in the egg (413, 338 or 108 mg/g for Val, Ile and Trp respectively). The AAI estimated by the model at 30 weeks were 853, 692 and 228 mg/day for Val, Ile and Trp, respectively. Our model incorporates the most physiologically relevant metrics, units, and values, which provide accurate estimates of amino acid requirements for broiler breeder hens.

Keywords: amino acid, broiler breeder hens, efficiency of utilization, egg output, mathematical model

Introduction

Mathematical models represent an important tool that incorporates variables of productivity to update nutritional requirements. These models also allow for greater flexibility than experimental methods in determining what variables might be more relevant to specific regions or even individual producers (Gous, 2014). Specifically regarding broiler breeder hens, the factorial models mostly rely on coefficients and data from commercial laying hens. This is clearly not an optimal situation. For example, laying hens have an amino acid utilization efficiency of 85% whereas for broiler breeder hens the amino acid utilization efficiency is 49% (Bowmaker and Gous, 1991; Silva et al. 2015). These differences arise from the egg-laying potential of the two types of hens, therefore, requiring specific coefficients for the model to predict nutritional requirements. Thus, the objective of this study was to determine amino acid utilization efficiency (k) and develop a factorial model to estimate valine (Val), isoleucine (Ile) and tryptophan (Trp) intakes.

Material and Methods

The experimental design consisted of eight treatments, eight replicates and one hen per cage. The diets were formulated by dilution technique using a summit diet and a nitrogen free diet, resulting in AA levels that ranged from 1.97 to 9.85g/kg for Val and Ile, and 5 to 2.5 g/kg for Trp. Each assay lasted nine weeks. Data obtained were AA intake (AAI), body weight (BW) and egg output (EO). Broken line with one inclination was used to evaluate the responses and with two inclinations to estimate *k*. Two models were compared, one from the literature (M1, from Bornstein et al. (1979)) and other model developed in this study (M2). The factorial model was $AAI=[AA_m\times(BW\times0.196)^{0.73}]+[(N_{egg}\times EO\times AA_{egg})/k]$, where AA_m is AA for maintenance (247, 134 or 37 mg/$BP_m^{0.73}$ for Val, Ile and Trp respectively) determined in previous studies at Unesp; $BP_m^{0.73}$ is mature body protein; *k* is 0.66 for Val, 0.60 for Ile or 0.56 for Trp determined in this study; N_{egg} is the N content in the egg (1.89 g/100g); AA_{egg} is the AA content in the egg (413, 338 or 108 mg/g for Val, Ile and Trp respectively). To predict valine, isoleucine, and tryptophan intake, the data of body weight and egg mass of 60 broiler breeder hens of Cobb (500) strain were used. These data were obtained from individual measurement of the hens during the period from 25 to 60 weeks of age. The statistical analyses were performed using SAS software (Statistical Analysis System, version 9.2).

Results

Fitted models for efficiency of utilization in function of amino acid intake were: $Efficiency_{Val}$ = 65.50-0.05×(X-654)+0.21×(654-X), $Efficiency_{Ile}$ = 59.65-0.05×(X-495)+0.27×(495-X), $Efficiency_{Trp}$ = 55.69-0.14×(X-171.2)+0.67×(171.2-X). Factorial models M1 and M2 were simulated with body weight and egg output from 60 individually monitored hens. The results of the simulation are shown in Table 1. The M1 model compared to M2 predicted 20% and 15% higher intakes for Val and Ile, respectively. The predicted intake for Trp was 50% lower in M1 than M2. The observed differences between the models remained during the whole period assessed.

Table 1. Prediction of valine, isoleucine, and tryptophan intake for broiler breeder hens from 25 to 60 weeks of age using two mathematic models with body weight (BW), body weight gain (BWG), and egg output (EO) data.

Age weeks	BW kg	BWG g/day	EO g/day	Valine intake		Isoleucine intake		Tryptophan intake	
				mg/day					
				M1[a]	M2[b]	M1[a]	M2[b]	M1[a]	M2[b]
25	3.89	6	37.1	790	645	601	507	111	166
30	4.10	6	53.9	1057	853	793	692	149	228
35	4.32	6	55.7	1098	883	824	716	155	236
40	4.54	4	55.6	1108	889	834	719	156	237
45	4.68	4	50.0	1032	828	781	662	145	217
50	4.82	0	48.7	1020	817	774	650	143	213
55	4.83	0	47.7	1005	805	764	640	141	209
60	4.83	0	45.6	974	780	741	617	137	202

[a]M1: $AAI=[(AA_m/0.85)\times BW]+[BWG\times(0.21\times AA_t)]+[EO\times(63\times AAy+158\times AA_t)]$ from Bornstein et al. (1979).
[b]M2: $AAI=[AA_m\times(BW\times 0.196)^{0.73}]+[((N_{egg}\times EO)\times AA_{egg})/k]$ developed in this study.

The average egg output and body weight values for 60 hens at 25, 30, 35, 40, 45, 50, 55, and 60 weeks of age were applied in the M2 model to predict Val, Ile, and Trp intake (Table 2). For egg output, the coefficient of variation (CV) at 25 weeks was high (35%) because of the low rate of lay, but after this age the CV of egg output stabilized at the average value of 19%. For body weight, the CV ranged between 7 and 10% of the average value of 4.5 kg. The average egg output value and standard deviation accounted for 46.55% of the population, whereas maximum egg output values accounted for 90.07% of the population. For body weight, the average values corresponded to 54.55% of the population and maximum values for 94.66% of the population. Predicted values for Val, Ile, and Trp intake varied by 544, 487, and 165 mg/hen per day, respectively (Table 2). Differences between the maximum and average intakes were 223 (Val), 197 (Ile), and 66 (Trp) mg/hen per day, yielding maximum values approximately 30% higher than average intake values.

Conclusions

The average efficiency of utilization for the three amino acids (Val, Ile, and Trp) was 66%. The AAI estimated by the model at 30 weeks were 853, 692 and 228 mg/day for Val, Ile and Trp, respectively. Our model incorporates the most physiologically relevant metrics, units, and values, which provide accurate estimates of amino acid requirements for broiler breeder hens.

Table 2. Data from a population of 60 broiler breeder hens and prediction of the minimum (min), average and standard deviation (μ±SD), and maximum (max) values for valine, isoleucine, and tryptophan intake based on the application of the factorial models.

	Population data					
Age weeks	Egg outptut (g)			Body weight (kg)		
	Min	μ±SD	Max	min	μ±SD	max
25	13	37±14	53	3.3	3.9±0.3	4.4
30	20	54±10	70	3.5	4.1±0.3	4.6
35	35	56±7	70	3.7	4.3±0.4	5.0
40	29	56±10	80	3.6	4.5±0.4	5.3
45	19	50±10	65	3.6	4.7±0.4	5.5
50	15	49±10	68	3.4	4.8±0.4	5.5
55	24	48±9	68	3.4	4.8±0.4	5.6
60	22	46±10	63	3.1	4.8±0.5	5.7

	Amino acid intakes predicted								
	Valine			Isoleucine			Tryptophan		
	Min	μ±SD	max	min	μ±SD	max	min	μ±SD	max
	mg/bird per day								
25	349	645±171	833	244	507±153	676	76	166±52	223
30	454	853±121	1051	332	692±109	869	105	228±37	288
35	641	883±87	1054	498	716±78	870	162	236±26	288
40	582	889±117	1186	439	719±106	982	141	237±36	326
45	458	828±122	1022	328	662±109	832	103	217±37	274
50	423	817±127	1063	292	650±113	865	91	213±38	285
55	533	805±113	1061	392	640±100	866	125	209±34	286
60	485	780±127	1011	358	617±119	816	114	202±38	268

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P-198 (ID 270)

MAINTENANCE REQUIREMENTS OF VALINE, ISOLEUCINE, AND TRYPTOPHAN FOR POULTRY

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Abstract

The objective of this study was to determine maintenance requirements of valine (Val), isoleucine (Ile), and tryptophan (Trp) for poultry. Each nitrogen (N) balance trial lasted five days and was conducted for each amino acid (AA). After 48 hours of fasting (receiving water + sucrose), 40 g of each diet limiting in Val, Ile, or Trp were fed by tube, once a day, for three days to give a range of intakes from 0 to 101, 0 to 119, and 0 to 34 mg for Val, Ile, and Trp per kg of body weight (BW_{kg}), respectively. A N-free diet was offered *ad libitum* during these three days. Excreta were collected during the last three days of the balance period and the N content of the excreta was analyzed. Nitrogen retention (NR) was obtained by the difference between N intake and N excretion. For each AA, a linear regression between NR and AA intake (mg/BW_{kg} per day) was performed. The equations obtained were: NR= -98.6+2.4×Val (R^2 = 0.90), NR= -46.9+2.3×Ile (R^2 = 0.92), and NR= -39.5+7.3×Trp (R^2 = 0.92). The Val, Ile, and Trp required to maintain the body at zero NR were calculated to be 41, 20, and 5 mg/BW_{kg} per day, respectively. Expressing mg per kg of metabolic weight, the intake of Val, Ile, and Trp was 59, 32, and 9, respectively. Considering the degree of maturity of the birds and body protein content ($BP_m^{0.73}$×*u*), the amount of Val, Ile, and Trp required for maintenance was calculated to be 247, 134, and 37 mg/$BP_m^{0.73}$×*u* per day. Maintenance requirement is more adequately expressed as body protein content.

Keywords: isoleucine, maintenance, nitrogen balance, tryptophan, valine

Introduction

Many studies to determine maintenance requirement express the values as mg of amino acid per kg of body weight or mg of amino acid per kg of metabolic body weight (Leveille and Fisher 1960; Baker et al., 1996). However, there are differences in body composition of birds, especially regarding the fat content. Therefore, it is more adequate to express amino acid maintenance requirement based on protein weight of the bird, which would allow comparisons between studies (Burnham and Gous, 1992; Bonato et al., 2011). Thus, the aim of this study was to determine the requirements of digestible valine, isoleucine, and tryptophan for maintenance considering different unit systems and using Cobb 500 roosters.

Material and Methods

One hundred forty four Cobb 500 roosters were used in three nitrogen balance studies. Forty two roosters were used in valine trial, 54 in isoleucine and 48 in tryptophan trials. The roosters were individually housed in metabolic cages with individual feeder and nipple type drinkers. After 48 hours of fasting (receiving water + sucrose), 40 g of each diet limiting in Val, Ile, or Trp were fed by tube, once a day, for three days to give a range of intakes from 0 to 101, 0 to 119, and 0 to 34 mg for Val, Ile, and Trp per kg of body weight (BW_{kg}), respectively. A N-free diet was offered *ad libitum* during these three days. Excreta were collected during the last three days of the balance period and the N content of the excreta was analyzed. Nitrogen retention (NR) was obtained by the difference between N intake and N excretion. The nitrogen retention responses to the amino acid intakes were expressed in three unit systems: 1) mg per kg of body weight 2) mg per kg of metabolic weight and 3) mg per unit of metabolic protein weight at maturity. Statistical analyses were performed using the PROC REG from SAS 9.2 software.

Results

The lowest feed intake was observed in valine trial (118 g/day), followed by isoleucine (143 g/day) and tryptophan (167 g/day) trials (Table 1). The experimental diets provided nitrogen retentions that ranged from negative to positive NR values. As the amino acid intake increased the NR values also increased. The responses to the control diets were limiting in the diet, because they provided retentions between levels 2 and 3, validating this study.

Table 1. Mean values for feed intake (FI), amino acid intake (AAI), and nitrogen retention (NR) in different systems of units.

Levels (%)	FI	AAI, mg			NR		
	g/d	kg BW d	kg $BW^{0.75}$	kg $BP_m^{0.73}$ d	kg BW d	kg $BW^{0.75}$	kg $BP_m^{0.73}$ d
Valine							
0.000	111.0	0.0	0.0	0.0	-96.3	-137.5	-577.0
0.235	124.5	21.8	30.5	129.8	-37.8	-53.0	-222.5
0.470	108.6	43.4	61.2	256.8	-10.4	-15.8	-66.0
0.705	110.8	60.0	86.2	362.4	55.6	79.0	332.0
0.940	126.3	81.8	116.0	488.3	102.8	145.5	611.0
1.175	125.8	100.8	143.2	601.6	141.6	199.8	839.0
Root MSE	16.1	18.3	13.9	42.6	40.1	54.9	230.4
Isoleucine							
0.000	112.3	0.0	0.0	0.0	-58.2	-89.7	-379.5
0.404	142.8	27.0	41.5	175.0	20.5	31.2	131.3
0.606	148.6	39.6	60.6	256.6	67.2	103.6	438.0
0.808	133.5	50.8	79.17	335.8	68.8	106.7	451.5
1.010	153.8	64.7	100.5	424.7	94.2	145.5	615.8
1.212	144.8	78.2	120.6	510.6	140.6	216.8	916.6
1.414	155.5	94.5	144.0	608.3	189.0	286.8	1211.5
1.818	156.2	118.6	183.2	774.4	221.4	342.0	1448.2
Root MSE	29.86	6.04	6.96	28.74	27.39	40.54	30.83
Tryptophan							
0.000	158.0	0.0	0.0	0.0	-48.8	-76.2	-322.0
0.095	154.0	6.0	9.8	40.5	13.8	21.3	89.5
0.190	162.8	12.0	18.8	79.8	61.2	95.7	405.5
0.285	162.7	17.5	27.7	118.0	82.8	129.8	550.0
0.380	166.2	23.2	36.8	155.8	121.2	191.2	810.2
0.475	172.3	29.7	46.3	196.5	182.8	287.0	1215.2
0.570	194.7	34.0	53.7	227.2	207.0	327.5	1388.3
Root MSE	33.5	1.5	1.7	7.0	29.2	44.4	187.7

BW = Body weight; $BP_m^{0.73}$ = Body protein at maturity; Root MSE = root mean square error.

The regression equations obtained for all the different systems of units regarding the valine, isoleucine, and tryptophan trials are shown in Table 2. The maintenance requirements determined for valine, isoleucine and tryptophan were 41, 20 and 5 mg/BW, respectively. The requirements determined considering the mg of amino acid per unit of metabolic protein weight at maturity were 24, 134, and 37 mg/$BP_m^{0.73}$ per day for valine, isoleucine, and tryptophan, respectively. Many researchers recommend requirements for maintenance using the system mg/BW per day or mg/$BW^{0.75}$ per day (Leveille and Fisher, 1960; Leveille et al., 1960). However, these systems do not consider the differences in body composition. According to Taylor (1980) and Emmans (1989), the requirement for maintenance should be related to protein weight of the bird and maturity rate of body protein (u), which is the ratio of the current protein weight of the bird divided by the mature protein weight. Because the scale used by these authors does not consider the fat content, it is suitable for avoiding imprecision in the definition of amino acid requirements due to variation of fat content in the body of the birds, which can vary widely in birds of the same genotype and body weight (Burnham and Gous, 1992). These authors suggested that the best way to express the amino acid requirement for maintenance in growing and adult birds is according to protein weight, because there is no demand for amino acid to maintain the adipose tissue.

Table 2. Maintenance requirements for valine, isoleucine, and tryptophan estimated from linear equations considering three units.

Amino acid	Regression equation[1]	R^2	Requirement
Valine			
mg/kg BW d	NR = -98.6 (±10.1) + 2.4 (±0.2) × Val	0.90	41
mg/kg $BW^{0.75}$ d	NR = -140.6 (±14.7) + 2.4 (± 0.2) × Val	0.90	59
mg/kg $BP_m^{0.73}$ d	NR = -591.7 (±62.2) + 2.4 (±0.2) × Val	0.90	247
Isoleucine			
mg/kg BW d	NR = -46.9 (±7.1) + 2.3 (±0.1) × Ile	0.92	20
mg/kg $BW^{0.75}$ d	NR = -72.6 (±11.1) + 2.3 (±0.1) × Ile	0.92	32
mg/kg $BP_m^{0.73}$ d	NR = -307.9 (±46.7) + 2.3 (± 0.1) × Ile	0.92	134
Tryptophan			
mg/kg BW d	NR = -39.5 (±7.7) + 7.3 (±0.4) × Trp	0.92	5
mg/kg $BW^{0.75}$ d	NR = -62.9 (±12.5) + 7.3 (±0.4) × Trp	0.92	9
mg/kg $BP_m^{0.73}$ d	NR = -266.7 (±52.4) + 7.3 (±0.4) × Trp	0.92	37

[1]The data from control diet was not used in the statistical analyses. BW = Body weight; BP_m = Body protein at maturity; NR = nitrogen retention; Val = valine; Ile = isoleucine; Trp = tryptophan.

Conclusions

Based on the concepts presented in this study, the requirement of valine, isoleucine and tryptophan for maintenance are 247, 134, and 37 mg/kg $BP_m^{0.73}$ d, respectively.

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P-199 (ID 84)

PARTIAL REPLACEMENT OF COMMERCIAL PROTEIN CONCENTRATE BY FEATHER MEAL IN BROILER DIETS

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Abstract

Two hundred and sixty four straight run day old chicks were reared for a period of 35 days to know the effect of inclusion of RFM and TFM as a replacement of CPC in broiler diets. The inclusion levels of RFM and TFM were 0, 2, 4 and 6% of the diets. The RFM and TFM respectively replaced 0, 25, 50, and 75 % of CPC in the diets where CPC content of control diet was 8%. A total of 24 healthy birds of either sex from non-feather meal dietary group were reared from 36 to 49 days for estimating the digestibility of CPC, RFM and TFM in metabolic trials. The crude protein contents of CPC, RFM and TFM were estimated as 53.7, 83.5 and 81.3%, respectively. Feed intake, live weight gain, feed efficiency and PER of the birds 2% RFM and TFM diet were considerably better than others. Feed intake and liveweight of broiler birds decreased linearly with the increase of RFM and TFM level beyond 2% level. Mortality of birds did not differ significantly among the tested diets. The percent digestibility was highest in CPC, intermediate in TFM and lowest in RFM diet. Inclusion of TFM in broiler diets may be suggested at 2% level by replacing similar amount of CPC.

Keywords: Commercial protein concentrate, feather meal, broiler diet

Introduction

Rapid growth in poultry production demands increased amount of protein concentrate along with other feed ingredients. Cheap and locally available alternative sources of proteins should be identified to meet the growing demand. Dale (1992) reported that feather meal contained a level of high energy (3092-3996 kcal/kg ME). Heating feather meal results in cleavage of disulfide bonds of cystine, which allow better digestion of this product in growing chicks (Moran *et al*., 1966). Due to differences in processing conditions, commercial feather meals vary widely in their protein digestibilities (Papadopoulos *et al***.,** 1985; Han and Parsons, 1991). Burgos *et al***.** (1974) reported that the amino acids in feather meal are almost completely digestible (95%). On the other hand, some other researchers (Parsons *et al.,* 1982; Sibbald, 1986) found much lower digestibility (about 32%) of these amino acids. However, the digestibility of feather meal could be improved through treatment. The present study has therefore been designed to examine the effects of the replacement of protein concentrate by graded levels of feather meal in broiler diets.

Materials and Methods

The study was conducted with 264 straight run Hubbard Classic broiler chicks and birds were reared on floor under almost similar management condition. Commercial protein concentrate (CPC) in diets were replaced by two different types of feather meals (raw and treated) with four different levels (0, 2, 4, and 6% of the diet). Twenty four separate pens having 3 replications in each dietary group were used for the trial. Seven iso-nitrogenous and iso-caloric diets were fed to the birds. Raw feather meal (RFM) was treated with alkali and autoclaved for 20 minutes under 15 PSI. Thereafter, the materials were oven dried and ground to produce treated feather meal (TFM).

Protocol of metabolic trial

After 35-day trial, 24 selected healthy birds of either sex (mean liveweight of 810±42g) from control pens were transferred to individual cages and reared for 14 days for metabolic trial. Ten days acclimatization period was provided for all birds with clean drinking water and standard broiler diet containing 22.04% CP and 2881 kcal ME/kg. Six birds were randomly allocated to each of the test feeds (i.e. CPC, RFM and TFM) and rest six birds for feeding glucose to determine endogenous loss. After 10 days adjustment period, individual bird was fasted for 24h. Birds of all groups were then drenched 40g of each test feeds into the crop through a 60ml syringe fitted with 18cm plastic tube of 3.85mm internal and 6.60mm external diameter. During this period, excreta were collected for 24h from individual bird. Fresh excreta was then weighed and preserved at -20°C until they were used for chemical analysis.

Chemical analysis, determination of endogenous losses and digestibility

All test materials and homogenized fresh excreta were subjected to chemical analysis and were determined by using proximate analysis procedure according to AOAC (1984). Proportion of nutrient found in excreta (abraded intestinal mucusa, bile, digestive fluid etc.) during glucose feeding period was considered as the endogenous loss and true digestibility was estimated according to Banerjee (1988).

Statistical analysis

All recorded and calculated data were for a 4 (FM levels) × 2 (FM types) × 3 (replication) in completely randomized design. The analysis of variance was done using SPSS 11.5 software (SPSS, 1999) and the significant differences between means were identified by Duncan's New Multiple Range Test.

Results and Discussion

Chemical analysis indicates that moisture, OM, CP and CF contents were much higher in feather meals (Table 1). Crude protein contents of CPC, RFM and TFM were 53, 83 and 81% respectively. Crude protein content of feather meals got support from earlier studies (Dale, 1992; Chubb, 1982 and Waldroup, 1982). On the other hand, just opposite scenario persisted for EE and Ash content.

Table 1. Chemical compositions of CPC, RFM and TFM

INGREDIENTS	Available nutrients (g / 100g)						
	Moisture	DM	OM	CP	CF	EE	Ash
CPC	4.61	95.39	75.12	53.74	1.43	9.02	20.27
RFM	8.76	91.24	90.25	83.50	2.96	2.79	0.99
TFM	9.09	90.91	89.04	81.25	2.32	4.49	1.87

The effect of feather meal types, inclusion levels and their interaction on feed intake, body weight, feed conversation, mortality, protein intake and protein efficiency ratio of broilers were calculated. Except final liveweight, none of the performance characteristics was affected by feather meal types, or by its interaction with level of inclusion. On the contrary, all of the parameters studied, other than mortality of birds, were remarkably influenced by levels of feather meals inclusion in broiler diets. Results indicate that birds fed 2% TFM diet consumed highest amounts (2009.85g) of feed, which was more or less close ($P>0.05$) to that of CPC diet (2006.33g). Replacement beyond 2% of CPC in broiler diets adversely affected ($P<0.01$) the feed intake of birds, which in turn lowered the growth performance of birds. The FM types, replacement levels and their interaction on body weight were statistically significant ($P<0.01$). The highest weight gain was achieved in 2% level of TFM (991g) followed by the 0% (928g), 4% (849g) and 6% (781g). Feed intake and live weight of broiler birds decreased linearly with increased RFM and TFM levels. But at 2% level of RFM and TFM birds ate more and gained higher liveweight. This superior growth was simply due to higher nutrient intake and better nutrient utilization by the birds. As a result, protein intake was also higher for the birds fed 2% FM diet. The feed conversion showed significant differences among levels; birds fed on 2% FM diet converted feed more efficiently than others. The PER decreased with the increasing level of RFM and TFM, that could be due to the poor digestibility of protein of FM. Sibbald (1986) also reported similar findings. However, results indicate that the feed intake, liveweight and feed conversion were superior with TFM at 2% inclusion level in broiler diet. The true digestibility of the CPC, RFM and TFM are shown in Table 2, which indicates that the digestibility of nutrients of CPC is much higher ($P<0.01$) than that of RFM and TFM. Low digestibility of FM may explain its naturally occurring carotinoid compounds and disulfide bonds of cystine. The findings got support from some other researchers (Liener, 1980). Digestibility of TFM was found superior to that of RFM except EE and Ash. Crude protein digestibility of FM increased from 43% to 47% which might be as a result of alkali treatment.

Table 2. True digestibility of different nutrients of CPC, RFM and TFM

Ingredients	Digestibility					
	DM	OM	CP	CF	EE	Ash
Protein concentrate	61±4.9 [b]	64±4.6 [b]	65±1.4 [c]	41±1.6 [c]	52±3.2 [c]	32±3.8 [c]
Raw feather meal	44±5.6 [a]	48±5.2 [a]	43±4.3 [a]	29±2.3 [a]	47±3.8 [b]	22±4.4 [b]
Treated feather meal	47±4.0 [a]	52±3.9 [a]	47±1.4 [b]	37±3.7 [b]	40±4.0 [a]	11±3.5 [a]
F value and sig. level	20.82**	19.75**	108.42**	31.11**	15.87**	39.19**

Values bearing different superscripts in the same row differ significantly; NS, Not significant; **, P<0.01

Conclusions

It may be concluded that TFM due to alkali treatment may loose some of its nutrient quantity but at the same time digestibility could be increased. Inclusion of FM in broiler diets may have a positive effect up to 2% level. Further study is necessary to overcome the factors that limiting the utilization of FM as feed ingredients for broiler.

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P-200 (ID 197)

EFFECT OF REPLACING SOYBEAN MEAL WITH DIFFERENT PROTEIN SOURCES DURING STARTER PERIOD ON PERFORMANCE AND FOOT PAD DERMATITIS IN BROILERS

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Abstract

Focus on early nutrition in boiler feeding has lately gained more attention. Fishmeal (FM), potato protein (PP), corn gluten (CG) and HP AviStart is common use as partly replacements of SBM. FM, PP and CG have all in common low K content in comparison to SBM. Too high K levels may leads to the consumption of large amount of water, and as a consequence there is an increase risk in litter moisture. That creates a predisposition for the development of FPD (foot pad dermatitis). In this study, we examined the effect of different sources of protein in starter feed on production performance and occurrence of FPD in broiler chickens. The experiment was conducted with 1,110 Ross 308 broiler chickens in duration of 42 days. The experiment was set up in five treatments with six replicates (37 chicks per replicate). Treatments were as follows: T1 soybean meal, T2 potato protein, T3 fishmeal, T4 HP AviStart and T5 corn gluten. All used starter feeds had the same energy content and similar protein content. Feeding with starter diets lasted for 11 days, after which all groups consumed the same feed - grower (from 12-28 days) and finisher (29-42 days). There were no statistically significant differences among treatments at day 42 in live weight. Scoring of foot pads lesions showed that the lowest level of foot pad damage for T4 group (0.52), and highest for T5 (0.89) and T1 (0.92) ($P<0.05$). Lowest FCR for T4 (1.74) and highest for SBM (1.81) ($P<0.05$) were found.

Keywords: protein sources, performance, FPD, broilers

Introduction

Factors such as minerals, crude protein level and quality, digestibility of raw materials, fat quality and feed form play a role in the consistency of the faeces (DE JONG et al., 2013) and thereby be a contributing cause for FPD. Prolonged contact of feet with fecal material and high moisture of the litter could contribute to the development of pododermatitis. Due to an all-vegetable diet typical will have higher content of K in the feed, the use of animal protein sources is widely seen as solution for dealing with the FPD, especially if only SBM is used a sole protein source. EICHNER et al. (2007) found that birds raised on an all-vegetable diet had a higher incidence of FPD than did birds rose on a mixed animal and plant based diet. EICHNER et al. (2007) found that addition of corn gluten meal to an all-vegetable diet reduced the incidence of FPD when compared with a vegetable - and animal-based diet. Studies on protein level and source have provided inconsistent results. The aim of the present study is to compare a new novel protein source HP AviStart to other protein source on its effect on performance and potential effect on FPD.

Material and Methods

The experiment was conducted at the Experimental farm of Faculty of Agriculture in Novi Sad, Serbia. The experiment was conducted with 1,110 Ross 308 broiler chickens in duration of 42 days. The experiment had five treatments with six replicates (37 chicks per replicate). Treatments were as follows: T1 soybean meal, T2 potato protein, T3 fishmeal, T4 HP AviStart and T5 corn gluten. All used starter feeds had the same energy content and similar protein content. Feeding with starter diets lasted for 11 days, after which all groups consumed the same feed - grower (from 12-28 days) and finisher (29-42 days). Measurements of body weight were performed weekly. Mortality and feed intake were recorded on a daily basis. Feed consumption was measured weekly. Feed conversion ratio and average weekly weight gain were calculated at the end. Foot pad lesions were recorded on all birds at 42days of age using a 0–3 scoring system. Briefly, the scores were 0: no lesion; 1: lesion on <25% of the pads; 2: lesion on 25 to 50% of the pads; 3: lesion on >50% of the pads (EICHNER et al., 2007). European Production Efficiency Factor (EPEF) was calculated using following equation:

$$\text{EPEF} = \frac{\text{body weight (g) x survival rate (\%)}}{\text{feed conversion x duration of trial (days)}}$$

Data were analysed by ANOVA using the GLM procedure and means separated by Duncans *post* hoc test using StatSoft computer package (STATISTICA 11).

Table 1. Feed composition

Ingredients,%	Starter (0-11)					Grover (12-28)	Finisher (29-42)
	T-1	T-2	T-3	T-4	T-5	All groups	All groups
Corn	52.00	59.23	60.10	54.99	54.89	57.55	59.77
Potato prot.	0.00	5.00	0.00	0.00	0.00	0.00	0.00
Corn gluten	0.00	0,00	0,00	0.00	5.00	0.00	0.00
HP AviStart	0.00	0.00	0.00	5.00	0,00	0.00	0.00
Fish meal	0.00	0,00	5.00	0.00	0.00	0.00	0.00
Full fat soya	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Soya 44%	34.40	24.20	24.40	27.20	28.00	29.00	26.30
Soybean oil	4.34	2.25	1.83	3.52	2.73	4.54	5.43
Rest [1]	4.25	4.42	3.78	4.29	4.42	3.89	3.53
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Calculated composition							
Protein, %	21.4	21.1	21.2	21.3	21.9	19.5	18.5
ME, MJ/kg	12.85	12.85	12.85	12.85	12.85	13.20	13.60
Lys	1.30	1.30	1.30	1.30	1.30	1.15	1.09
Met+Cys	0.97	0.97	0.97	0.97	0.97	0.88	0.83
K	0.98	0.82	0.85	0.95	0.86	0.89	0.83

1Lys, Met, Thr, MCP, NaHCO3, CaCO3, NaCl and premix

Results

No significant difference among treatments for body weight at day 42 was found (Table 2). Lowest FCR for T4 (1.74) and highest for SBM (1.81) (P<0.05) were found. For neither mortality or EPEF no significant difference among treatments was found.

Table 2. Performance of broiler chicken

Item	T1	T2	T3	T4	T5
Average body weight at 42 day (g)	2,238	2,207	2,188	2,224	2,290
Feed conversion ratio	1.81^{b}	1.80^{b}	1.76^{ac}	1.74^{a}	1.80^{bc}
Mortality (%)	5.4	3.6	1.8	0.9	4.1
European Production Efficiency Factor	279	282	292	302	292

$^{a-c}$ Values among rows with no common superscript are significaly different (P<0.05)

Significant difference among treatments were found, with lowest level of foot pad damage for T4 group (0.52), and the highest for T5 (0.96) and T1 (0.92) (P<0.05) (Table 3). These results are similar with those noted by EICHNER et al. (2007) with the birds fed all CG showing the lowest incidence of foot pad dermatitis (P ≤ 0.05).

Table 3. Footpad lesion score at day 42

Treatments	Total broilers	Number of broilers with lesion score				% of broilers with lesion score				Average score
		0	1	2	3	0	1	2	3	
T1	210	59	105	46	0	28.1	50.0	21.9	0.0	0.92^{c}
T2	214	94	98	22	0	43.9	45.8	10.3	0.0	0.66^{b}
T3	218	91	109	18	0	41.7	50.0	8.3	0.0	0.76^{b}
T4	220	114	97	9	0	51.8	44.1	4.1	0.0	0.52^{a}
T5	213	61	115	37	0	28.6	54.0	17.4	0.0	0.89^{c}

$^{a-c}$ Values between colum with no common superscript are significaly different (P<0.05)

VIERIA and LIMA (2005) found that feeding a corn-soybean meal diet formulated with animal feed component (3% pork by-product, 2.5% poultry by-product and 1.5% feather meal) and 0.14% less K produced litter with less moisture than diets formulated exclusively with corn and soybean meal.

Conclusions

Usage of different alternative protein sources like FM and PP, together with HP AviStart offer solution for reduction in occurrence and intensity of damage to the foot pads in broilers.

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P-201 (ID 46)

ESTABLISHING AN AMINO ACID PATTERN AND IDEAL RATIO FOR MAINTENANCE OF POULTRY BY DELETION METHOD

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Abstract

This study aimed to estimate the essential amino acid (EAA) profile and the ideal ratio (IAAR) for maintenance of poultry by deletion method. A nitrogen balance (NB) trial was conducted using 138 adult roosters, housed individually in metabolic cages. The treatments were 23 purified diets being 11 diets with an amino acid mixture providing high protein intake of 500 mg N/$BW_{kg}^{0.75}$ per day and 11 diets providing low protein intake of 250 mg N/$BW_{kg}^{0.75}$ per day (in each diet one EAA was diluted 50%) and one nitrogen free diet. Each treatment had six replicates. After 48 h of fasting receiving water plus sucrose, the roosters were fed 40 g of the diets by tube once a day for three days. The excreta were collected within 72 h after the first feeding. The diets and excreta were analyzed for nitrogen content. For each EAA studied, a linear regression was fitted by NB and amino acid intake (AAI). The maintenance requirements were estimated as the AAI to maintain the NB equal to zero. The daily EAA requirements for maintenance were estimated to be: Lys 11, Met 40, Thr 30, Trp 7, Arg 54, Val 29, His 6, Gly 57, Phe 50, Leu 82 and Ile 25 mg/$BW_{kg}^{0.75}$ per day. Therefore, the IAAR for maintenance was concluded to be Lys 100, Met 372, Thr 274, Trp 66, Arg 498, Val 264, His 55, Gly 522, Phe 460, Leu 760 and Ile 235% independent of the scale. The EAA profile and the IAAR for maintenance of poultry estimated in this study contribute to improve factorial model for estimating EAA requirements for poultry.

Keywords: amino acid, maintenance, nitrogen balance, purified diet, rooster

Introduction

Maintenance requirement is an important component in factorial models because in young animals the maintenance requirement is lower in relation to the total requirement and assumes greater importance as the poultry reaches maturity due to the lower need for essential amino acids (EAA) for growth (OWENS and PETTIGREW, 1989). One alternative approach used to determine the maintenance requirement for each EAA is the deletion method, based on monitoring the nitrogen balance (NB) that arises as the EAA are reduced from the diets. This approach allows determining the requirements for all EAA in one set of experiments and is generally accepted as an efficient and rapid tool to estimate the ideal EAA profile. Thus, in the present study, the deletion method was used to update and obtain new coefficients for maintenance requirements for poultry to derive an ideal EAA ratio for maintenance.

Material and Methods

A NB trial was conducted using 138 adult Cobb roosters. The treatments consisted of two protein levels (low and high) where 11 EAA were evaluated (lysine, methionine, threonine, tryptophan, arginine, valine, phenylalanine, histidine, glycine, isoleucine, and leucine) and a nitrogen-free (NF) diet, totalling 23 treatments with six replicates each. The purified diets were formulated to meet the requirements for energy and other nutrients for roosters (ROSTAGNO et al., 2011), except protein and EAA. The EAA composition of the diets with low (LP) and high protein concentration (HP) was based on the EAA profile for maintenance established by LEVEILLE et al. (1960). The series of HP diets were made, each providing one of the EAA tested in the quantity required for maintenance but with twice the other EAA required for maintenance, resulting in a daily nitrogen intake (NI) of 500 mg/$BW_{kg}^{0.75}$. The series of LP diets were made, each providing half of the EAA tested in the quantity required for maintenance and the other EAA in adequate quantities to meet their requirements for maintenance, totalling a daily NI of 250 mg /$BW_{kg}^{0.75}$. The birds underwent a fasting period of 48 h, receiving 60 ml of water with sucrose (50:50), once a day, by intubation. In the subsequent 72 hours the birds received 40 g of the diet by intubation. The NF was made available ad libitum during the trial. The excreta were collected daily and, at the end of the trial, leftover feed was quantified to determine feed intake. Diets and excreta were analysed for total nitrogen content to quantify NI, nitrogen excretion (NEX) and NB (NI-NEX). Data from NB trial were subjected to a linear regression, considering the amino acid intake (AAI) as the independent variable and the NB as dependent variable. The maintenance requirements were estimated as the AAI to maintain the NB equal to zero (in mg/$BW_{kg}^{0.75}$)

Results

The mean values and the standard deviations (±SD) from NB assay are presented in Table 1. The SD of the data was low considering the small amount of data obtained for each EAA. The roosters fed diets limiting in lysine and tryptophan in the LP diets presented higher NB than other EAA, which were above the maintenance requirement. However, roosters fed limiting diets in valine and histidine in LP diets had the NB close to zero, which were closer to nitrogen equilibrium in the LP diets. The linear equations obtained using the NB data, daily maintenance requirements expressed in $mg/BW_{kg}^{0.75}$ and the ideal EAA ratio are presented in Table 2. The difference between the EAA pattern required for growth and that required for maintenance concerns lysine (JANSEN, 1974). The same authors also explains that of all EAA, lysine turnover is quite low and this implies that lysine required for maintenance is also quite low (JANSEN, 1974). This low requirement was also observed. Consequently, the ideal ratio relative to lysine (Table 2) was quite different from those established for growth (ROSTAGNO et al. 2011), with higher values. However, the EAA ratio presented in Table 2 was similar to that presented by LI et al. (2003).

Table 1. Mean of daily values (±SD) resulting from the nitrogen-free diet (NF) and individual amino acid (EAA) deletions in the low (LP) and high protein (HP) diets, in $mg/BW_{kg}^{0.75}$

EAA	Diets[1]	AA Intake	Nitrogen		
			Intake	Excretion	Balance
	NF	0.0± 0.0	66.1 ± 2.7	258.7 ± 3.3	-192.6 ± 1.5
Lys	LP	17.7 ± 0.6	312.0 ± 9.9	234.2 ± 6.0	77.8 ± 6.1
	HP	35.8 ± 0.8	557.4 ± 15.5	200.7 ± 6.9	356.7 ± 15.0
Met	LP	54.5 ± 1.2	231.1 ± 5.5	194.3 ± 6.8	36.9 ± 7.7
	HP	109.1 ± 2.1	550.7 ± 11.6	219.3 ± 9.3	331.4 ± 9.0
Thr	LP	45.0 ± 0.7	298.9 ± 4.7	231.8 ± 7.9	67.1 ± 7.6
	HP	89.6 ± 1.4	542.8 ± 8.9	181.7 ± 12.2	329.4 ± 15.3
Trp	LP	11.6 ± 0.1	286.6 ± 5.3	204.6 ± 10.1	82.0 ± 5.8
	HP	23.1 ± 0.4	560.8 ± 9.7	191.8 ± 5.3	369.0 ± 9.3
Val	LP	34.5 ± 0.4	285.4 ± 5.3	290.9 ± 9.4	-5.5 ± 4.4
	HP	67.6 ± 1.2	457.7 ± 7.1	239.2 ± 7.0	218.5 ± 5.4
Arg	LP	67.5 ± 1.1	273.7 ± 4.2	261.7 ± 7.1	12.0 ± 9.0
	HP	139.9 ± 2.4	486.4 ± 9.8	210.9 ± 4.7	275.5 ± 11.0
Ile	LP	41.5 ± 0.4	265.0 ± 3.3	203.8 ± 6.4	61.3 ± 4.6
	HP	80.1 ± 1.3	460.7 ± 6.9	185.8 ± 18.4	274.9 ± 7.1
Leu	LP	69.7 ± 0.6	263.6 ± 3.6	240.8 ± 5.8	22.8 ± 4.5
	HP	138.4 ± 1.0	441.3 ± 3.5	200.9 ± 13.3	240.4 ± 1.8
His	LP	4.5 ± 0.0	308.3 ± 2.6	305.7 ± 5.4	2.6 ± 6.4
	HP	8.9 ± 0.1	442.0 ± 5.6	253.6 ± 7.4	188.5 ± 5.1
Phe	LP	33.9 ± 0.3	243.9 ± 1.6	265.6 ± 6.9	-21.6 ± 6.0
	HP	67.0 ± 0.5	436.0 ± 3.5	241.7 ± 5.2	194.3 ± 5.7
Gly	LP	47.5 ± 0.5	280.9 ± 2.8	255.7 ± 3.7	25.1 ± 4.3
	HP	95.2 ± 1.2	499.5 ± 3.8	253.7 ± 6.6	245.8 ± 4.7

Table 2. Linear equations obtained from nitrogen balance (NB) trial with their coefficient of determination (R^2), calculated requirements and the ideal ratio for maintenance (IAAR)

Amino acid	Equations from linear regression	Requirement ($mg/BW_{kg}^{0.75}$)	IAAR	R^2	Probability
Lys	NB=-171.9(±4.16)+16.1(±0.32)×Lys	11	100	0.98	<0.001
Met	NB=-198.4(±6.25)+5.0(±0.16)×Met	40	372	0.96	<0.001
Trp	NB=-188.4(±5.17)+25.1(±0.61)×Trp	7	66	0.96	<0.001
Thr	NB=-178.0(±5.33)+6.0(±0.17)×Thr	30	274	0.96	<0.001
Arg	NB=-167.3(±5.15)+3.1(±0.10)×Arg	54	498	0.96	<0.001
Val	NB=-156.8(±5.36)+5.4(±0.23)×Val	29	264	0.94	<0.001
Ile	NB=-165.8(±3.64)+6.6(±0.13)×Ile	25	235	0.97	<0.001
Leu	NB=-171.4(±2.60)+2.1(±0.05)×Leu	82	760	0.98	<0.001
Phe	NB=-164.0(±4.63)+3.3(±0.20)×Phe	50	460	0.96	<0.001
Gly	NB=-158.3(±3.13)+2.8(±0.09)×Gly	57	522	0.98	<0.001
His	NB=-163.0(±4.57)+26.0 (±1.48)×His	6	55	0.93	<0.001

Conclusions

The deletion method was valid to update the EAA requirements and establish an IAAR for maintenance of poultry, which can contribute to improve the factorial models.

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P-204 (ID 282)

STOCHASTIC MODEL TO PREDICT LYSINE REQUIREMENT AND EGG PRODUCTION OF BROILER BREEDER HENS

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Abstract

The aim of this study was to develop a stochastic model to estimate lysine (Lys) requirements for broiler breeder hens, depending on body weight (BW) and egg production (EP). A population of broiler breeder hens was generated considering average BW of 2.34 (±0.23) kg. The age from first egg (AFE) adopted was 193d, which was estimated from the lighting program applied during the growing phase by Bristol-Reading Model. The model incorporated BW, AFE, internal cycle length ($ICL=25.40+8.99\times(0.75^{AFE})+0.07\times AFE$) to determine the EP (ICL/24), yolk ($YW=18.03\times[1-e^{-0.015\times(AFE-103.4)}]\times e^{-0.001\times AFE}$), albumen ($AW=14.38\times YW^{0.375}$) and shell ($SW=0.358\times(YW+AW)^{0.687}$) weights. These data were used to simulate EP and developing a model to calculate Lys requirements (Lys_r): $Lys_r=((600*BP^{0.73}*BP)+((Lys_y*(0.03*YW)+(Lys_a*(0.02*AW)/0.6)/EP)$ where BP is body protein content calculated from BW, Lys_y and Lys_a are the Lys contents of yolk and albumen, 0.03 and 0.02 are nitrogen content YW and AW, respectively, and 0.6 is efficiency of utilization. The maximum EP estimated was 87% at 28 weeks of age and declined to 57% at 60 weeks of age. The mean egg weight (EW =YW+AW+SW) increased from 63 to 77g, YW from 15 to 23g and AW from 40 to 47g. Considering the BW and EP, the Lys intake required by the flock increased to 838 mg/d at 28 weeks then declined to 753 mg/d at 60 weeks of age. The recommendation of Lys would be 5.3 g/kg of diet for broiler breeder hens at peak production for a daily feed intake of 160 g/d.

Keywords: broiler breeder, modelling, requirements

Introduction

Feed properly broiler breeder flocks has become essential to ensure the increasing production of hatching eggs. Thus, mathematical models have been used as a tool in poultry nutrition to adjust the nutritional levels. This approach has raised a number of issues that were previously ignored by researchers or were subjects to isolated studies that were previously unrelated to model development. Such models have been the basis of several trials, which the measurements considered the growth rates of ovary and oviduct prior to the onset of lay (Bowmaker and Gous, 1989; Ferreira et al., 2015), changes in internal cycle length over time (Johnston and Gous, 2003), and allometric relationships between yolk, albumen and shell weight (Johnston and Gous, 2007). However, issues still need to be studied in order to update and improve mechanistic models. Moreover, the modelling process could be used to identify gaps in the knowledge on physiological systems. Therefore, the aim of this study was to develop a stochastic model to predict the lysine requirements of broiler breeder flocks during the production of eggs, which can be calculated according to the requirements for different conditions such as BW and the lighting program used during growing period.

Material and Methods

The model described here simulates the potential for egg production and hence lysine requirements for a flock of broiler breeder hens. The information required for developing this simulation model was age and BW at the beginning of the laying period, the potential rate of EP, EW, changes in ICL, yolk, albumen and shell content, and body composition over the laying period. These variables were obtained from various sources, including a research reported by Ferreira et al. (2015). A population of broiler breeder hens was generated in Excel spreadsheet, considering the average BW of 2.34 (±0.23) kg. The age from first egg (AFE) adopted was 193d, which was estimated from the lighting program applied during the growing phase by Bristol-Reading Model. The model incorporated BW, AFE, internal cycle length ($ICL=25.40+8.99\times(0.75^{AFE})+0.07\times AFE$) to determine the EP (ICL/24), yolk ($YW=18.03\times[1-e^{-0.015\times(AFE-103.4)}]\times e^{-0.001\times AFE}$), albumen ($AW=14.38\times YW^{0.375}$) and shell ($SW=0.358\times(YW+AW)^{0.687}$) weights.

Results and discussion

The mean reproductive performance (yolk, albumen and shell weight, EW, rate of laying and egg output), and the minimum, mean and maximum daily intakes of Lys required by the simulated population of broiler breeders hens are summarized at four-weekly intervals in Table 1. The maximum rate of lay of 87% was achieved at 28 weeks of age and declined up to 57% at 60 weeks of age. The laying performance simulated for a flock of broiler breeder hens in this exercise was similar to the Cobb-Vantress target breeding performance, validating the mechanistic method used to simulate rate of lay (Figure 1).

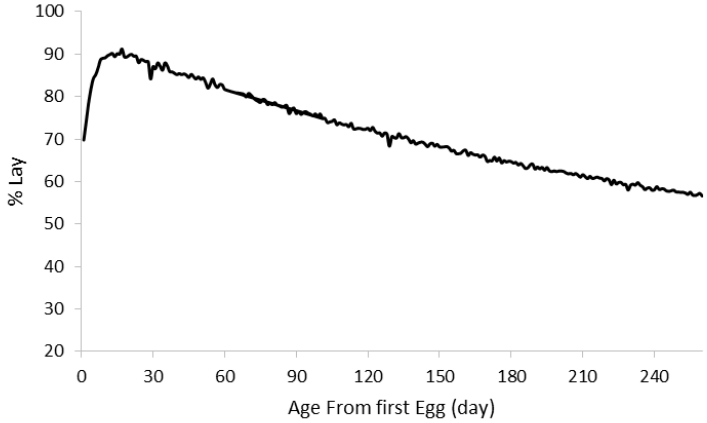

Figure 1 Percentage of lay of a theoretical population of fifty broiler breeder hens over the 260 days after the first egg.

Similarly, the increase in egg weight over time was similar to the changes that are expected in a commercial broiler breeder flock. It was observed that the requirements for the yolk and albumen increased over the weeks due to the increase in the size of the egg formed. However, the egg output decreases with the age and the requirements of daily amino acid also decreases (Gous and Nonis, 2010). The daily intake of Lys required by the flock increased to 838 mg/d and then declined to 753 mg/d by 60 weeks. Ekmay et al. (2013) estimated the amino acid requirements for broiler breeders at peak production, and recommend for Lys (916 mg/g), values above those found in this study compared to 28 weeks, probably due to BW difference.

The production of eggs, the body weight of the birds and the quality of feed, will directly influence the results of the requirement and consequently the concentration of nutrients. The concentration of nutrients estimated in this study is lower than that recommended by the breed manual. By considering the nutritional recommendations of the manual, the amount of feed offered (DFI) would be less than 160 g/d which was similar to the results of Gous and Nonis (2010). These authors reported that the DFI estimate is often considerably lower than the daily allowance, which is uncommon for broiler breeder hens. In this case, the model predicts a decrease in consumption as the supply of nutrients increases.

Table 1. The mean weight of yolk, albumen and shell (g), percentage of lay and egg output (g/d), and the minimum (Min), mean and maximum (Max) daily intakes of lysine (mg/d) required at four-weekly intervals of age for theoretical population of broiler breeders.

Age	Yolk	Albumen	Shell	Egg weight	Lay	Egg Output		Lysine	
Week	g	g	g	g	%	g/d	Min	Mean	Max
24	15.0	39.7	5.6	60.3	84	50.4	360	773	979
28	17.1	41.7	5.9	64.6	87	56.0	767	838	939
32	18.6	43.0	6.1	67.7	81	55.1	748	827	895
36	19.7	44.0	6.2	69.9	77	53.8	717	819	884
40	20.6	44.7	6.3	71.6	73	52.2	694	765	854
44	21.3	45.3	6.4	73.0	69	50.2	668	730	777
48	21.9	45.8	6.5	74.1	66	48.6	712	734	751
52	22.4	46.2	6.5	75.1	62	46.8	738	739	740
56	22.9	46.5	6.6	76.0	60	45.3	745	746	747
60	23.4	46.9	6.6	76.9	57	43.9	752	753	754

It should be taken into consideration that in the nutrients partition, maintenance has priority, followed by the deposition of yolk and albumen. Besides, the egg output is dependent on the nutrient intake, since the requirements for maintenance and growth of the yolk are met. However, if the intake of the limiting nutrient for the day cannot sustain this minimum egg size, EP does not occur (Gous and Nonis, 2010). Therefore, to feed a flock of broiler breeders it is necessary to follow a daily pattern, even when no egg laying, the bird consumes the diet. This causes the energy consumed in excess to be deposited on the body.

Conclusions

The modelling approach presented here has the potential to help nutritionists to provide a more efficient feeding program for broiler breeder hen flocks. Moreover, the proposed model is dynamic and these parameters can be adapted to meet the requirement of the day. Improvements can be made in the model and also need to be re-evaluated regularly to keep pace with genetic advancements.

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P-205 (ID 318)

PROTEIN NUTRITION OF COMMERCIAL LAYING HENS

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Abstract

Two experiments were conducted to investigate the reduction of the protein level in the diet of laying hens from 24 to 40 weeks of age. In each experiment, 240 laying hens were distributed in a completely randomized design with 3 treatments (3 levels of CP: 17.4%, 16.4% and 15.4%), 10 replications and 8 birds per pen each. It was used Bovans White and Bovans Brown laying hens in the first and second trial, respectively. The parameters evaluated were performance, egg weight and shell quality (specific gravity, shell thickness and breaking strength) and internal egg quality (albumen height, Haugh unit and yolk color). Birds were fed *ad libitum* and parameters were evaluated in 4 cycles of 28 days each. In experiment I, egg shell quality, albumen height and egg weight were not affected by dietary treatments. Hens fed 16.4% CP did not have their performance reduced. In experiment II, only yolk color was affected by treatments, with higher yolk color in eggs of hens fed diets with 15.4% of CP. Considering these results, it is concluded that laying hens fed diets with 16.4% CP showed better results for egg production and feed conversion. However, the reduction of protein to 15.4% reduced egg production. In addition, for semi-heavy hens it is possible to reduce protein to 15.41% without reduce egg production and egg quality.

Keywords: Amino acids, egg production, egg quality, protein

Introduction

The modern laying hen has high egg production and laying efficiency. Layer hens are able to digest protein that will supply the amino acids (AA) needed for maintenance or for producing new proteins for growth or egg production; however, only less than half of the nitrogen in AA can be utilize by chickens (Latshaw and Zhao, 2011). Because of that, it is convenient to decrease the level of crude protein (CP) in the diet, since synthetic AA are supplemented to meet the requirements of limiting AA. On the other hand, it is necessary to determine the extent to which the CP content of laying hen diets can be reduced without decrease hen performance. Thus, the aim of this study it was to investigate the reduction of the protein level in the diet of laying hens from 24 to 40 weeks of age.

Material and Methods

A total of 240 hens (Bovans White), in the first trial, and 240 hens (Bovans Brown), in the second trial, were randomly assigned to 1 of 3 dietary treatment groups varying in protein level (17.4%, 16.4% and 15.4%). Each of the 3 treatments was assigned to 10 replicate cages with 8 hens per cage. Hens were placed in an open side house with feed and water provided for *ad libitum* consumption. Diets were based on corn, wheat bran, and soybean meal. Calculated levels of nutrients of experimental diets are in Table 1. Evaluation of performance and egg quality parameters, started 4 wk after the trial began. The evaluation was carried out in 4 intervals of 28 d from 24 to 40 wk of age. The evaluated performance parameters were feed intake (g/bird-day), egg production (% hen-day), egg weight (g), feed conversion (kg of feed intake/number of eggs produced). The number of eggs per cage was recorded each day and summarized by 28 d periods. The mean production of 2 consecutive days, after each 28 d period, was used for estimating the other parameters. Egg and eggshell quality traits were evaluated by egg weigth (g), albumen height, Haugh units, yolk color, eggshell thickness (mm), egg breaking strength (kgf), and egg specific gravity (g/mL of H2O). The results were analyzed using the statistical program SAS (2012). The research was conducted under the approval of the Ethics Committee on Animal Use of the School of Veterinary Medicine and Animal Science of the University of Sao Paulo.

Table 1. Diets compositions

	Protein levels (%)		
	17,4	**16,4**	**15,4**
Corn	62,450	63,380	64,320
Soybean meal	26,200	22,800	19,500
Wheat bran	0,000	2,400	4,500
Dicalcium phosphate	1,300	1,300	1,300
Fine limestone	5,210	5,166	5,188
Coarse limestone	4,000	4,000	4,000
Sodium chloride	0,350	0,350	0,350
DL – Methionine	0,190	0,220	0,250
L-Lysine	0,000	0,000	0,100
L-Threonine	0,000	0,058	0,103
L- Tryptophane	0,000	0,003	0,020
L-Valine	0,000	0,023	0,073
Mineral/vitamin mixture	0,300	0,300	0,300
Total	100,000	100,000	100,000
	Nutrient calculated		
ME, kcal/kg	2.756	2.756	2.756
CP, %	17,4	16,4	15,4
Calcium, %	4,04	4,04	4,04
Available P, %	0,46	0,46	0,46
Sodium, %	0,16	0,16	0,16
Methionine + cystine (%)	0,77	0,77	0,77
Lysine, %	0,91	0,83	0,83
Threonine, %	0,67	0,67	0,67
Tryptophane, %	0,21	0,20	0,20
Valine, %	0,83	0,80	0,80

Results

In Table 2, are presented means of performance parameters and egg quality traits measured in Bovans White hens. Different levels of dietary protein did not affect (P > 0,05) egg shell quality, albumen height and egg weight. However, hens fed 16.4% CP did not have their performance reduced when compared with those fed 17,4 CP.

Table 2. Performance and egg quality parameters of Bovans White from 24-40 weeks of age.

	Protein levels (%)				
	17,4	16,4	15,4	SEM	P
Egg production, %	93.05[a]	93.45[a]	90.05[b]	1,17	0,048
Feed intake, g/bird/day	125[b]	114[a]	111[a]	6	0,001
Feed conversion, kg/eggs	2,32[c]	2,11[a]	2,20[b]	0,07	0,032
Specific gravity, g/mL of H2O	1,096	1,096	1,095	0,002	0,721
Egg weight, g	58	58	56	3	0,070
Albumen height, mm	6.1	6.3	6.5	0,2	0,139
Yolk color	5.8[c]	6.0[b]	6.1[a]	0,05	0,034
Haugh units	78[b]	79[b]	82[a]	2	0,038
Egg breaking strength, kgf	4.87	5.19	5.05	0,37	0,276
Eggshell thickness, mm	0.45	0.43	0.43	0,01	0,245

[a,b] Means within a row not sharing a common superscript differ significantly based on Tukey test (P<0,05)

Performance and egg quality parameters measured in Bovans Brown hens are summarized in Table 3. The results show that different levels of dietary protein did not affect (P<0,05) performance, egg shell quality, and internal egg quality except yolk color with higher yolk color in eggs of hens fed diets with 15.4% of CP.

Table 3. Performance and egg quality parameters of Bovans Brown from 24-40 weeks of age.

Protein levels (%)					
	17,4	16,4	15,4	SEM	P
Egg production, %	94.48	94.23	93.26	2,24	0.690
Feed intake, g/bird/day	116	113	112	30	0,845
Feed conversion, kg/eggs	2,05	2,03	2,04	0,12	0,712
Specific gravity, g/mL of H2O	1,095	1,094	1,096	0,003	0,684
Egg weight, g	60	59	59	3	0,239
Albumen height, mm	5.5	5.0	5.2	0,4	0,182
Yolk color	4.9[c]	5.3[b]	5.5[a]	0,1	0,001
Haugh units	71	69	70	3	0,393
Egg breaking strength, kgf	4.14	4.21	4.35	0,24	0,466
Eggshell thickness, mm	0.40	0.40	0.41	0,02	0,114

[a,b,c] Means within a row not sharing a common superscript differ significantly based on Tukey test (P<0,05)

Conclusions

Light laying hens fed diets with 16.4% CP improved feed conversion. For semi-heavy hens, it is possible to reduce protein to 15.41% without reduce egg production and egg quality.

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P-207 (ID 224)

EFFECT OF DIFFERENT DIETARY ENERGY AND PROTEIN LEVELS ON PERFORMANCE AND CARCASS CHARACTERISTICS OF JAPANESE QUAIL

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Abstract

To investigate the effect of different levels of energy and protein on growth indices in Japanese quail, an experiment was carried out using 360 quail in a two way factorial arrangement on completely randomize design with two levels of energy (2800 and 2900 kcal/kg) and three levels of protein (22, 24 and 26 %). Four replicates with 15 quails were allocated to each experimental treatment and birds were reared for 45 days. Weekly measurements of feed intake, weight gain and feed conversion ratios were calculated. At the end of experiment, two birds from each experimental unit were slaughtered to determine carcass characteristics. The results showed that there were significant difference in weight gain, feed intake and feed conversion ratio between experimental treatments ($p<0.05$). Quails were fed rations containing 2900 ME and 26 % crude protein had highest body weight and lowest feed conversion ratio. The effects of interaction among energy and protein on carcass, breast, thigh and heart percentage were significant ($p<0.05$). The highest breast weight was observed in quails fed 2900 ME and 26 % crude protein. It can be concluded that using diets containing 2900 kcal ME/kg and 26% crude protein is recommendable to achieve a desirable performance in Japanese quails.

Keywords: Japanese quail, Energy, Protein, performance and Carcass characteristics

Introduction

Japanese quails (*Coturnix coturnix japonica*) are being raised for the purpose of producing eggs and meat. Due to the increasing demand for quail's meat and eggs, and economical production of these products, it is expected that quail farming will develop in the future. It is blessed with the unique characteristics of fast growth, early sexual maturity, high rate of egg production, that makes it suitable for diversified animal agriculture (KAUR *et al*., 2008). The metabolizable energy and crude protein are the major components of poultry diet and on the other hand feeding cost is usually considered the most expensive item, but the energy and protein requirements as well as the efficiency of feed utilization are still poorly documented, especially for quails (SIYADATI *et al*., 2011). In the number of studies on the effect of energy and protein levels in Japanese quail, different responses have been reported (SOARES *et al*., 2003 and ATTIA *et al*., 2012). Today, in addition to the quantity of poultry production, carcass quality is important. In studies on carcass characteristics of Japanese quail, different responses to different levels of energy and protein were observed (SIYADATI *et al*., 2011, PARVIN *et al*., 2010). Therefore, due to the genetic selection to achieve maximum production, the importance of diet energy and protein in poultry and lack of accurate data to estimate the nutritional requirements of Japanese quail, the present study was performed to investigate the effect of dietary levels of protein and energy on the performance and carcass characteristics of growing Japanese quail.

Material and Methods

The study included three hundred and sixty Japanese quails (5-day-old). A factorial design arrangement (2 x 3), using 6 treatments with four replicates of 15 chicks each was performed included two levels of metabolizable energy (2800, 2900 kcal ME/Kg) and three levels of crude protein (22, 24 and 26%), based on comletly randomize design on growth performance and carcass traits of Japanese quail. The dietary nutrients requirement (except energy and protein) was regulated based on the NRC (1994) tables and diets were balanced by UFFDA software. Quail chicks were grown on the floor and brooding temperature gradually decreased from 36 °C during the initial 7 days to 27°C by 21 days of age. The data of body weights and feed intake were recorded at weekly intervals and at the end of experiment (42 days), two birds from each experimental unit were slaughtered to determine carcass characteristics. The weight of the carcass, breast, thighs, back and neck of each quail (as a percentage of live weight) was measured separately. Data obtained from the experiment with GLM procedure of SAS software were analyzed; In addition differences among treatment means were tested using turkey's test.

Results

The effect of treatments on the performance of Japanese quail during the growing period is provided in Table 1. A statistically significant difference was found in the productive performance on whole the growing period (5-42 days old) between the treatments (P <0.05). The highest weight gain, feed intake and also the highest feed conversion ratio was observed in quails were fed with diets containing 2900 kcal ME/kg and 26% crude protein. The growing quail metabolizale energy and crude protein requirements in some resources 2900 and 24% and 28% is mentioned, which is partly consistent with the results obtained in this study (LEESON and SUMMERS, 2008 and NRC, 1994). In the present study feed intake were increased with increasing levels of energy and protein, which is in contrasts with the findings of SOARES *et al.* (2003).This researchers reported that the dietary protein level had no effect on feed intake (P >0.05). It is likely due to the higher level of oil which was used in our diet, therefore feed intake were increased in this group. Since the oil reduces dust and increased palatability of the diet, this were lead to a higher feed intake in this treat (KESHAVAREZ and JACKSON, 1992).In the study by SIYADATI *et al.* (2011), improved BWG and FCR in the quails were fed with diets containing higher metabolizable energy and crude protein was reported and also in another study (STERLING *et al*., 2005) the best feed conversion ratio was obtained with feeding diets containing 26 % crude protein, Which corresponded with the findings of the present investigation.

Table 1. Live weight gain, feed intake (g bird^{-1}) and feed conversion ratio of growing quails in 5-42

Treatments	Live weight gain	Feed intake	FCR
2800×22	193.807^{c}	649.768^{b}	3.356^{ab}
2800×24	178.824^{d}	641.206^{b}	3.586^{a}
2800×26	196.543^{bc}	693.592^{a}	3.530^{ab}
2900×22	192.395^{c}	659.972^{b}	3.430^{ab}
2900×24	206.258^{ab}	689.874^{a}	3.346^{ab}
2900×26	213.622^{a}	709.604^{a}	3.324^{b}
SEM	2.422	5.704	0.053
P_value	<.0001	0.0071	0.0123

^{a,b,c} Means within each column with uncommon superscript are significantly different (P <0.05)

The effect of treatments on carcass, breast, thighs and back and neck as a percentage of live weight is reported in Table 2. Carcass weight in birds were fed diets containing 2800 kcal ME/kg and 24% crude protein was lower than the other treatments. The results of this study are in agreement with YAZERLOU *et al*. (2013), Those reported reduce carcass in quail fed diets containing lower energy (2750 kcal ME/kg) and 24% crude protein. The average weight of the thigh and breast were significantly different between experimental treatments (P <0.05). Better thigh and breast weight were observed in quails fed high levels of energy and protein. Which is in consistent with the result of SIYADATI *et al*. (2011) and CORREAL *et al*. (2008.)

Table 2. Average carcass characteristics in different experimental treatments (as percentage of live weight)

Treatments	Carcass	Thigh	Breast	Back and neck	Heart
2800×22	69.598^{abc}	15.758^{ab}	26.573^{ab}	26.860^{a}	0.847^{a}
2800×24	66.856^{c}	14.801^{b}	25.743^{b}	26.401^{a}	0.748^{b}
2800×26	67.665^{bc}	15.049^{ab}	27.390^{a}	25.099^{b}	0.836^{a}
2900×22	70.878^{a}	15.925^{a}	27.363^{a}	26.246^{ab}	0.844^{a}
2900×24	69.379^{abc}	14.910^{b}	26.852^{ab}	26.207^{ab}	0.839^{a}
2900×26	70.494^{ab}	15.742^{ab}	27.417^{a}	27.335^{a}	0.730^{b}
SEM	0.643	0.224	0.262	0.288	0.007
P-value	0.0019	0.0051	0.0015	0.0010	<0.0001

^{a,b,c} Means within each column with uncommon superscript are significantly different (P <0.05)

Conclusions

Due to higher weight gain, feed conversion ratio and good carcass, in general conclusion can be stated that a diet containing 2900 kcal ME/kg and 26% CP leads to a desirable performance in Japanese quails.

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P-209 (ID 81)

EFFECT OF WHITE LUPINE SEEDS INCLUSION ON BROILER CHICKENS PERFORMANCE, DIGESTIBILITY AND VILLI STRUCTURE

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Abstract

480 male broiler chickens were used in a 35-day study to determine the influence of white lupin inclusion (0, 100, 150, 200, 250 and 300 g/kg) on their performance and diet utilization as well as on ileal villi height and crypt depth. One-day-old male Ross 308 chicks were randomly assigned to six dietary treatments (ten replications per treatment). Birds fed diets with 150 g/kg white lupine were characterized by lower BWG, FI and higher FCR than the control birds. The use of 300 g/kg of white lupine decreased BWG by 400 g in comparison with the control (P<0.05). White lupine seeds were found to exert a negative effect on dietary AMEn and ileal crude fat digestibility as well as on crude protein (P<0.05). Ileal digesta viscosity increased together with the increase of white lupine seed content in experimental diets. The inclusion of 150 g/kg of white lupine seeds in experimental diet increased digesta viscosity from 1.76 cP (control treatment) to 2.41 cP (P<0.05). The highest digesta viscosity was determined when 300 g/kg of white lupine seeds was used – 3.74 cP. Villi height and villi surface area decreased as white lupine seeds content increased (P<0.05). The shallowest (P<0.05) crypts were determined in the control birds.

Keywords: broiler, villi, protein, viscosity

Introduction

Soybean meal (SBM) is the most common vegetable protein source in poultry diets but the inclusion of homegrown legume seeds in diets for non-ruminant animals as an alternative to soybean products has been receiving growing attention in countries that are not climatically suited to soybean production (Nalle et al., 2012). Good nutritional value of white lupine for broilers was reported previously by many authors. The AME of white lupine was determined to range from 8.0 to almost 15 MJ/kg. White lupine protein and amino acid (AA) ileal digestibility are similar to those obtained for soybean meal SBM (Nalle et al., 2012). Nalle et al. (2012) reported that average digestibility for white lupine AA ranged from 0.85 to 0.87. Ravindran et al. (2005) showed that white lupine AA digestibility ranged from 0.75 to 0.88.

On the other hand, whole lupin seeds contain substantial concentrations of oligosaccharides, phytate or NSP limiting the use of white lupin in poultry feeding. Phytate in plant feedstuffs can interact electrostatically with dietary (and possibly endogenous) proteins and amino acids which negatively influences protein utilization and increases endogenous protein loss (Selle et al., 2012). Additional, non-starch polysaccharides may increase viscosity of digesta affecting negatively nutrients absorption and consequently AME.

The aim of the study was to determine the effect of white lupine seeds dose on growth performance, nutrient utilization and villi structure in broiler chickens.

Material and Methods

The experiment was conducted with 480 broiler (Ross 308) chicks (initial individual weight: 40±2 g) divided into six dietary treatments, including a maize –SBM control diet (CON) and five experimental diets containing; 100, 150, 200, 250 and 300g/kg white lupine seeds. Each comprised 10 replications of 8 birds. Apparent total tract digestibility and AME_N value of the diets were calculated relative to the ratio of TiO_2 to the content of the nutrient in question in the feed, digesta or excreta. Data were analyzed by ANOVA using GLM procedure of R environment. The cage was the experimental unit and orthogonal contrasts were used to establish the difference between treatments. The contrasts used were the CON treatment versus the remaining 5 dietary treatments (100, 150, 200, 250 or 300 g of lupine seeds per kg of feed) as well as the contrast for linear (L) and quadratic (Q) effects. All data are presented as means.

Results

It was evident that feeding diets containing 200 g/kg WL seeds had a negative effect on the BWG and FCR of birds (0-35d), (BWG: CON – 2100 g, 200 – 1917 g, P<0.05). These could have been caused by a quadratic decrease in feed intake when WL seeds were introduced into the diet (FI: CON – 3053 g/bird, 200 – 2934 g/bird). Similarly, VIVEROS et al. (2007) reported that WL seeds had deleterious effects on the performance of broiler chickens. On the other hand, NALLE et al. (2012) found that broiler diets could contain up to 200g/kg of WL without a negative effect on growth. These discrepancies between above studies may reflect differences in cultivars or feed formulation strategies (NALLE et al., 2012). In our study, the observed decrease in the feed intake after WL addition could be partially explained by probable high insoluble NSP content. JØRGENSEN et al. (1996) reported that digesta retention time and nutrient digestibility are adversely affected by insoluble NSP content. The depression in birds' performance observed in our study may be attributed to determined increase in ileal viscosity. (Table 1). It is generally accepted that the high viscosity of digesta is associated with soluble NSP content in the diet. Adverse effects of soluble NSP are primarily associated with the viscous nature of these compounds. The modes of action include altered intestinal transit time, and changes in microflora composition and nutrient absorption (CHOCT and ANNISON, 1992).

Table 6. Apparent metabolisable energy of diet, ileal digestibility (CF - crude fat, crude protein and starch), viscosity (Vi) of ileal digesta, excreted NANA, villi height (VH) and area (VA) of broiler chickens fed diets with different doseges of WL.

WL dose g/kg	AME_N [MJ/kg]	Apparent ileal digestibility:			Ileal Vi [cP]	Total NANA [µmol/gTiO_2]	Ileum	
		CF	CP	S			VH [µm]	VA [µm^2]
0 [CON]	12.8	0.74	0.74	0.99	1.76	219	861	415 800
100	11.5*	0.68	0.74	0.94	2.18'	282*	935	350 966'
150	11.6*	0.68	0.74	0.96	2.41*	314*	703*	301 265*
200	11.2*	0.68	0.73	0.96	3.06*	308*	698*	265 664*
250	10.9*	0.56*	0.70*	0.90*	3.08*	310*	721*	286 118*
300	10.4*	0.56*	0.68*	0.90*	3.74*	316*	704*	280 905*
Contrast								
L	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.0006	<0.05
Q	NS	NS	NS	NS	NS	<0.05	0.187	<0.05

* - different from control treatment at P<0.05, ' - different from control treatment at P<0.1
L - linear contrast, Q - quadratic contrast, NS – P>0.05

The increase in ileal viscosity following WL addition determined in our study is in agreement with the findings of OLKOWSKI et al. (2005) who reported that 400g/kg addition of WL to the diet caused an increase in digesta viscosity (from 1.8 to 4.2 cP). The diet utilization in our study was negatively affected by relatively low (100g/kg) WL inclusion. The increase in ileal digesta viscosity and high insoluble NSP content were probably the main factors affecting nutrient AID. NSP content was not determined in this study but the observed increase in ileal digesta viscosity after WL inclusion indicate that soluble and insoluble NSP content was increasing together with the increasing content of WL seeds in experimental diets. Ether extract, crude protein and starch AID (Table 1) were affected only by 250 and 300g/kg WL inclusion. The EE and CP AID could have been depressed by increasing ileal viscosity as well as insoluble NSP content. The use of over 150 g of WL depressed VH and VA (Table 1) with may explain the depression in nutrient utilization and, consequently, AME_N reduction. Additionally, we observed a quadratic increase in excreted NANA together with WL inclusion. These increase in NANA excretion may have been caused by leveed mucin secretion, resulting in higher endogenous losses. The increased mucin production associated with diets containing WL meal may be due to an increase in bacterial numbers in the distal gut linked to an increase in substrate availability. As suggested by COWIESON et al. (2003), diets that increase mucin secretion are nutritionally expensive for birds with may explain the depression in birds' performance determined in our experiment.

Conclusions

It could concluded that the inherent variability of seeds may partially explain why birds did responded differently across trials (including this experiment) after similar dose of WL seeds. The NSP present in WL seeds caused high intestinal viscosity affecting nutrient absorption and, probably, endogenous losses.

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P-210 (ID 185)

INTERACTION BETWEEN THREONINE AND GLYCINE AT LOW DIETARY CRUDE PROTEIN AND THE EFFECT ON PRODUCTION PERFORMANCE, MEAT QUALITY AND PLASMA METABOLITES IN BROILERS

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Abstract

This trial was designed to study the interaction between Thr and Gly in 7-28d broilers. A total of 2,040 broilers were allocated to 17 treatments. A positive control (PC) diet (20.5% CP) was formulated to be adequate in essential AA. A negative control (NC) diet (18.5% CP, deficient in Thr and Gly) was supplemented with L-Thr and Gly to obtain a 4 Thr x 4 Gly design. The PC was compared to the other treatments by Student's t-test and the Thr x Gly interaction was tested by interactive regression analysis. Reducing CP increased feed intake and BWG ($P<0.05$) except for the NC diet. The four most deficient diets in Thr showed higher FCR than the PC diet irrespective of the Gly level. A Thr x Gly interaction was found for FCR for the 7-17d. Linear response of FCR to Thr was significant only at 2 levels of Gly and was stronger with the lowest Gly level. Plasma uric acid concentration was higher in the PC than in all the other diets and plasma triglyceride concentration was linearly decreased by Thr supplementation, but not by Gly. White striping and wooden breast occurrence at 28d were not affected. In conclusion, there is an interaction between Thr and Gly, and Thr is able to spare Gly in low CP diets.

Keywords: threonine, glycine, protein, performance, plasma metabolites

Introduction

Lowering the dietary crude protein (CP) content in broiler feeds is an efficient tool to improve intestinal health, and to reduce foot pad lesions and the excretion of nitrogen to the environment (MEDA et al., 2011; WIDYARATNE, 2012). When CP content is reduced, the animal performance will be limited by the indispensable amino acids (IAA) but dispensable AA (DAA) and especially Gly might also become limiting (DEAN et al., 2006). However, Thr, the third limiting IAA in broilers, is a known precursor of Gly via the Thr dehydrogenase and aldolase pathways (DAVIS AND AUSTIC., 1982) and it was earlier demonstrated that Thr could spare the Gly requirement (OSPINA-ROJAS et al., 2013; SIEGERT AND RODEHUTSCORD, 2013). In this trial, the effects of Gly and Thr on production performance, meat quality and plasma metabolites were investigated in low CP diets.

Material and Methods

A total of 2,040 male Ross 708 broilers were housed on litter in small pens at the experimental broiler facilities of Schothorst Feed Research, Lelystad, the Netherlands. Birds were fed a commercial starter diet from day 0 to 7, an experimental diet from day 7 to 28 and a finisher commercial diet from day 28 to 35. The experiment was performed with 17 treatments and 6 replicates, with 20 broilers per replicate. Treatment 1 was the positive control (PC) diet with a CP level of 20%, an apparent faecal digestible (AFD) Thr:Lys of 64% and an AFD Gly+Ser:Lys of 156 %. Treatment 2 was the negative control (NC) diet with a CP level of 18.5%, an AFD Thr:Lys ratio of 55% and an AFD Gly+Ser:Lys ratio of 135%. On top of the NC diet, the addition of 3 supplemental levels of L-Thr and Gly gave the treatments 2 to 17 (55, 58, 61, and 64% AFD Thr:Lys x 135, 142, 149, and 156% AFD Gly+Ser:Lys). The animals were fed an AFD Lys of 1.0% and AMEn of 3,000 kcal/kg. Performance parameters were measured at day 0, 7, 17, 28, and 35. Plasma sampling was performed at day 28 on 10 birds per treatment and analysed for uric acid, glucose, triglycerides and total AA levels. White striping and wooden breast incidence was evaluated in 5 treatments (PC + the 4 highest Thr treatments). The PC diet was compared to the other treatments by a 2-by-2 Student's t-test (PROC GLM, SAS 9.2). Quadratic and linear regressions were performed to test the effect of Thr and Gly at each level of Gly and Thr. Interactive regression analysis tested the interaction between Thr and Gly (PROC REG, SAS 9.2).

Results

Reducing dietary CP resulted in higher feed intake (FI) and body weight gain (BWG) in most treatments (data not shown, P<0.05). FCR was degraded when dietary CP was low but only when dietary Thr level was 55% AFD Thr:Lys. Broilers fed the PC diet showed higher plasma uric acid concentration than any of the low CP treatments (data not shown, P<0.05).

Table 1: Effect of AFD Gly+Ser:Lys (%) and AFD Thr:Lys (%) on body weight gain (BWG, g/d), feed intake (FI, g/bird), feed conversion ratio (FCR), plasma uric acid (UA, mg/L), tryglycerides (g/L), glucose (Glu, g/L) and total amino acids (AA, mg/mL) circulating levels[1]

AFD Gly+Ser: Lys (%)	AFD Thr:Lys (%)	BWG (g/bird)		FI (g/bird)		FCR		UA, mg/L	TG, g/L	Glu, g/L	AA, mg/mL
		D7-17	D7-28	D7-17	D7-28	D7-17	D7-28	D28	D28	D28	D28
	55	486	1511	671	2165	1.377	1.430	48.6	1.12	2.23	0.53
	58	499	1575	684	2220	1.373	1.410	50.3	1.10	2.19	0.51
	61	489	1548	664	2181	1.359	1.402	50.9	1.11	2.28	0.50
	64	489	1541	658	2158	1.351	1.400	52.0	0.95	2.37	0.48
135		490	1545	672	2179	1.372	1.411	49.9	1.08	2.28	0.48
142		484	1539	663	2177	1.366	1.409	49.1	1.08	2.27	0.48
149		497	1552	673	2200	1.354	1.413	50.9	1.07	2.28	0.54
156		491	1538	669	2167	1.367	1.409	51.8	1.05	2.24	0.52
***P*-values**											
	ALL[2]	NS	**0.089**	NS	NS	**0.001**	**<0.001**	NS	**0.004**	**0.03**	**0.003**
	135[3]	NS	**0.037**	NS	NS	**<0.001**	**0.001**	NS	NS	NS	NS
Linear effect of Thr	142[3]	NS	NS	**0.089**	NS	NS	**<0.001**	NS	**0.059**	NS	NS
	149[3]	NS	NS	NS	NS	**0.016**	NS	NS	NS	NS	**<0.001**
	156[3]	NS	NS	NS	NS	NS	**0.020**	NS	NS	**0.083**	NS
	ALL[4]	NS	NS	NS	NS	NS	NS	NS	NS	NS	**<0.001**
	55[5]	**0.063**	**0.017**	**0.038**	NS	**0.058**	NS	NS	NS	NS	**<0.001**
Linear effect of Gly	58[5]	NS	NS	NS	NS	NS	NS	NS	**0.057**	NS	NS
	61[5]	NS	NS	NS	NS	NS	NS	NS	NS	NS	**0.047**
	64[5]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Interaction		NS	NS	NS	NS	**0.088**	NS	NS	NS	NS	NS

[1] Data presented as means (n = 6 pens/treatment for performance criteria, n = 10 birds/treatment for plasma metabolites).
[2] Linear regressions to threonine irrespective of glycine plus serine level.
[3] Linear regressions to threonine at each glycine plus serine level (135, 142, 149, or 156 % AFD Gly+Ser:Lys).
[4] Linear regressions to glycine plus serine irrespective of threonine level.
[5] Linear regressions to glycine plus serine at each threonine level (55, 58, 61, or 64 % AFD Thr:Lys).
NS (P-value > 0.1).

Supplementation of Thr linearly affected BWG but only when Gly was low (135 AFD Gly+Ser:Lys; Table 1). FCR was linearly decreased with increasing dietary levels of Thr in both periods of age (P<0.05). Addition of Thr also linearly decreased TG and total AA plasma levels and increased glucose plasma levels (P<0.05). On the contrary, Gly supplementation did not affect any of the parameters studied except when Thr level was the lowest where it resulted in an

increase of BWG, FI, and total AA plasma level (P<0.05). Quadratic regressions confirmed the linear effects (data not shown). White striping or wooden breast incidence were not affected by dietary CP or Gly level (data not shown).

Statistically, a tendency was detected for the interaction between Thr and Gly for FCR from day 7 to 17 but not for any other parameter or later in age (P=0.088). A response contour was performed using SAS 9.2 (PROC RSREG) to graphically represent this interaction (Figure 1). At low AFD Thr:Lys levels, the animals needed more Gly to show similar performance. At high Thr level, FCR was the lowest indicating that at least 64% AFD Thr:Lys is required for optimal feed efficiency. When dietary Thr was adequate, Gly addition had no effect on FCR.

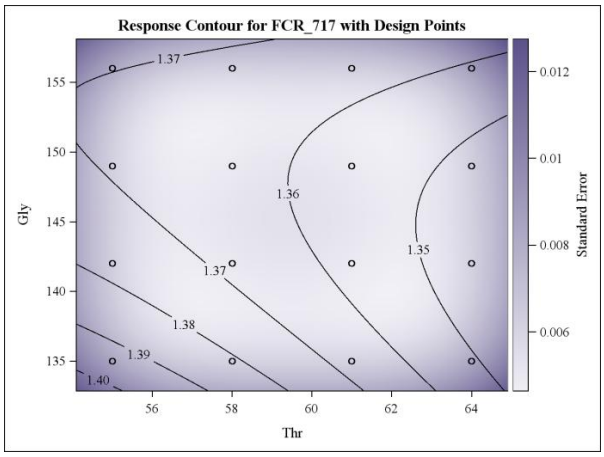

Figure 1: Response contour with design points for feed conversion ratio from 7 to 17d (FCR_717). Gly = AFD Gly+Ser:Lys (%), Thr = AFD Thr:Lys (%).

Conclusions

Lowering dietary CP contributes to more sustainable broiler production. In this experimental trial, Gly was not limiting for growth at low CP levels unless Thr was deficient. In conclusion, the interaction between Thr and Gly is confirmed by this trial and Thr is able to spare the Gly requirement in low CP diets.

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P-211 (ID 188)

IS META-ANALYSIS AN EFFECTIVE TOOL TO ESTIMATE THE GLYCINE PLUS SERINE REQUIREMENT OF BROILER CHICKENS?

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Abstract

A meta-analysis was conducted to predict the Gly+Ser requirement of broilers. Dose-response trials from literature were entered in our database if they 1) were published after 1980, 2) provided specific information such as age or sex and 3) tested a basal diet supplemented with at least 3 levels of Gly. The database consisted of 14 trials and 76 data lines from 7 publications. The dietary energy, CP and AA levels were recalculated based on INRA (2004) tables. To be able to express the Gly+Ser requirement in % of the diet, 2 dose-responses (21 to 35d broilers) were excluded to obtain an homogeneous set of studies over the period 0 to 22d (n=12). A curvilinear-plateau model was then used to model the response to Gly+Ser. According to this model, the standardized digestible (SD) Gly+Ser requirement of 0-21d broilers was 1.78 % and 2.13 % for ADG and G:F, respectively. These results agree with previously published meta-analyses. However, in literature, low levels of Thr or Cys have been shown to give rise to metabolic interactions with Gly or Ser, thereby causing a reduced endogenous synthesis or an increased need of Gly or Ser. In the dose-response trials used in this meta-analysis, dietary Thr and Cys levels were often lower than suggested requirements. Due to the low number of dose-responses available and the influence of AA deficiencies, it is up to now difficult to give a reliable Gly+Ser requirement for broiler chickens.

Keywords: meta-analysis, glycine, requirement, interactions

Introduction

Even though Gly is often considered as a dispensable AA (DAA), it is becoming a much studied AA in broiler research. Glycine requirement is always expressed as the sum of the Gly and Ser requirements as Ser to Gly conversion is equimolar and reversible. According to ROSTAGNO (2011), the digestible Gly+Ser requirement in ratio to Lys is higher for young broilers (147%, 0-21d) than for older broilers (134%, 21-42d). WU (2014) even suggested levels of 245% digestible Gly+Ser:Lys. Considering the conflicting evidence in literature, a meta-analysis was conducted to estimate the SD Gly+Ser:Lys requirement of broiler chickens.

Material and Methods

Dose-response trials from literature were entered in the database if they 1) were published after 1980, 2) provided specific information such as age or sex and 3) tested a basal diet supplemented with at least 3 levels of Gly. The database consisted of 14 trials and 76 data lines from 7 publications. Respectively, 36, 36 and 28% of the trials were carried out in European Union, the United States and Brazil. The published diets were recalculated using INRA (2004) tables (SAUVANT et al., 2004). Trials were eliminated of the database if the diets were not sublimiting in digestible Lys (based on the average of two breeder recommendations) or deficient in one or more other indispensable AA (according to ROSTAGNO (2011) recommendations). From the 14 trials of the database, only 2 were matching the aforementioned criteria. However, 12 trials shared the same studied period of age (0 to 22 days) and could be used to model the response to Gly. Considering that the dietary Lys was not sublimiting in all trials, only the absolute SD Gly+Ser (% of the diet) requirement was expressed and not the ratio to Lys. In order to describe the response of ADG, ADFI and G:F to supplemental Gly, a curvilinear-plateau (CLP) model was used (PROC NLIN, SAS 9.2). The model based on SIMONGIOVANNI et al. (2012) was the following: $\boldsymbol{Y_{ij}} = \boldsymbol{A_i}\left(\boldsymbol{1} + \boldsymbol{U}\left(\boldsymbol{R} - \boldsymbol{X_{ij}}\right)^2\right) + \boldsymbol{\varepsilon_{ij}}$ *for* $\boldsymbol{X_{ij}} < R$;$\boldsymbol{Y_{ij}} = \boldsymbol{A_i} + \boldsymbol{\varepsilon_{ij}}$ *for* $\boldsymbol{X_{ij}} \geq \boldsymbol{R}$ *where* $\boldsymbol{Y_{ij}}$ is the response criterion (i.e. ADG, ADFI, G:F) for observation j of experiment i, $\boldsymbol{X_{ij}}$ is the SD Gly+Ser supply, A_i is the maximum response for experiment i, R is the minimum SD Gly+Ser supply required to reach the plateau (requirement) and U is the parameter describing the response to the SD Gly+Ser supply before the plateau value. The residual error term $\boldsymbol{\varepsilon_{ij}}$ is assumed to be independent and normally distributed.

Results

Based on the 12 studies with young broilers, ADFI did not respond to Gly supplementation and is therefore not presented in this paper. ADG and G:F positively responded to SD Gly+Ser level (Figure 1a,b).

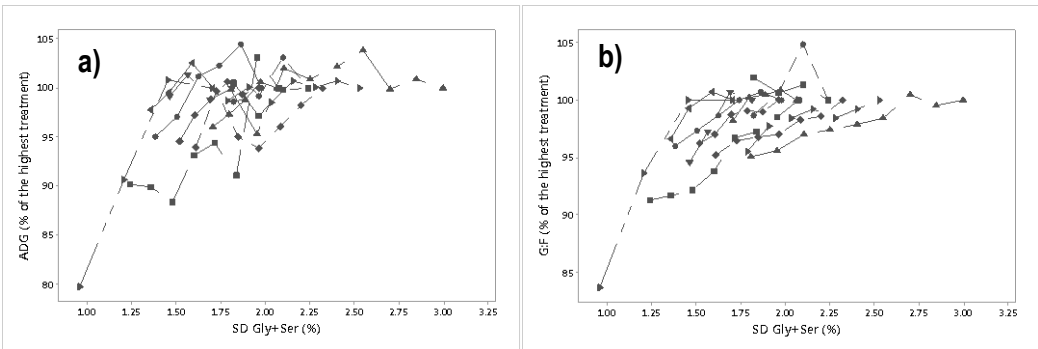

Figure 1: a) Average daily gain (ADG, g/d) and b) gain to feed (G:F; % of the highest treatment) according to the dietary SD Gly+Ser (%) level for the selected trials (n=12).

The CLP model estimated that the Gly+Ser requirement was 1.78% SD Gly+Ser for ADG and 2.13% for G:F in 0 to 22 days old broilers (Table 1). However, the increase from 1.78 to 2.13% SD Gly+Ser only further improved G:F by 1.2% suggesting a weak response above 1.78% SD Gly+Ser. The requirement found in this meta-analysis is close to the recommendation based on other meta-analyses (AKINDE, 2014; SIEGERT et al., 2013). Similarly to AKINDE (2014), the present meta-analysis predicted a higher requirement for G:F than for ADG and the estimated requirements were also quite comparable between the 2 studies (in total Gly equimolar Ser). In contrast, SIEGERT et al. (2013) estimated a total Gly equimolar Ser requirement of 2.06% for ADG, higher than in AKINDE (2014; 1.93%) and the present meta-analysis (Table 1).

Table 1: Parameter estimates and error of estimates of the CLP model for average daily gain (ADG, g/d) and gain to feed (G:F) as response criteria (n=12).

	ADG	G:F
Plateau (A)	35.1	0.758
Slope (U)	-0.2393	-0.1004
SD Gly+Ser requirement (R, %)	1.78 (0.09)	2.13 (0.09)
Total Gly equimolar Ser requirement (R, %)	1.88 (0.12)	2.08 (0.10)

Even though predicting the Gly+Ser requirement and response is theoretically possible, several factors in the protocols of the dose-response studies might have affected the estimation. First, the present requirement is only valid for young broilers (0-22d) and broilers are known to have a higher requirement in younger ages (ROSTAGNO, 2011; [1] in Table 2). Second, low dietary CP levels were used and convincing evidence suggested a higher Gly requirement at low CP levels (DEAN et al., 2006; [2]). Third, Gly is rich in N and increasing dietary levels of Gly logically increased dietary CP levels. Diets were therefore not isonitrogenous and the response to Gly might have been confounded with the response to protein [3]. Four, dietary Cys level was low on average and response to Gly may be increased at low dietary Cys (POWELL et al., 2011; [4]). Five, dietary Thr levels were low compared to recently published Thr requirement and response to Gly may be increased when Thr is low (OSPINA-ROJAS et al., 2013; [5]). To finish, the majority of the studies in the meta-analysis (9 out of 12) were deficient in one or more AA [6].

Table 2: Key features of the trials used to estimate the Gly+Ser requirement (n=12).

	Key features of the trials (n=12)
Age (days) [1]	0 to 22
CP of the lowest Gly treatment (average, %) [2,3]	18.7
CP of the highest Gly treatment (average, %) [2,3]	19.4
SD Cys:Lys (%) [4]	21
Deficient in SD Thr:Lys (<67%; number of studies) [5]	7
At least one IAA limiting (Number of studies) [6]	9

Conclusions

Theoretically, modelling the response of production performance to Gly supplementation and estimating a Gly+Ser requirement is possible. Experimental factors (dietary CP, AA levels) of the dose-response studies favored the estimation of a high Gly requirement as predicted in this paper. Until more knowledge on Gly is released, it is up to now difficult to give a reliable Gly requirement for broilers chickens.

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P-212 (ID 186)

THREONINE REQUIREMENT OF BROILER CHICKENS: INTEGRATED PREDICTION BY META-ANALYSIS

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Abstract

Due to its incorporation into mucus glycoproteins and immunoglobulins and its role as glycine precursor, Thr is an effective nutrient in enhancing gut health, immune response and growth. However, the use of different strains, sexes, ages, AA systems or regression models to estimate Thr requirement, has contributed to the discrepancy in the published Thr recommendations. A meta-analysis was therefore conducted to estimate the standardized digestible (SD) Thr:Lys requirement. Dose-response trials from literature were entered in our database if they 1) were published after 1980, 2) provided specific information such as age or sex and 3) tested a basal diet supplemented with at least 3 levels of L-Thr. The database consisted of 81 trials and 469 data lines from 43 publications. The dietary energy, CP and AA levels were recalculated based on INRA (2004) tables. The diets had to provide sub-limiting Lys and adequate essential AA:Lys levels. Twenty-one trials were finally selected and a curvilinear-plateau model was used to describe the response of ADG and G:F to Thr. According to this model, the SD Thr:Lys requirement of broilers was 67.3% for ADG and 67.6% for G:F. The response between 60% SD Thr:Lys and the predicted requirement was estimated to result in an increase by 4.8 and 4.1 % for ADG and G:F, respectively. In conclusion, a dietary level of 67% SD Thr:Lys seems a reasonable compromise.

Keywords: meta-analysis, threonine, requirement, response

Introduction

Thr is the third limiting AA in broiler diets and has been widely studied since feed grade L-Thr has become available in the animal feed market. Due to its incorporation into mucus glycoproteins and immunoglobulins and its role as glycine precursor, Thr is an effective nutrient in enhancing gut health, immune response and growth. However, the use of different strains, sexes, ages, AA systems or regression models to estimate Thr requirement, has contributed to the discrepancy in the published Thr recommendations. For instance, MACK et al. (1999), ROSTAGNO (2011) and WU (2014) recommended levels of 63, 65 and 67% SD Thr:Lys, respectively, for maximizing performance. A meta-analysis was therefore conducted to estimate the standardized digestible (SD) Thr:Lys requirement of broiler chickens.

Material and Methods

Extensive review of the available literature was performed to collect trials studying the effect of Thr supplementation on growth performance. Dose-response trials from literature were entered in our database if they 1) were published after 1980, 2) provided specific information such as age or sex and 3) tested a basal diet supplemented with at least 3 levels of L-Thr. In total, 81 trials and 469 data lines from 43 publications were entered in the database. A majority (61.7 %) of the trials was performed in the United States, 66.7 % used male broilers and 67.9 % fed broilers with corn-based diets. The published diets were recalculated using INRA (2004) tables (SAUVANT et al., 2004). Trials were eliminated of the database if the diets were not sublimiting in digestible Lys (based on the average of two breeder recommendations) or deficient in one or more other indispensable AA (according to ROSTAGNO (2011) recommendations). In order to describe the response of ADG, ADFI and G:F to supplemental Thr, a curvilinear plateau (CLP) model was used based on SIMONGIOVANNI et al. (2012): $\boldsymbol{Y_{ij}} = \boldsymbol{A_i}\left(\boldsymbol{1} + \boldsymbol{U}\left(\boldsymbol{R} - \boldsymbol{X_{ij}}\right)^{\boldsymbol{2}}\right) + \boldsymbol{\varepsilon_{ij}}$ *for* $\boldsymbol{X_{ij}} < R$;$\boldsymbol{Y_{ij}} = \boldsymbol{A_i} + \boldsymbol{\varepsilon_{ij}}$ *for* $\boldsymbol{X_{ij}} \geq \boldsymbol{R}$ *where* $\boldsymbol{Y_{ij}}$ is the response criterion (i.e. ADG, ADFI, G:F) for observation j of experiment i, $\boldsymbol{X_{ij}}$ is the SD Thr: Lys supply, A_i is the maximum response for experiment i (i.e. plateau), R is the minimum SD Thr:Lys supply required to reach the plateau and U is the parameter describing the response to the SD Thr:Lys supply before the plateau value. The residual error term $\boldsymbol{\varepsilon_{ij}}$ is assumed to be independent and normally distributed. The modelling of the response trials was performed with the PROC NLIN of SAS 9.2.

Results

From the 81 trials of the database, 35 had non-sub-limiting levels of Lys and 24 presented deficiencies in AA (Met, Val, Trp). After the modelling of the 22 selected trials, the analysis of the residuals revealed that 2 points out of 4 of one dose-response were outliers and this dose-response was therefore eliminated, resulting in a final selection of 21 trials.

Based on these 21 dose-response studies, the response of ADFI, ADG and G:F to supplemental Thr was modelled again. Average daily feed intake did not respond to Thr supplementation and is therefore not presented in this paper. Average daily gain and G:F positively responded to SD Thr:Lys level (Figure 1a,b).

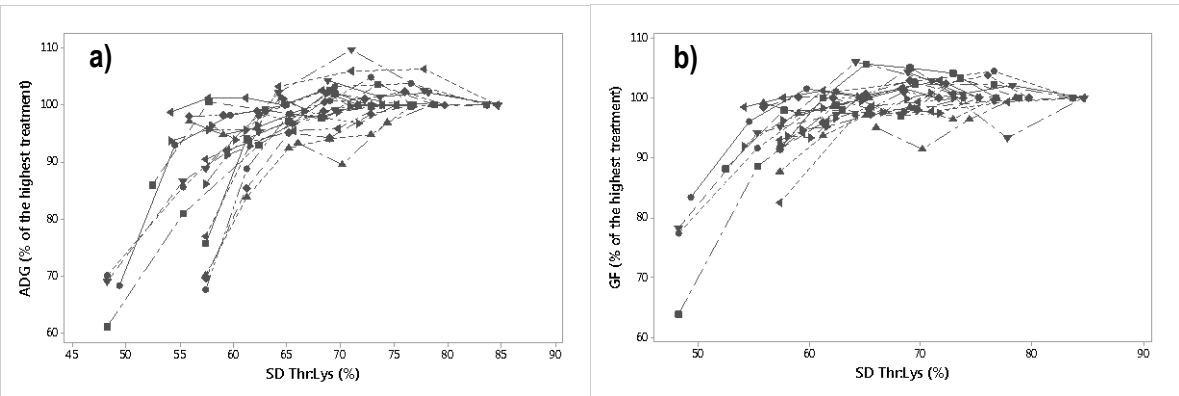

Figure 1: a) Average daily gain (ADG) and b) gain to feed (G:F) according to the dietary SD Thr:Lys level for the selected trials (n=21).

According to the CLP model, the SD Thr:Lys requirement of broilers is 67.3% for ADG and 67.6% for G:F (Table 1). Recent studies (JIANG et al., 2014; MELOCHE et al., 2014) also support a SD Thr:Lys ratio of at least 68% depending on the growth criteria, close to the conclusion of this meta-analysis.

Table 1: Parameter estimates and error of estimates of the CLP model for average daily gain (ADG, g/d) and gain to feed (G:F) as response criteria (n=21 trials).

	ADG	G:F
Plateau (A)	51.3	0.624
Slope (U)	-0.00086	-0.00067
SD Thr:Lys requirement (R, %)	67.3 (2.9)	67.6 (8.0)

WECKE and LIEBERT (2013) recently proposed an optimal Thr:Lys of 60 % for starter and 62 % for grower broilers based on nitrogen balance studies. However, recent studies have shown that challenged broilers require higher dietary Thr levels as Thr is the first AA in mucins and immunoglobulins (STAR et al., 2012). In the dose-response studies used in our meta-analysis, broilers are raised in good environmental conditions and results are based only on production performance. It is suspected that in practical conditions, in contrast to experimental facilities, broilers might require even more Thr for optimal health and growth.

Based on our estimation with the CLP model, ADG and G:F were represented from a deficiency of 10% (60% SD Thr:Lys) corresponding to the requirement proposed by WECKE and LIEBERT (2013). The CLP model predicts that an increase from 60% to the predicted SD Thr:Lys requirement improves ADG by 4.8% and G:F by 4.1%.

Conclusions

According to the present meta-analysis, growing broilers require at least 67% SD Thr:Lys for optimal growth and feed efficiency. When SD Thr:Lys is increased from 60% to the predicted requirement, ADG is improved by 4.8% and G:F by 4.1%. In practice, Thr requirement might be even higher in case of challenging environmental conditions.

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P-214 (ID 25)

IMPACT OF DIFFERENT DIETARY L-ARGININE CONCENTRATIONS ON GROWTH OF PUREBRED LAYING HENS OF FOUR GENOTYPES DIFFERING IN PHYLOGENY AND PERFORMANCE LEVEL

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Abstract

In order to examine the impact of graded dietary L-arginine supply on growth of four purebred layer lines, two high performing genotypes (WLA - White Leghorn; BLA - Rhode Island Red) from LTZ were contrasted to two low performing genotypes (R11 - White Leghorn; L68 - New Hampshire) from FLI. Three diets containing 70 %, 100 % and 200 % of recommended L-arginine (NRC, 1994) were fed *ad libitum* to each genotype (n = 32 chicks per genotype and diet) from hatch to the end of 41st week of age. Growth data were fitted to Gompertz function by non-linear regression. Estimated parameters were compared by mixed procedure and Tukey-Kramer test. Asymptotic body weight and age at maximum body weight gain (t_{max}) were significantly affected by genotype and diet, while no interaction occurred. Brown hens achieved higher asymptotic body weight than white hens. The diet supposed to supply 70 % of recommended Arg induced significantly lower asymptotic body weight and delayed t_{max} in all genotypes. Although asymptotic body weight did not differ between diets of 100 and 200 % arginine, 100 % arginine caused a lower t_{max} in genotypes. WLA even showed lowest t_{max} independent of diet, while the other genotypes did not differ from each other. In conclusion, insufficient dietary arginine caused growth restriction and retardation, but sufficient Arg induced fastest growth in all genotypes. Brown hens grew stronger than white hens, whereas white ones grew faster.

Keywords: amino acids, L-arginine, genotypes, Gompertz function, growth

Introduction

Due to bird's uricotelism, L-arginine (Arg) is considered as essential amino acid in poultry. It plays a decisive role in physiological processes such as growth and feathering and serves as precursor of proteins, creatine, polyamines, L-proline, various hormones and nitric oxides. The magnitude of required Arg depends on a large variety of environmental and genetic factors (reviewed in: KHAJALI and WIDEMAN, 2010). The objective of the present study was to examine the impact of graded dietary L-arginine supply on growth of purebred layer lines differing in performance and phylogenetic origin in order to calculate genotype specific growth curves from hatch to the end of the 41st week of age.

Material and Methods

Two high performing, commercial genotypes from LTZ (WLA and BLA) were contrasted to two low performing ones from FLI (R11 and L68). White layer lines (WLA and R11) were of White Leghorn origin and phylogenetically closely related, but distant from the Rhode Island Red line BLA and its counterpart L68 (New Hampshire). Three diets were calculated for chicks (hatch to week 7), pullets (week 8 to 16) and laying hens (week 17 to 41) each. Diets of each age-group comprised of a basal diet with no Arg supplementation (low Arg, **LA**), a low Arg (adequate Arg, **AA**) and a high Arg (high Arg, **HA**) supplemented diet equivalent to 70, 100 and 200 % Arg of recommended level (NRC, 1994). Each group (genotype x diet) comprised 32 one-day-old female chicks equipped with individual wing-tags housed in floor-range pens. After rearing pullets were moved to floor-range systems of a layer facility. During the entire trial diets and water were provided *ad libitum* and chickens' body weight was recorded as follows: from hatch to week 4 once a week, from week 6 to 16 every second week and from week 16 to 41 every 28 days. Based on recorded body weights daily weight gain was calculated. The growth function of GOMPERTZ (1825) was fitted regressively to time-dependent individually recorded growth data from hatch to the end of 41st week of age using procedure "nonlinear regression" of STA-

TISTICA 12.0 (STATSOFT INC., 2014). Parameters of growth function were estimated using iterative Quasi-Newton method.

$$y(t) = a \cdot e^{-b \cdot e^{-c \cdot t}}$$

Where y(t) = hen's body weight (g) at time t, expressed as function of a; a = hen's adult body weight (g; asymptotic limit); b,c = regression coefficients of the function; and t = time (weeks). The age at maximum daily weight gain (t_{max}), equivalent to the point of inflection of the cumulative, sigmoid growth curve, was calculated by the second derivative of the cumulative growth function:

$$t_{\max} = \frac{\ln b}{c}$$

Maximum daily weight gain was computed by inserting group-specific calculated t_{max} in the derivative of the corresponding cumulative growth function.

Statistical analysis of calculated growth function parameters was carried out by means of a two factorial ANOVA with genotype, diet and their interaction as fixed effects. The Tukey-Kramer test was applied for multiple comparisons of means. Data were reported as least square mean values and pooled standard error of mean. Differences were considered to be statistically significant for $P < 0.05$. ANOVA of growth curve parameters were performed using procedure MIXED of the software package SAS 9.4 (SAS INSTITUTE INC., 2012).

Results

Table 1 summarized results of nonlinear regression. Genotype and diet significantly affected calculated parameters of growth function, t_{max} and corresponding maximum daily weight gain. The asymptotic limit of growth curves, equal to estimated adult body weight, received higher values in brown hens than in white ones ($p < 0.001$) and **HA** induced higher adult body weight in brown hens than **LA** ($p < 0.01$). WLA fed with **AA** reached t_{max} firstly and BLA fed with **LA** grew slowest ($p < 0.05$). Independent of diet, t_{max} of WLA differed significantly from that of the other genotypes. Latter ones showed no statistically differences between each other. In all genotypes **AA** caused fastest growth and differed significantly from **HA** and **LA**.

The derivatives of growth curves, identical to daily weight gain course showed a significant increase until group-specific t_{max} with its corresponding estimated maximum daily weight gain (graphs not shown). In the following, daily weight gain strongly decreased and approached a nearly zero-gain asymptotically after 40th week of age. Brown genotypes achieved higher maximum daily weight gain than white ones ($p < 0.001$). Yet, **LA** induced lower maximum daily weight gain at t_{max} and led to flatter curves than supplemented Arg diets ($p < 0.001$).

Conclusions:

The present study confirmed the essential role of Arg in growing poultry. Insufficient dietary Arg led to a significant growth reduction and retardation compared to an adequate or surplus Arg supply. Negative effects caused by insufficient dietary Arg, such as reduced feed intake and secondary growth depression, might be the result of adverse effects of dietary imbalances between Arg and lysine as well as Arg and methionine (reviewed in: KHAJALI and WIDEMAN, 2010). A lack of metabolisable Arg might cause a deficit of endogenous L-ornithine, the precursor of growth-promoting polyamines. Genotype specific interactions with Arg were only found in WLA and L68 that led to the assumption that genetically determined differences in Arg utilization and metabolism occur. HEGSTEDT et al., 1941 reported on different rapidity in feathering that induced a higher Arg requirement in fast feathering genotypes than in slow feathering ones. The fast and well growing of WLA with additional Arg indicated that WLA belonged to fast feathering breeds, while L68 has been selected for slow feathering (K 30%, k 70%) consciously and should require less Arg during feathering.

Table 1. Effect of genotype and Arg supply on results of time-dependent individual growth data fitted to Gompertz function (LSMeans, PSEM, n = 32 chicks/experimental groups)

	WLA			BLA			R11			L68				ANOVA (p values)		
	LA	AA	HA	LA	AA	HA	LA	AA	HA	LA	AA	HA	PSEM	GT	DIET	GTxDIET
a (g/chick)	1503 ^{d}	1492 ^{d}	1537 ^{d}	1756 ^{c}	1770 ^{bc}	1819 ^{b}	1342 ^{e}	1422 ^{e}	1404 ^{e}	1887 ^{b}	1964 ^{ab}	1977 ^{a}	29	< 0.001	< 0.05	n.s.
b	3.67 ^{bc}	3.66 ^{bc}	3.61 ^{c}	3.85 ^{a}	3.58 ^{c}	3.57 ^{c}	3.74 ^{b}	3.65 ^{bc}	3.63 ^{bc}	3.78 ^{ab}	3.74 ^{b}	3.91 ^{a}	0.04	< 0.001	< 0.01	< 0.001
c	0.140 ^{c}	0.157 ^{a}	0.147 ^{b}	0.133 ^{d}	0.136 ^{cd}	0.133 ^{d}	0.135 ^{cd}	0.142 ^{bc}	0.138 ^{c}	0.135	0.143 ^{bc}	0.142 ^{bc}	0.002	< 0.001	< 0.001	n.s.
t_{max} (weeks)	9.36 ^{bc}	8.33 ^{d}	8.76 ^{cd}	10.22 ^{a}	9.44 ^{bc}	9.65 ^{b}	9.82 ^{ab}	9.17 ^{c}	9.35 ^{bc}	9.94 ^{ab}	9.30 ^{bc}	9.60 ^{bc}	0.16	< 0.001	< 0.001	n.s.
R^2	0.998	0.998	0.998	0.997	0.998	0.997	0.998	0.998	0.998	0.998	0.998	0.998				
RSD	24	22	20	25	26	22	22	20	19	26	24	30				
Estimated MDWG (g/chick/d)	11.1 ^{e}	12.3 ^{cd}	11.9 ^{d}	12.3 ^{cd}	12.6 ^{c}	12.7 ^{c}	9.5 ^{g}	10.6 ^{ef}	10.2 ^{f}	13.4 ^{b}	14.8 ^{a}	14.7 ^{a}	0.2	< 0.001	< 0.001	< 0.01
Achieved MDWG (g/chick/d)	11.0 ^{e}	12.1 ^{d}	12.1 ^{d}	12.9 ^{c}	12.7 ^{cd}	13.2 ^{c}	9.5 ^{f}	11.0 ^{e}	10.0 ^{f}	14.6 ^{b}	15.5 ^{a}	15.0 ^{ab}	0.3	< 0.001	< 0.001	n.s.

$^{a-g}$ LSMeans values with PSEM in the same row with different superscripts are significantly different ($p < 0.05$)

R^2 = coefficient of determination; RSD = residual standard deviation; PSEM = pooled standard error of mean; MDWG = maximum daily weight gain; n.s. = not significant

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P-215 (ID 66)

INTERACTION OF GRADED DIETARY L-LYSINE CONCENTRATIONS AND GENOTYPE ON PERFORMANCE AND EGG QUALITY OF PUREBRED LAYING HENS DIFFERING IN PHYLOGENY AND PERFORMANCE LEVEL

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Abstract

In order to examine the impact of graded L-lysine supply on performance and egg quality of five purebred layer lines, two high performing genotypes (WLA - White Leghorn; BLA - Rhode Island Red) from LTZ were contrasted to three low performing genotypes (R11 and G11 - White Leghorn; L68 - New Hampshire) from FLI. Three diets containing 80 %, 100 % and 120 % of recommended L-lysine (NRC, 1994) were fed *ad libitum* to each genotype (n = 8 hens per genotype and diet). From the 22nd to 61st week of age body weight, daily feed intake and laying performance were monthly recorded and in the 30th, 45th and 60th week of age egg quality was examined. Analysis of variance was performed using mixed procedure and Tukey-Kramer test. Body weight, daily feed intake and laying performance were significantly affected by genotype, age and their interaction, but not by dietary lysine. Depending on their genetic background WLA and BLA showed significantly higher daily feed intake, laying performance and albumen proportion as well as significantly lower feed to egg mass ratio than low performing genotypes. Eggs of the latter ones had higher yolk proportion, yolk to albumen ratio and more intensive coloured yolks. In contrast to the absent influence of dietary lysine on performance, relative weights of egg components varied significantly due to dietary lysine. In conclusion, dietary lysine level failed to alter performance, but modified egg proportions in genotypes equally. The differences in performance related traits traced back to the determined performance potential of genotypes.

Keywords: amino acids, egg quality, genotypes, L-lysine, performance

Introduction

L-lysine (Lys) is an essential amino acid in poultry and associated with protein synthesis. In order to achieve their genetically determined performance potential, chickens depend on sufficient dietary supply with amino acids such as Lys. As most dietary plant proteins show relatively low amounts of Lys, a dietary supplementation with free L-lysine is frequently necessary in poultry nutrition. To approach the hypothesis that high performing genotypes are less capable to respond adequately to environmental changes such as insufficient nutrient supply compared to low performing ones, we conducted a performance trial with five lines of purebred laying hens differing in performance and phylogenetic origin. In order to examine interacting effects of genotype and Lys on laying performance and egg quality, hens were fed graded levels of dietary Lys.

Material and Methods

Two high performing, commercial genotypes from LTZ (WLA and BLA) were contrasted to three low performing ones from FLI (G11, R11 and L68). White layer lines (WLA, G11 and R11) were of White Leghorn origin and phylogenetically closely related, but distant from Rhode Island Red line BLA and its counterpart L68 (New Hampshire). To enable individual records of performance and feed intake, 24 hens of each genotype were kept in single cages of a three-floor cage-system randomly. Each cage was equipped with a feeding trough, nipple drinker and perch. Feed and water were provided *ad libitum*. Hens were equally distributed to diets containing 80 %, 100 % (7.2 g Lys/kg diet) and 120 % of recommended Lys (NRC, 1994). The trial lasted from the 22nd to the 61st week of age and was subdivided into ten 28-day laying periods.

Hens were weighed at the end of each 28-day period. Eggs were recorded daily and egg weight was monitored by collecting laid eggs of each hen on four consecutive days in a two-week interval. Feed not consumed was recorded weekly. Based on recorded feed intake and egg mass the feed to egg mass ratio was calculated. Additionally, in the

30th, 45th and 60th week of age eggs of each hen were collected on three consecutive days, weighed and egg yolk and albumen were separated. Weight of shell and yolk were recorded and that of albumen was determined by difference. Egg components are presented as relative weights of total egg weight and the yolk to albumen ratio was calculated. Yolk colour was estimated by using a Roche-fan (15 fans, F. Hoffmann-La Roche Ltd., Basel, Switzerland).

Statistical analysis of examined traits was carried out by means of a three factorial analysis of variance (ANOVA) with genotype, diet, age and their interactions as fixed effects using procedure MIXED of SAS 9.4 (SAS INSTITUTE INC., 2012). As traits were measured repeatedly on the same animal a "repeated" statement was considered in the statistical model to account for similarities within subjects. The Tukey-Kramer test was applied for a multiple comparison of means and data were reported as least square mean values and pooled standard error of the mean. Differences were considered to be statistically significant for $p < 0.05$.

Results

Table 1 summarizes average body weight and laying performance of genotypes during the entire trial. All examined traits were affected by genotype, age and their interaction ($p < 0.001$), while dietary lysine level failed to alter body weight and laying performance.

Table 1. Effects of genotype and lysine on the average performance of ten 28-day periods (LSMeans, PSEM, n = 8).

Genotype	L-Lysine (%)	Body weight at week 22 (g)	Body weight at week 61 (g)	Daily feed intake (g/hen/d)	Laying intensity (%)	Egg weight (g/egg)	Daily egg mass (g/hen/d)	Feed to egg mass ratio (kg/kg)
WLA	80	1470 ^{b}	1448 ^{c}	96 ^{b}	92.0 ^{a}	56.1 ^{b}	51.5 ^{b}	1.87 ^{a}
	100	1499 ^{b}	1485 ^{c}	99 ^{b}	90.9 ^{a}	56.0 ^{b}	50.7 ^{b}	1.96 ^{a}
	120	1425 ^{b}	1518 ^{c}	96 ^{b}	94.9 ^{a}	55.7 ^{b}	52.8 ^{b}	1.82 ^{a}
BLA	80	1624 ^{a}	1846 ^{b}	109 ^{a}	89.9 ^{a}	61.9 ^{a}	55.6 ^{a}	1.96 ^{a}
	100	1647 ^{a}	1856 ^{b}	111 ^{a}	95.5 ^{a}	61.2 ^{a}	58.4 ^{a}	1.91 ^{a}
	120	1641 ^{a}	1815 ^{b}	109 ^{a}	93.0 ^{a}	61.9 ^{a}	57.7 ^{a}	1.89 ^{a}
R11	80	1239 ^{c}	1415 ^{d}	80 ^{c}	67.5 ^{b}	51.3 ^{c}	34.8 ^{c}	2.30 ^{b}
	100	1232 ^{c}	1381 ^{d}	76 ^{c}	63.5 ^{b}	49.3 ^{c}	31.3 ^{c}	2.43 ^{b}
	120	1205 ^{c}	1279 ^{d}	75 ^{c}	62.2 ^{b}	49.7 ^{c}	30.9 ^{c}	2.43 ^{b}
L68	80	1735 ^{a}	2014 ^{a}	94 ^{b}	63.0 ^{b}	54.4 ^{b}	34.3 ^{c}	2.74 ^{c}
	100	1694 ^{a}	1915 ^{a}	95 ^{b}	62.6 ^{b}	55.2 ^{b}	34.6 ^{c}	2.75 ^{c}
	120	1752 ^{a}	2151 ^{a}	96 ^{b}	65.1 ^{b}	53.2 ^{b}	34.6 ^{c}	2.77 ^{c}
G11	80	1224 ^{c}	1364 ^{d}	76 ^{c}	67.4 ^{b}	45.9 ^{d}	30.9 ^{c}	2.46 ^{b}
	100	1172 ^{c}	1344 ^{d}	78 ^{c}	68.5 ^{b}	45.9 ^{d}	31.4 ^{c}	2.48 ^{b}
	120	1191 ^{c}	1371 ^{d}	74 ^{c}	64.5 ^{b}	45.5 ^{d}	29.3 ^{c}	2.53 ^{b}
PSEM		60	60	3	2.7	1.0	1.4	0.38

a-d: LSMeans within columns with no common superscripts are significantly different ($p < 0.05$)

Table 2 summarizes the average results of egg quality examinations in 30th, 45th and 60th week of age. The proportions of shell, yolk and albumen as well as the calculated yolk to albumen ratio were influenced by genotype, diet and age, but yolk colour was only affected by genotype and age ($p < 0.001$).

Table 2. Effect of genotype and lysine on the average egg quality (LSMeans, PSEM)[1]

Genotype	L-Lysine (%)	Proportion (%) of			Yolk to albumen ratio	Yolk colour (Roche fan scale)
		Egg shell	Egg yolk	Egg albumen		
WLA	80	12.7 [a]	29.7 [d]	57.6 [b]	0.518 [d]	12.4 [c]
	100	12.7 [a]	29.4 [d]	57.9 [b]	0.511 [d]	12.5 [bc]
	120	12.3 [ab]	30.0 [d]	57.7 [b]	0.522 [d]	12.4 [c]
BLA	80	12.0 [b]	26.0 [e]	62.0 [a]	0.422 [e]	12.2 [c]
	100	12.3 [ab]	25.4 [e]	62.3 [a]	0.409 [e]	12.4 [c]
	120	11.8 [b]	25.8 [e]	62.4 [a]	0.414 [e]	12.2 [c]
R11	80	12.5 [a]	31.4 [c]	56.1 [cd]	0.562 [c]	12.8 [b]
	100	13.2 [a]	30.0 [cd]	56.8 [bc]	0.432 [e]	12.5 [bc]
	120	11.6 [bc]	31.3 [c]	57.1 [bc]	0.551 [c]	13.0 [b]
L68	80	11.2 [c]	33.1 [ab]	55.7 [d]	0.598 [b]	13.7 [a]
	100	11.3 [c]	32.2 [bc]	56.5 [bc]	0.573 [c]	13.6 [a]
	120	11.6 [bc]	33.3 [ab]	55.1 [d]	0.609 [b]	13.7 [a]
G11	80	12.0 [b]	34.4 [a]	53.6 [e]	0.647 [a]	12.3 [c]
	100	11.6 [bc]	33.5 [ab]	54.9 [de]	0.613 [b]	12.6 [bc]
	120	11.9 [b]	33.8 [a]	54.9 [de]	0.627 [ab]	12.4 [c]
PSEM		0.1	0.3	0.3	0.008	0.1

[1] eggs collected on three consecutive days of n = 8 hens per group in 30th, 45th and 60th week each
a-e: LSMeans within columns with no common superscripts are significantly different ($p < 0.05$)

Conclusions

Due to their genetically determined performance potential, high performing layer lines achieved a significantly higher performance than their low performing counterparts. In the present experiment graded levels of dietary Lys failed to alter body weight, feed intake and laying performance independent of genetic background. Therefore, it can be assumed that the requirement for Lys is equal to or less than the lowest level used in this study, which was 80 % recommended Lys (NRC, 1994). The oversupply of dietary Lys (120 % Lys) showed neither enhancing nor depressing effects on hens' performance. This result indicates that high and low performing hens tolerated surplus Lys equally. However, lysine supply caused shifts in yolk to albumen ratio, while total egg weight remained constant. As amino acid pattern of egg proteins is genetically determined, they are hardly to influence by diet (LEESON, 1993; HUSSEIN and HARMS, 1994). That leads to the assumption that laying hens compensate dietary limitation and excess of Lys with a shift of egg proportions. In order to find an appropriate nutritional stressor for our animal model, further research with other amino acids or greater dietary imbalances seems to be necessary.

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P-216 (ID 290)

EFFECT OF DIGESTIBLE ARGININE TO DIGESTIBLE LYSINE (DARG:DLYS) RATIOS ON PERFORMANCE OF MALE ROSS 308 BROILERS

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Abstract

Arginine (Arg) plays an important role on protein deposition in broilers. A review of the ratio of digestible Arg to digestible Lysine (dArg:dLys) is needed considering the genetic improvement on feed conversion rate (FCR). Ross 308 male broilers were fed crumbled starter (S; 0-10d), pelleted grower (G; 11-24d) and pelleted finisher (F; 25-46d) diets. Three dArg:dLys ratios were provided to birds by phase, (94, 107, 120), in a 3x3x3 factorial design (5 reps/trt - 20 birds/pen). Diets were formulated to meet Aviagen 2007 recommendations, except for dArg. At 10d, birds fed 94 dArg:dLys diets were lighter, and had lower feed intake and high FCR than those fed higher dArg:dLys ($p<0.001$). At 25d, no interaction between the S and G phase was observed. During this phase, birds fed both the 107 and 120 ratios of dArg:dLys had the highest liveweight (LWT) and the lowest FCR ($p<0.001$). At 46d, no interaction between feeding phases was observed. During this phase, birds fed 107 and 120 ratios of dArg:dLys resulted in the heaviest birds with the lowest FCR ($p<0.001$). However, the 120 dArg:dLys trt was comparable to the 94 dArg:dLys trt on LWT. Regression analysis of cumulative intake of dArg showed positive correlation (r^2 =0.83 for FCR and 0.58 for LWT) suggesting that a dArg:dLys feeding strategy of 120, 107 and 120 during the S, G and F phases, respectively, was optimal for FCR. At the same time, maintaining a ratio of 107 across all feeding phases was optimal for growth, in line with the factorial data.

Keywords: broilers, nutrition, amino acid, digestible arginine, ratio

Introduction

Arginine (Arg) is an essential amino acids for broilers, playing an important role in protein deposition rate, and key metabolic pathways that are necessary for maintenance, growth, reproduction, and immune status. Calculations from the more recent optimum amino acid balances studies suggested the optimum ratio of digestible Arg to digestible Lysine (dArg:dLys) to be in the range from 90 to 118% (Balnave and Brake, 2002). The following study aimed to review the ratio of dArg:dLys in light of the constant genetic improvement in growth rate and feed conversion rate (FCR).

Material and Methods

A total of 2700 male Ross 308 broilers were studied over starter (0-10 days), grower (11-24 days) and finisher (25-46d days) feeding phases. Three different dArg:dLys levels were studied per phase (94, 107 and 120 dArg:dLys) in a 3 x 3 x 3 factorial design to give a total of 27 treatments, with 5 reps per treatment. Diets were wheat and soybean meal based, crumble form at starter, and pellet in grower and finisher phases. All diets were formulated according to 2007 Aviagen Nutrient Recommendations, except dArg.

Results and Discussion

At day 10, birds fed 94 dArg:dLys diets were lighter and had lower feed intake with increased FCR compared to those fed higher dArg:dLys ($p<0.001$). The low feed intake of birds fed 94 dArg:dLys during first 10 days negatively affected their performance throughout the trial. The lack of difference in body weight (BW) and FCR between birds receiving 107 or 120 dArg suggests that despite receiving less Arg, birds on 107 dArg:dLys trt were not compromised. Therefore in the first 10 days the optimum for dArg is likely to be between 107 - 120.

At 25 days, no interaction between the starter and grower phase was observed for bird performance parameters. The heaviest birds were those that had been fed to one of the higher ratios of dArg:dLys in the starter ($p=0.02$) and grower phase ($p<0.001$). In terms of FCR, exposure to the highest ratio in the starter resulted in birds with lower FCR at 25d compared to birds fed to a ratio of 97 ($p=0.03$). Considering the grower phase independently, the most feed efficient birds were those fed both the 107 and 120 ratios of dArg:dLys ($p<0.001$). The improved performance of birds from the

107 and 120 treatment ratios was due to an increased Arg intake, suggesting a ratio of 107 is optimal for growth and feed efficiency.

At 32 and 46 days, no interactions between feeding phases were observed for FCR or BW. At 32 days, the heaviest (p=0.01) and most feed efficient (p<0.01) birds were those that had been fed a ratio of 107 dArg:dLys over the starter and grower feeding phases, comparable to birds fed higher ratios of dArg:dLys. At 46 days, the influence of the starter period on BW and FCR had diminished, with only a tendency (p=0.09) for improved BW in birds exposed to the two higher ratios of dArg:dLys in starter phase, suggesting that for grow outs over 32 days, dArg does not have to be at a ratio of 107 to dLys in the starter feed to maximise growth. However, the two higher ratios in the grower phase did positively influence FCR (p<0.001) and ratios of 107 dArg:dLys in the grower phase yielded heavier birds (p=0.049) at 46 days than birds from the lowest treatment. Considering the influence of dArg:dLys in the finisher independent of the preceding phases, the 107 and 120 dArg:dLys treatments resulted in the heaviest birds with the lowest FCR (p<0.001), but the 120 dArg:dLys treatment was comparable to the 94 dArg:dLys treatment in terms of BW. Therefore the performance results at 46 days suggest, in line with the previous phases, that a ratio of 107 dArg:dLys is optimal.

Results of the regressions (Figures 1 and 2) of accumulative dArg inatke on FCR and BW at 46 days significantly showed a strong positive correlation (r^2 =0.83 and 0.58 FCR and BW, respectively). The models run suggested that a dArg:dLys strategy of 120, 107 and 120 starter, grower, and finisher, respectively, was optimal for FCR while maintaining a ratio of 107 across feeding phases was optimal for growth, in line with the factorial data.

Since the interaction effects between phase and levels were not significant for the most processing carcass variables at 46 days, independent effects of arginine ratios in each phase are presented in the Table 1. The highest two ratios of dArg:dLys in the grower and finisher rations resulted in an increase in % eviscerated carcass, and this increase was linear in the finisher phase (p<0.001). Abdominal fat was lower in birds exposed to either the 107 or 120 treatments in the finisher period (p=0.012). However birds exposed to a ratio of 94 or 120 dArg:dLys in the starter had similar abdominal fat % (p<0.029). Birds fed the highest ratio in the finisher period displayed a significant increase in % breast meat, with the 107 dArg:dLys treatment yielding birds with a lower % breast but higher than those birds fed the 94 dArg:dLys treatment (p<0.001). The impact of the grower phase on breast was less dramatic, with both of the highest ratio treatments resulting in an increase in breast yield (p<0.001). The starter phase had no influence on breast meat yield at 46 days. Breast yield % was also measured at 32 days and the results were in line with the 46 day processed birds.

Table 1. Carcass traits at 46 d

	Eviscerated Carcass %				Abdominal Fat %				Breast Yield %			
	94	107	120	*p*	94	107	120	*p*	94	107	120	*p*
S	73.2	73.97	73.95	0.088	1.58^{b}	1.49^{a}	1.55^{ab}	0.029	21.76	21.8	21.85	-
G	73.52^{b}	74.10^{a}	74.02^{a}	<0.001	1.59	1.51	1.53	-	21.38^{b}	22.1^{a}	21.97^{a}	<0.001
F	73.43^{c}	73.97^{b}	74.24^{a}	<0.001	1.59^{b}	1.45^{a}	1.49^{a}	0.012	21.31^{c}	21.87^{b}	22.23^{a}	<0.001

Results of the regression suggest that the ideal strategies for breast yield are ratios of 94, 120 and 107 for starter-grower-finisher, respectively; the lower ratio treatment in the starter is in line with the lack of effect of ratio observed from the Anova results. However the grower and finisher ratio treatments are slightly conflicting with the factorial results.

Conclusions

Based on accumulative dArg intake a dArg:dLys strategy of 120, 107 and 120 in the starter, grower, and finisher, respectively, is optimal for FCR while maintaining a ratio of 107 across feeding phases was optimal for growth.

A dArg:dLys ratio of 107 or 120 in the grower and finisher rations increased the % of eviscerated carcass and reduced the % of abdominal fat at 46d days.

A dArg:dLys ratio of 120 in the finisher increased the % of breast meat.

To maximize performance traits, a minimum ratio of 107 dArg:dLys should be applied, in line with 2014 Aviagen Nutrient Recommendations

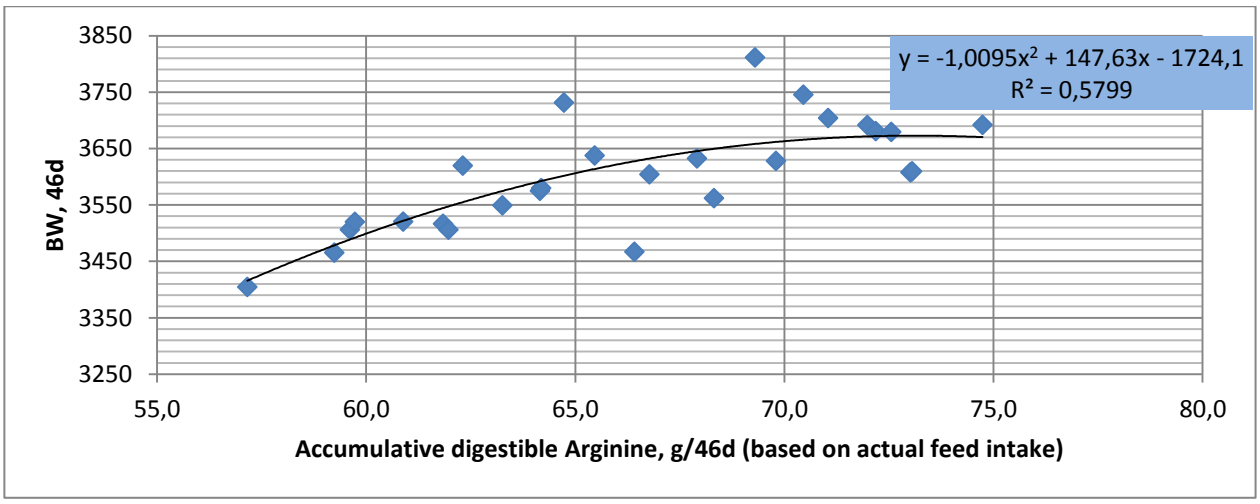

Figure 1: Response of 46 day BW to accumulative intake of digestible Arginine.

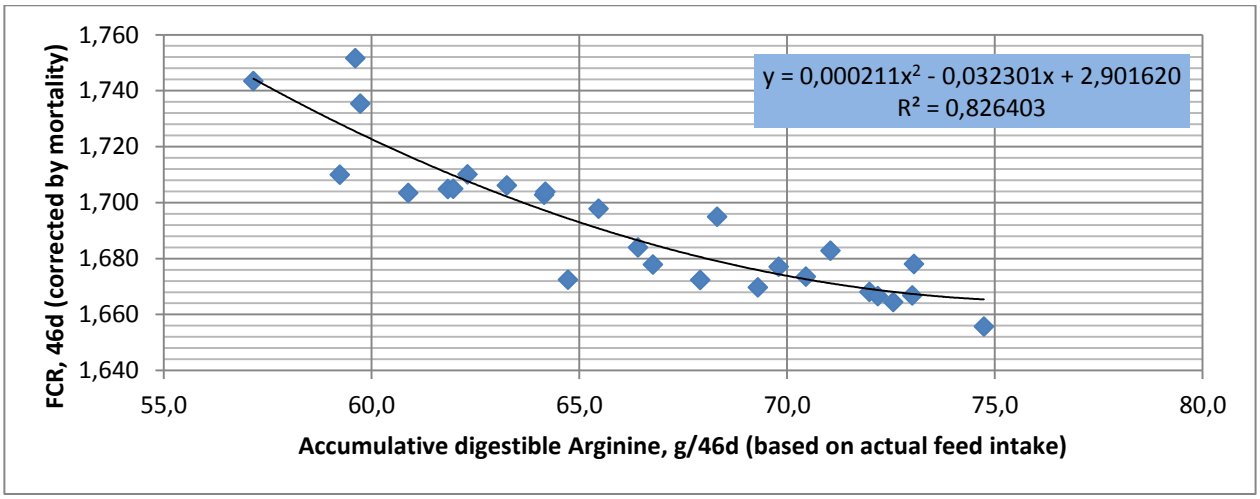

Figure 2: Response of 46d FCR to accumulative intake of digestible Arginine.

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P-218 (ID 321)

THE USE OF HERBAL METHIONINE AS AN ALTERNATIVE TO DL-METHIONINE IN BROILER CHICKENS

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Abstract:

An experiment was conducted to evaluate the effects of graded DL-methionine replacement by herbal methionine on performance and carcass characteristics in broiler chickens. A total of 280 day-old male Ross 308 broiler chickens were used in 7 treatments, each treatment has 4 replicates, and 10 birds per replicate. Experimental treatments were as following: Basal diet, in which total methionine requirement was supplied from DL-methionine (control), and in treatments 2 to 7, DL-methionine in basal diet was replaced by 20, 40, 50, 60, 80 and 100% of Herbal methionine (Metaherb), respectively. Results indicated that BW in birds fed diets in which 20, 40 and 50% of DL-methionine were replaced by herbal methionine did not significantly differ than control group. No significant treatment impact were observed in feed intake, carcass percent and relative weights of breast, legs, liver, heart, thymus and bursa. In conclusion, it could be feasible to use herbal methionine in broiler diets as a replacement to supply part of the methionine requirement without affecting performance or carcass traits, while reducing production cost.

Keywords: Herbal methionine, Synthetic methionine, Broiler, Performance

Introduction

Methionine is an essential amino acid for healthy and productivity of poultry. It is considered to be the first limiting amino acid in corn soybean based diets, followed by lysine and arginine. Synthetic methionine sources commonly used in the poultry industry are DL-methionine and methionine hydroxy analogue (MHA-FA). Today, the greatest resource that used is DL- methionine, that produced by chemical synthesis from acrolein, methyl mercaptan, and hydrogen cyanide. The increase in demand for meat has given rise to the use of these synthetic compounds in feed. Therefore, the prices of this amino acid changes with changes in the prices of the precursors. Recently, commercial sources of herbal methionine in some countries, including Sudan, India and recently in Iran be used, which is an additives plant and mixture of several plants rich in methionine, containing herbal ingredients that mimic the activity of methionine. Some researchers reported that broiler chickens fed on herbal methionine had similar performance in terms of body weight gain and feed conversion efficiency in comparison with birds that received DL-methionine (KALBANDE *et al*. 2009; HALDER and ROY. 2007). In contrast, some other researchers found that herbal methionine is not effective substitute for DL- methionine (YUAN *et al*. 2012; ITOE *et al*. 2010). The aim of this experiment was to evaluate the effects of graded DL-methionine replacement by herbal methionine on performance and carcass characteristics in broiler chickens.

Materials and methods

The research protocol was approved by the Razi University animal care and use committee. A total of 280 one-day old male Ross 308 broiler chickens randomly assigned to 7 dietary treatments with 4 replicates per treatment (10 birds per replicate). The Experimental treatments were as follows: basal diet, in which 100% methionine requirement was supplied from DL-methionine (control), and DL-methionine in basal diet was replaced by 20 (HM 20), 40 (HM 40), 50 (HM 50), 60 (HM 60), 80 (HM 80) and 100% (HM 100) Herbal methionine (Metaherb), respectively. Herbal methionine (Metaherb) was supplied form Arosol Indian company. All diets were the same, only difference was the methionine source (Synthetic or herbal). Feed intake (FI), feed conversion ratio (FCR) body weight (BW) were weekly measured. Mortality was determined on a daily basis. At 42 days of age, one bird from each pen was randomly selected and slaghtered by cervical dislocation, and live BW, carcass percentage, abdominal fat pad, liver, breast, legs, heart, bursa, thymus and lien weight were determined. All data were analyzed with SAS software (version 9.2), with GLM procedure. Output data are given as means ± SD. Duncan's multiple range test was used to compare means.

Results

The effects of experimental treatments on performance of broiler chickens are shown in Table 1. Results indicated that at 21 and 35 days of age diets supplemented with herbal methionine until 60% replacement, did not significantly affect BW, whereas 80 and 100% replacement significantly reduced BW in compare to control group (P≤0.05). At 42 days of age, until 50% replacement synthetic methionine with herbal methionine showed same BW with control group, while levels of 60, 80, 100% herbal methionine significantly decreased BW. Experimental treatments had significant effect on BWG of broiler throughout the experiment. Feeding diets supplemented until 60% replacement herbal methionine did not influence BWG until 21 days of age, but in treatments 80 and 100% replacement, it was significantly decreased than control group (P≤0.05). Whereas data of BWG during 22-42 days of ages indicated that there was no significant effect, until 40% replacement, but other treatments significantly reduced BWG (P≤0.05). Overall, Statistical analysis of body weight gain during 8-42 days of ages showed that no negative effects were observed from feeding herbal methionine supplemented diets up to 40% replacement on BWG, whereas replacement level higher than 40%, reduced BWG. Experimental treatments did not affect FI throught the trial. During 8-21 days, treatments including 20 and 60% herbal methionine did not change FCR, whereas other treatments (40, 50, 80 and 100% replacement), significantly increased FCR (p≤0.05). During 22-42 and whole experimental period (8-42 days), FCR in all replacement levels were increased, except for the 20% replacement level that did not change FCR. Effect of herbal methionine supplementation on carcass characteristics at 42 day of age are shown in Table 2. No differences observed in carcass percent and relative weights of breast, legs, liver, heart, thymus and bursa in the control and experimental groups.

Conclusions

Results showed that herbal methionine replacement with DL-methionine had no significant effect in terms of BW, BWG and FCR until levels of 50, 40 and 20%, respectively. But levels of 80 and 100% herbal methionine replacement significantly reduced the performance of broiler chickens. The results of the present study indicated that it could be feasible to use herbal methionine in broiler diets as a replacement to supply part of the methionine requirement without affecting performance or carcass traits, while reducing production cost.

Table 1. Effect of herbal methionine supplementation on growth performance in broiler chickens

Treats		Control	20 HM	40 HM	50 HM	60 HM	80 HM	100 HM	P-value
BW(g)	21 day	901.1±41.1^{a}	906.3±33^{a}	914.3±25.9^{a}	906.8±31.3^{a}	887.6±5.4^{a}	835.9±70.2^{b}	808.2±15^{b}	0.005
	35 day	2189±56.2^{a}	2186.6±61.4^{a}	2139±75.8^{ab}	2154.7±39.3^{ab}	2114.3±96.8^{ab}	2010.5±139.9^{bc}	1944.6±105.8^{c}	0.02
	42 day	2767.7±127.5^{a}	2732±92.4^{a}	2699±49.8^{a}	2665.4±69.4^{ab}	2573.3±54.5^{bc}	2577.9±13.6^{bc}	2472±116.8^{c}	0.001
BWG(g)	8 - 21	732.4±35.4^{a}	730.1±29.3^{a}	739.3±22.6^{a}	732.5±21.8^{a}	708.1±46.7^{ab}	677.3±76.1^{bc}	630.8±15.8^{c}	0.005
	22 - 42	1852.9±119.9^{a}	1821±110.4^{ab}	1758.7±42.9^{abc}	1732.2±30.1^{bcd}	1680.7±22.4^{cd}	1762.3±62.7^{abc}	1638±94.6^{d}	0.001
	8 – 42	2585.3±111.8^{a}	2551.1±103.7^{ab}	2498±35.1^{abc}	2455.7±34.2^{bc}	2388.8±46.9^{c}	2429.6±63.1^{bc}	2268.8±95^{d}	0.001
FI(g)	8 - 21	1050.1±40.7^{ab}	1075±34.1^{b}	1096±34.8^{a}	1081.7±35.5^{ab}	1046.5±49.9^{ab}	1029.6±79.2^{b}	1025.5±27.4^{b}	0.02
	22 - 42	3481.2±95.2^{ab}	3504.7±29.7^{ab}	3599.1±118.6^{a}	3503.8±39.9^{ab}	3512.2±71.8^{ab}	3530.2±119.5^{ab}	3405.1±76.6^{b}	0.001
	8 – 42	4531.4±87.7^{bcd}	4579.7±45.5^{abc}	4695.2±140.8^{a}	4585.6±53.3^{ab}	4558.7±76.6^{abcd}	4438±87.7^{cd}	4430.6±102.9^{d}	0.001
FCR	8 - 21	1.42±0.05^{d}	1.47±0.05^{cd}	1.5^{bc}	1.5^{bc}	1.47±0.05^{cd}	1.55±0.05^{ab}	1.6^{a}	0.0004
	22 - 42	1.87±0.09^{c}	1.9±0.14^{c}	2.05±0.1^{a}	2^{ab}	2.1^{a}	2±0.11^{ab}	2.07±0.09^{a}	0.0007
	8 – 42	1.75±0.05^{c}	1.8±0.08^{bc}	1.9±0.08^{ab}	1.9^{ab}	1.9^{ab}	1.87±0.05^{ab}	1.97±0.05^{a}	0.001

All values represent the mean±SD of 4 replicates

Means within columns with different superscripts were significantly different (P≤0.05)

Table 2. Effect of herbal methionine supplementation on carcass characteristics (relative weight) in broiler chickens.

Treats	Carcass, %	Breast, %	Legs, %	Liver, %	Heart, %	Thymus, %	Bursa, %
Control	63.5±2.02	22.2±2.9	20.1±1.3	1.9±0.14	0.52±0.08	0.34±0.1	0.25±0.03
20 HM	63.06±2.9	21.2±2.6	20.9±0.82	2.1±0.29	0.51±0.08	0.52±0.3	0.22±0.08
40 HM	63.2±1.9	23.1±0.72	20.5±1.2	2.18±0.34	0.5±0.03	0.41±0.2	0.29±0.03
50 HM	63.26±1.4	22.6±2.9	21±0.46	1.9±0.2	0.5±0.04	0.51±0.2	0.27±0.11
60 HM	63.2±3.3	23.8±0.76	19.5±0.78	2.02±0.08	0.47±0.02	0.46±0.07	0.27±00.09
80 HM	63.06±2.9	21.6±1.1	20.4±1.1	2.09±0.2	0.53±0.02	0.56±0.2	0.180±0.1
100 HM	63.06±2.9	21.3±1.9	21.2±1.5	2.1±0.17	0.52±0.07	0.62±0.2	0.28±0.04
P-value	0.99	0.2	0.56	0.02	0.3	0.74	0.82

All values represent the mean±SD of 4 replicates

Means within columns with different superscripts were significantly different (P≤0.05)

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P-223 (ID 63)

VARIATIONS IN SOYBEAN MEAL AND EFFICIENCY OF PROTEIN HYDROLYSIS BY PANCREATIC PROTEASE AND MICROBIAL PROTEASE

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Abstract

Variation in trypsin inhibitor activity (TIA) and protein solubility within commercial soybean meals (SBM) is believed to affect animal performance. The objective of this research was to investigate the proteolytic effect of purified pancreatic trypsin/chymotrypsin and a purified protease from *Nocardiopsis prasina* on 9 commercial SBM samples varying in chemical composition, TIA and protein solubility characteristics. SBM was incubated with or without protease (pH 7, 3 hours, 40°C) and the supernatants were analyzed by SDS-page and for level of free soluble amino ends using the o-phthal-dialdehyde method. SDS-page analysis showed differences in the amount and composition of the soluble protein fraction of the SBM. The proteolytic efficiency of the lower dosages of pancreatic protease varied extensively between SBM batches whereas at the highest dose the efficiency was comparable for all SBM. The difference could not be explained by differences in protein solubility and TIA values as isolated variables, but the data strongly suggest that the hydrolysis of soya protein by pancreatic proteases depends on the commercial batch of SBM used. Incubation with *N. prasina* protease showed similar response for the different SBM, the main difference being a somewhat lower effect at high protease dose for one of the SBM batches, which might be in part explained by its distinct lower protein solubility.

Keywords: protease, soybean meal, trypsin inhibitor

Introduction

Soybean meal (SBM) is used in diets for non-ruminants because of its high protein content, superior amino acid profile, availability and cost. The crude protein fraction of SBM consists roughly of 80% storage proteins, 5% anti nutritional proteins including trypsin inhibitors (TI) and 15% of other proteins. Although it has been demonstrated, that commercial SBM varies significantly in chemical composition and nutritive value (de Coca-Sinova *et al.*, 2008), due to the lack of simple reliable analytics the feed compound industry often accepts that the digestible amino acid content per unit of protein is constant irrespective of variables such as origin, genotype, processing, and storage conditions. TI inhibits the activity of the pancreatic digestive enzyme trypsin and high levels of TI activity in SBM have been associated with impaired animal performance (e.g. Perilla *et al.*, 1997). The commercial availability of microbial proteases for inclusion in diets for non-ruminants offers an opportunity to complement the animals own digestive proteases. If these commercial proteases are not affected by TI it might be of particular interest to use such proteases in diets with moderately elevated TI levels to ensure sufficient amount of active protease in the intestinal tract to digest dietary protein.

Material and Methods

The 9 SBMs were obtained at the port at the arrival of the vessels by qualified personnel. All analysis was conducted at the University of Madrid. TI activity was assayed according to Hammerstrand et al. (1981). Two different enzyme preparations were used for the experiment: A purified preparation of pancreatic trypsin and chymotrypsin with approximately 90% trypsin and 10% chymotrypsin on a protein:protein basis and a purified preparation of an S1 protease from *Nocardiopsis prasina* (both Novozymes A/S, Denmark).

Each SBM was incubated (0.06 g DM/mL) in a pH 7 buffer (100mM succinic acid, 100 mM HEPES, 100 mM CHES, 100 mM CAPS, 12.5 mM $CaCl_2 \cdot 2H_2O$, 150 mM KCl, 0.01% Triton X-100) either without or with the addition of protease diluted in an acetate buffer at pH 6 (100 mM Acetate, 5 mM CaCl2, 0.01% BSA, 0.01% Tween 20). Samples were incubated with magnetic stirring (350 rpm) for 3 hours at 40°C. After incubation a stop reagent (220 mM TCA, 440 mM $NaAc \cdot 3H_2O$, 570 mM glacial acetic acid) was added at a 2:1 volume and samples were centrifuged (10 min, 4,000 rpm, 5°C). Supernatants were recovered and stored on ice bath until analysis. Protein composition of the non-protease

treated supernatants was analyzed by SDS-page. Supernatants were diluted 5 times, mixed 1:1 with SDS-page loading buffer and incubated 5 minutes at 95°C and then cooled on ice bath before SDS-page analysis according to standard procedure. Protein bands were visualized by staining with Coomassie Blue. The effect of protease on release of soluble free amino groups was determined using a colorimetric assay based on the o-phthal-dialdehyde (OPA) method according to Nielsen et al. (2001). Supernatants were diluted 3-5 times in de-ionized water. To each 25 µL diluted sample, 200 µL of OPA reagent was added and samples were mixed by shaking (2 min, 700 rpm) before absorbance was read at 340 nm.

Results

Analysis of the SBMs (Table 1) showed similar dry matter and crude protein content but considerable variation in solubility characteristics. SBM #7 from Brazil stood out as having lower solubility in water (PDI) and KOH as compared to all other samples. TI content ranged from 2.39 to 3.75 mg/g DM with the samples from the United States generally having higher TI activity than those from Brazil and Argentina. Urease activity was low in all samples indicating that urease activity is not suitable to predict TI activity when operating in the low end. Low protein solubility in KOH and water was correlated with high Heat Damage Index (R^2=0.52 and 0.58 for KOH and PDI). Likewise high protein solubility in KOH and water was correlated with high TI activity (R^2=0.50 and 0.76 for KOH and PDI).

Table 1. Analysed values for dry matter (DM, %), crude protein (CP, %), protein dispersibility index (PDI, %), protein solubility in KOH (KOH, %), urease activity (mg N/g DM), TI activity (TIA, mg/g DM) and Heat Damage Index (Aminored-Evonik)

SBM batch	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**#9**
Origin	**USA**	**USA**	**USA**	**Arg**	**Arg**	**Arg**	**Bra**	**Bra**	**Bra**
DM, %	87.9	88.7	89.0	88.6	89.8	88.4	87.7	87.1	88.5
CP, %	53.8	52.4	52.9	53.6	52.9	52.8	53.7	54.2	53.2
PDI, %	25.6	24.8	9.7	10.4	11.7	10.1	7.0	15.2	12.5
KOH, %	80.2	79.7	78.6	75.4	77.3	71.4	67.9	77.2	81.4
Urease act., mg N/g DM	0.02	0.00	0.02	0.01	0.00	0.00	0.05	0.05	0.01
TIA, mg/g DM	3.75	3.49	3.15	2.71	2.67	2.49	2.39	2.76	2.71
Heat Damage Index	5	2	8	11	12	15	25	14	16

Evaluation of the non-protease treated SBM incubations by SDS-page showed that the samples had different amounts of solubilized protein. Protein band intensities were generally higher for those SBM batches with higher protein solubility and low Heat Damage Index.

The efficiency of pancreatic trypsin/chymotrypsin to hydrolyze protein varied markedly among SBMs. Protein in SBM 2, 4, 5 and 6 was hydrolyzed effectively at lower pancreatic protease levels compared to protein in SBM 1, 3, 7, 8 and 9. However, with sufficiently high inclusion level of protease, all SBMs were hydrolyzed to approximately the same extent. The difference in response to pancreatic protease could not be explained by differences in protein solubility and TI activity as isolated parameters. The efficacy of the protease from *N. prasina* was very similar on all SBMs except for SBM#7, which was hydrolyzed to a lower degree. The lower efficiency on SBM#7 might be explained, at least in part by its lower protein solubility as compared to the other batches, meaning that less protein was readily available for hydrolysis.

Conclusions

The results clearly showed that the efficiency of pancreatic trypsin/chymotrypsin varied among SBMs. The differences observed could not be explained by differences in protein solubility and TI activity as isolated parameters. Incubation with the *N. prasina* protease resulted in effective and very similar responses for the different SBMs and did not appear to be affected by TI activity. Hence, the *N. prasina* protease seems to represent a promising tool to ensure a sufficient amount of active protease in the intestinal tract of animals fed diets with moderately elevated levels of TI.

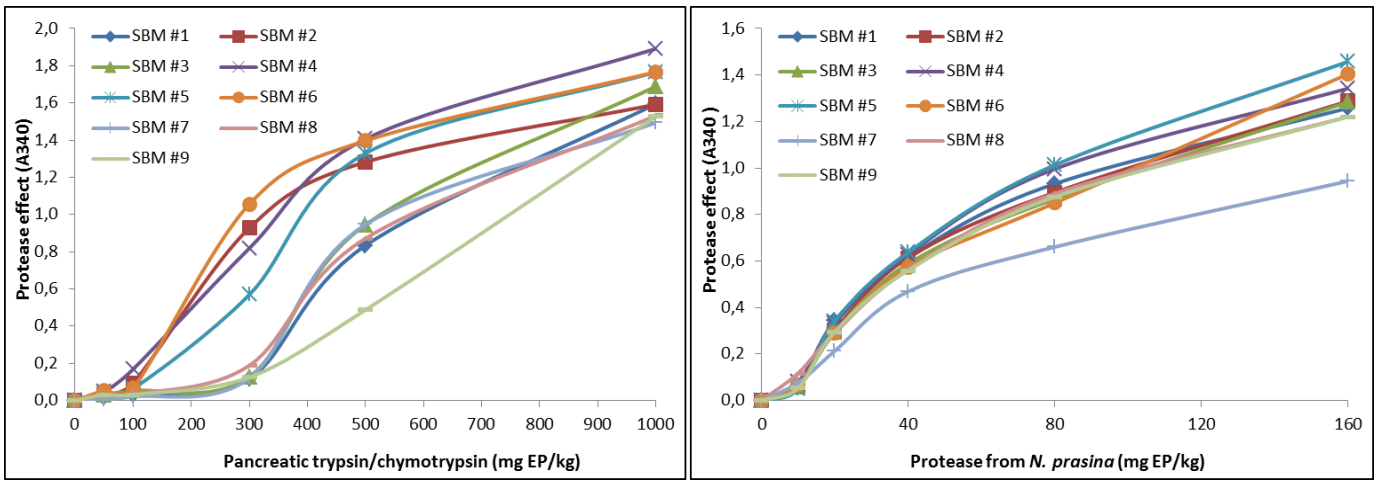

Figure 2. Protease effect measured as release of soluble free amino groups after incubation of the different SBMs at pH 7, 3 h, 40°C with different proteases.

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Other topics

P-232 (ID 297)

STABILITY OF SECONDARY PLANT COMPOUNDS BASED PRODUCTS IN FEED PROCESSING

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Abstract

Supplementing feed with plant compound based products is gaining more and more attention since livestock industry demands alternatives for growth promoting antibiotics to increase performance and animal health. Most feed supplements based on secondary plant compounds (SPCs) are offered as free flowing, granulated products. But many secondary plant compounds are highly volatile and thus do not reach the animal's digestive tract to unfold their positive effects.

Encapsulation can protect SPCs from evaporation, but the products on the market differ greatly in design, technology, and performance.

To determine the stability during feed processing, we compared the recovery rate of the leader substance of 4 competitor products to Activo®, a matrix encapsulated blend of SPCs. For each product the recommended dosage was added to a standard pig or poultry diet, respectively. After mixing, pellets were produced in a feed press using different temperature conditions (70°C and 90°C; 3 min. heating). After the pelletizing process, 10 samples for each temperature were collected and analyzed by a certified laboratory in Germany. "Leader substances" were determined through GC/MS-analysis and recovery rate in the finished feed was calculated.

Our data show that the content of SPCs in feed can be considerably influenced by the processing temperature. Activo® maintained the highest content of active compounds in the feed after processing (94,7% compared to 53,3 – 85,7% in the competitor products at 70°C and 82,1 % vs. 50,5-79,9% at 90°C).

Micro droplet encapsulation with the appropriate matrix offers the most effective and economic solution for optimal use of SPCs in animal nutrition.

Keywords: secondary plant compounds, stability, micro droplet encapsulation, feed processing

Introduction

Secondary plant compounds are incorporated into diets to improve productivity of livestock by ameliorating feed properties, promoting the animals' production performance and improving the quality of food derived from these animals. The commonly used SPC products are offered as free flowing and granulated products. On farm, this standard use of SPCs has a pleasant side effect namely the smell. However, the economic sense has to be questioned, since substances that reach our nose are no longer available in the product to find their way into the digestive tract of the animal.

To prevent these losses many products containing SPCs are encapsulated and have entered the market over the last few years. These products and the processes that generate them differ greatly in design, technology, and unfortunately, in terms of performance. The question is if the product is stable enough to overcome production steps like grinding, pelletizing or extrusion.

Encapsulation is a method of enclosing the active components within a carrier material in order to protect them from harmful environmental influences. With this innovation developed more than 50 years ago the delivery of veterinary and medical drugs has largely improved (Avgenaki, 2002). As the structure of the carrier material determines the degree of protection against volatilization, aspects like good film formation, emulsion stability, low viscosity, hygroscopicity, lack of an own taste or smell, and the probabilityof release have to be considered (Salzer and Siewek, 2014; Avgenaki, 2002). The inclusion in an amorphous carbohydrate-based matrix for example, provides protection against oxidative changes. However, the limiting factor of this method is the insufficient water resistance releasing the encapsulated substances upon contact with it.

For many applications utilizing the physical methods of spray drying, spray granulation or extrusion, the commonly used techniques are involving core-shell encapsulation (Coating) or matrix / micro droplet encapsulation (Figure 1).

Core shell encapsulation (Coating)

Core shell encapsulation applies a layered coating around the outside of the active ingredient e.g. essential oils to form a protective barrier. The most commonly used substances to form such a barrier or shell are ethyl cellulose, polyvinyl alcohol, gelatin or sodium alginate.

One of the major disadvantages of core shell encapsulation is the limited stability it provides during feed processing like pelleting, grinding, extruding. The high temperature and pressure applied through such processing technologies very often leads to the damage of the outer shell and thus the release of liquid and easily volatile secondary plant compounds into the environment. This can significantly reduce the content of active compounds in final feed but can also change odor and taste.

Matrix / Micro Droplet Encapsulation

Matrix encapsulation technology allows enclosing minute droplets of a complete mixture in different matrices like hydrocolloids, proteins or fat.

Micro droplet encapsulation with fat emerged as an effective and economical method to protect secondary plant compounds. One of its major advantages compared to core shell encapsulation is the increased protective capacity during feed processing. In the event of damage, only smallest amounts of substance leak out and higher quantities of the active substance remain in the feed. Thus, losses in active compounds can be reduced and a negative influence on odor and taste is minimized. The use of a combination of different sources of fats can optimize enzymatic degradation of the matrix through lipases. This allows the slow release of secondary plant compounds to exert their full potential in promoting digestion, reducing pathogenic load and inflammatory processes, and acting as antioxidants along the gastrointestinal tract.

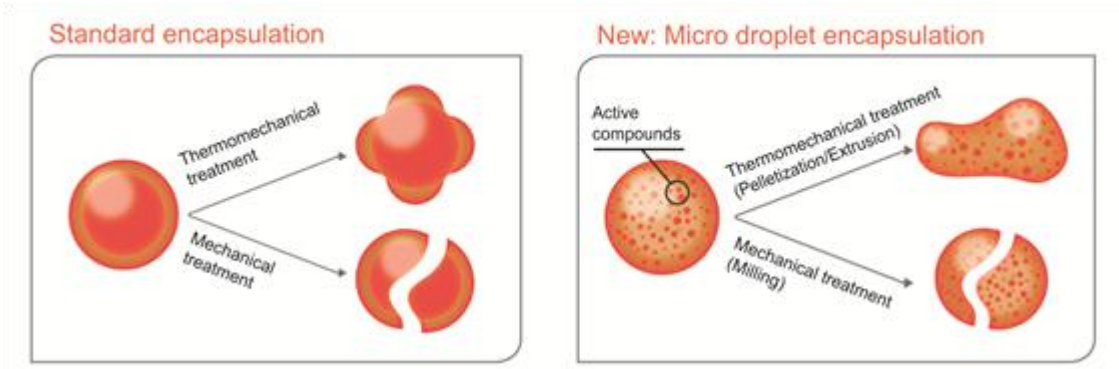

Figure 1: Coating and micro droplet encapsulation and the results after processing

Material and Methods

The recovery rate of the leader substance in Activo® was compared to 4 competitor products after mixing and feed processing. The amount of recovered leader substance was analyzed through GC/MS analysis by a certified laboratory in Germany.

For each product the recommended dosage was added to a standardized feed (the ingredients of this feed formulation are characteristic for pig feed as well as for poultry feed). After mixing, pellets were produced in a feed press using different temperature conditions (70°C and 90°C). The heating time for each temperature was approx. 3 minutes. In the process, steam was added into the conditioner before the feed entered the shaping die. Subsequently, the pellets were cooled down with a belt cooler and for each temperature 10 samples were collected and analyzed. The amount of leader substance after mixing, but before heat processing was set as 100%.

Results

As shown in figure 1, the critical point of the process is heating. Feed processing at 70°C can lead to losses in the leader substance of 5,3% in Activo® with 94,7% of the leader substance still remaining in the pelleted feed to 46,7% in the non-encapsulated product of competitor C. An increase in the processing temperature to 90°C decreases the resid-

ual content in leader substance from 82,1% (Activo®) to 50,5% in competitor C. In general, the encapsulated products provide a better protection against volatilization than the non-encapsulated products. An exception is competitor D that is offered as dried herbs, providing a better protection against heat related losses than the non-encapsulated extracts.

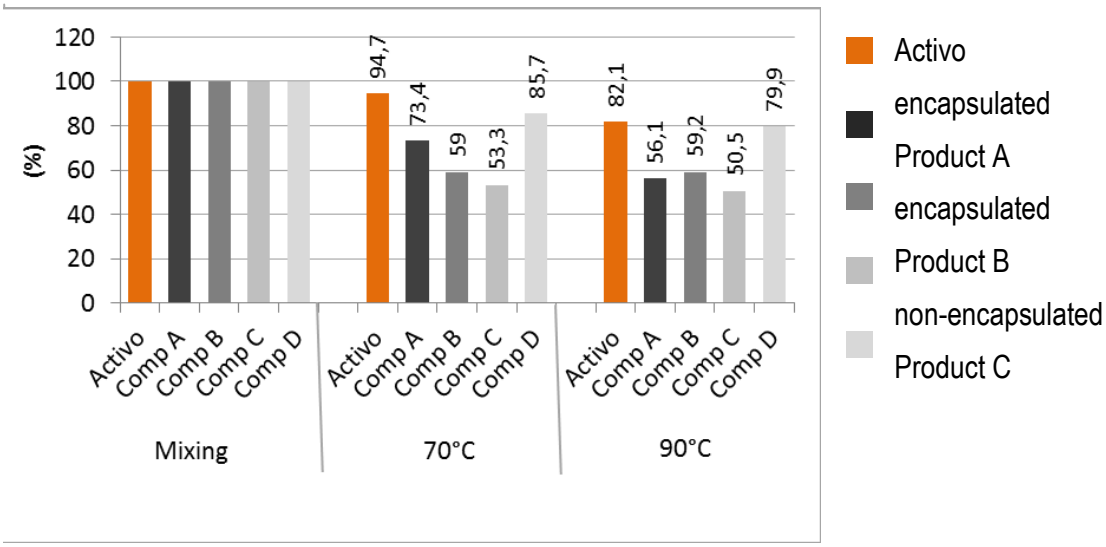

Figure 2: Recovery rate of the respective marker substances

Conclusions

Encapsulation is a good tool to protect the often volatile secondary plant compounds from volatilizing. The kind of encapsulation, however, is very important. Core-shell encapsulation can only protect, if the product is not undergoing extensive processing and is thus not damaged by milling. The more sophisticated method of micro-droplet encapsulation shields the mixtures in case of heating but also of milling. If damaged, only smallest amounts of the active components can lack, but the main part is ingested. If a fat matrix composed of different fats is used, release in the gastrointestinal tract can be regulated up to a certain degree. So, if secondary plant compounds are used in animal nutrition, an adequate method of encapsulation should be implemented to tap the full potential of these useful substances.

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P-234 (ID 134)

EFFECT OF YEAST AS A SOURCE OF NUCLEOTIDE SUPPLEMENTED IN BROILER BREEDERS DIETS AND ITS CARRYOVER EFFECT ON PROGENY

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Abstract

In order to study the effects of dietary supplementation of nucleotides on broiler breeders' and progenies' performance, 4 trials were conducted. The 1st and 2nd trials with 80 broiler breeder females (Cobb) in each, distributed in a completely randomized design (CRD) with 2 treatments; 0 and 5kg/MT of nucleotide source (NU) from 25-45 weeks of age. Each treatment consisted of 10 reps, 4 breeders each. Breeders were inseminated at 35 and 45 weeks of age and the eggs incubated. Egg production, fertility and hatchability were evaluated. On 3th trial, 300 male chicks were distributed in CRD in 2 groups (progeny from breeders supplemented or not) and fed with same feed, with 10 reps, 15 chicks each. On 4th trial, 240 male chicks were distributed in CRD in a factorial arrangement 2x2 (progeny from broiler breeders supplemented or not, and progeny supplemented or not, at 5kg/MT of NU), resulting in 4 treatments with 5 reps, 12 birds each. Study criteria included BWG, FI and, F/G at 21 and 42 days for both trials. The data were analyzed using the GLM (SAS) and means were compared by Tukey (P=0.05). The 1st and 2nd trails demonstrated that supplementation of NU to broiler breeders increases the number of live chicks by 6 and 13%, respectively. The 3th and 4th trials showed a positive carryover effect on progeny performance for BWG (+5.6 and 4.3%) and F/G (+4.5% and 9.5), however, the NU supplemented broilers from the 4th trial had improved F/G (6 and 3.5%) in both breeders age.

Keywords: egg production, broilers, feed conversion

Introduction

Although the nucleotides are not considered as essential nutrients, they play an important role in several metabolic processes, in particular, in some body tissues and stages of animal life. The free nucleotides and nucleosides can be readily absorbed by the enterocytes in the intestine, and are especially important in tissues of rapid cell proliferation and limited capacity for de novo synthesis (major route of nucleotide production), such as intestinal epithelial cells, blood cells, hepatocytes and cells of the immune system. Then, the free nucleotides can be used by the salvage pathway, where the body can synthesize nucleotides with less energy consumption, as result of recycling bases and nucleotides from metabolic degradation of nucleic acids from dead cells or from the diet. However, when the endogenous supply is insufficient, exogenous (dietary) nucleotide sources become semi-essential or "conditionally essential" nutrients (CARVER and WALKER, 1995). This especially occurs in animals in rapid growth phases (early stages), reproduction, stress, and disease challenges.

The nutrition of broiler breeders is directly linked to egg production and quality of hatched chickens. Based on this, two studies were conducted with broiler breeders in order to evaluate the effects of supplementation of *Saccharomyces cerevisiae* yeast as a source of nucleotides on the production performance and over their progeny.

Material and Methods

Two trials were conducted with 80 broiler breeder females (Cobb) in each, distributed in a completely randomized design (CRD) with 2 treatments; 0 and 5kg/MT of nucleotide source (NU - *Saccharomyces cerevisiae* yeast, equivalent to 0.3kg/MT of free nucleotides) from 25-45 wks of age. Each treatment consisted of 10 reps of 4 breeders each. Breeders were inseminated at 35 and 45 wks of age and the eggs incubated. Egg production (*EP%)*, egg fertility (*EF%)* and hatchability of incubated eggs (*HIE%)* and fertile eggs (*HFE%)* were evaluated in both trials; and total embryonic mortality (*TEM*%) and chick weight (*CW*,g) were evaluated only in 2nd trial. On 3th trial, 2 groups of 150 male chicks; one group originated from breeders fed nucleotide supplemented diets, and other group originated from the breeders not given dietary nucleotides, were distributed in CRD, with 10 reps of 15 birds in each. All broiler groups received the

same growout diets over a 45 day grow out period (1 to 21, 22 to 35 and 36 to 45d). On 4th trial, 240 male chicks were distributed in CRD in a factorial arrangement 2x2 (being, progeny derived from broiler breeders which were supplemented or not, and progeny supplemented or not, at 5kg/MT of NU, up to 10 d), resulting in 4 treatments, 5 reps of 12 birds each. Study criteria included FI (g), BWG (g) and, F/G (g/g) at 45 d for 3th trial and 42 d for 4th trial. The data were analyzed using the GLM (SAS) and means were compared by Tukey at 5% of significance.

Results

Nucleotide supplementation in the diet of broiler breeders improved (P<0.05) EP (1.6%), EF (1.7%), HIE (4.1%) and HFE (2.3%), in the 1st trial (Table 1). On the 2nd trial, nucleotide supplementation of the breeder diet improved (P<0.05) EP (2.6 and 2.9%), EF (2.5 and 2%), HIE (6.7 and 2.6%), HFE (9 and 6.9%), TEM (36.5 and 34.5%) and CW (2.2 and 4.4%), respectively, for hens from 25-35 and 36-45 wks (Table 2).

Table 1. Broiler breeder's performance from 25 to 45 weeks (1st trial)

Treatments	Egg production (%)	Fertility (%)	Hatchability incubated eggs (%)	Hatchability fertile eggs (%)
Control	74.3^{b}	95.1^{b}	85.2^{b}	89.2^{b}
Nucleotide Source	75.8^{a}	96.8^{a}	89.3^{a}	91.4^{a}
Probabilities	0.018	0.023	0.011	0.006

Means followed by different letters in the same column statistically differ by the Tukey test (P<0.05).

Table 2. Broiler breeder's performance from 25 to 45 weeks (2nd trial)

Parameters / Treatments	Breeders - 25 to 35 weeks			Breeders - 36 to 45 weeks		
	Control	Nucleotide	P	Controle	Nucleotide	P
Egg production (%)	81.14^{b}	83.27^{a}	0.041	76.43^{b}	78.66^{a}	0.011
Fertility (%)	94.49^{b}	96.88^{a}	0.037	95.81^{b}	97.74^{a}	0.012
Hatchability incubated eggs (%)	85.07^{b}	90.74^{a}	0.035	87.90^{b}	90.22^{a}	0.022
Hatchability fertile eggs (%)	86.02^{b}	93.78^{a}	0.044	89.28^{b}	95.45^{a}	0.019
Total embryonic mortality (%)	2.82	1.03	0.281	2.18	0.76	0.344
Chick weight (g)	45^{b}	46^{a}	0.006	45^{b}	47^{a}	0.003

Means followed by different letters in the same row statistically differ by the Tukey test (P<0.05).

Offspring from 35 and 45 wks old breeders (3th trial) fed diets supplemented with 5% of nucleotides had improved (P<0.05) BWG and F/G versus chicks from breeders not supplemented dietary nucleotides (Table 3). On the 4th trial, there was no interaction (P>0.05) between breeders and offspring supplementation, for either breeder age. Offspring from 35 and 45 wks old breeders fed nucleotide-added diets had better (P<0.05) BWG and F/G versus chicks from unsupplemented hens. The supplemented offspring groups had better (P<0.05) F/G compared to non-supplemented broilers from 35 and 45 wks breeders (Table 4 and 5).

Table 3. Progeny performance from breeders with 35 and 45 weeks (3th trial) at 45 days

Treatments	FI (g)	BWG (g)	F/G (g/g)	FI (g)	BWG (g)	F/G (g/g)
	Breeders – 35 weeks			Breeders – 45 weeks		
Control	5.48	3.30^{b}	1.66^{b}	5505	3244^{b}	1.70^{b}
Nucleotide source	5.58	3.45^{a}	1.61^{a}	5514	3457^{a}	1.60^{a}
Probabilities	0.327	0.042	0.04	0.417	0.027	0.035

Means followed by different letters in the same column statistically differ by the Tukey test (P<0.05).

Table 4. Progeny performance from breeders with 35 weeks (4th trial) at 42 days

Progeny		Breeders		Average	Probabilities		
		Control	Nucleotide		Breeder	Progeny	Breeders x Progeny
FI (g)	Control	5056	4769	4911	0.008	0.111	0.773
	Nucleotide	4900	4581	4703			
	Average	4978A	4675B				
BWG (g)	Control	2554	2757	2646	0.029	0.259	0.466
	Nucleotide	2663	2711	2688			
	Average	2604B	2703A				
F/G (g/g)	Control	1.98	1.73	1.86b	0.012	0.048	0.92
	Nucleotide	1.84	1.69	1.75a			
	Average	1.91B	1.71A				

Means followed by different letters in the same row (uppercase) and column (lowercase), statistically differ by the Tukey test (P<0.05).

Table 5. Progeny performance from breeders with 45 weeks (4th trial) at 42 days

Progeny		Breeders		Average	Probabilities		
		Control	Nucleotide		Breeder	Progeny	Breeders x Progeny
FI (g)	Control	4755	4734	4745	0.109	0.320	0.624
	Nucleotide	4733	4712	4723			
	Average	4744	4723				
BWG (g)	Control	2668	2835	2752	0.008	0.063	0.715
	Nucleotide	2784	2856	2820			
	Average	2726B	2856A				
F/G (g/g)	Control	1.78	1.67	1.73b	0.036	0.049	0.414
	Nucleotide	1.70	1.65	1.67a			
	Average	1.74B	1.66A				

Means followed by different letters in the same row (uppercase) and column (lowercase), statistically differ by the Tukey test (P<0.05).

Conclusions

This study demonstrated that supplementation of nucleotides to broiler breeders increases the number of live chicks by 6 and 13% (1st and 2nd trials) and appears to have a positive carryover effect on their progeny performance for both BWG and F/G (3th and 4th trials). In addition, the nucleotide-supplemented broilers from the 4th trial had improved F/G (6 and 3.5%) in both breeders age.

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P-235 (ID 274)

EFFECTS OF FEEDING ORIGINAL XPC DURING A *CAMPYLOBACTER* CHALLENGE IN BROILERS AND TURKEYS

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Abstract

Two studies were conducted to test the effects of feeding XPC during a *Campylobacter* challenge: 1) Cobb broiler chicks (n=1200) in Georgia, USA or 2) Hybrid hen poults (n=288) in North Carolina, USA. Both were placed in barns with 24 floor pens (50 chicks or 12 poults per pen). Three treatments were fed in the chicken study: 1) Control, 2) 1.25 kg XPC/tonne from 0-21d and 0.62 kg XPC/tonne from 22-42d, and 3) 1.25 kg XPC/tonne from 0-42d. Turkeys were fed one of 2 dietary treatments: Control or XPC (1.25 kg/tonne from 0-42d and 0.62 kg/tonne from 43-84d). At 14d (chickens) or 70d (turkeys), 25 or 5-tagged birds/pen (respectively) were orally gavaged with a gentamicin-resistant strain of *C. coli* (log 10^6 CFU/ml). The remaining birds per pen were not tagged or challenged and were used to assess horizontal transmission (HT). Fifteen chickens at 42d or 10 turkeys at 84d were sampled per pen (5 tagged birds) and the ceca removed for *C. coli* analysis. FCR was statistically improved ($P < 0.05$) in chickens and turkeys from the addition of XPC. *C. coli* prevalence (% positive) in HT turkeys and chickens was reduced by feeding XPC ($P < 0.05$). HT chickens fed XPC had lower *C. coli* colonization ($P = 0.09$). In the turkeys, *C. coli* numbers were reduced ($P < 0.05$) from 4.5 to 3.5 Log_{10} CFU/g in Control vs. XPC fed birds, respectively. Based on these results, XPC-fed chickens and turkeys had improved FCR and lower prevalence and numbers of *Campylobacter*.

Keywords: Campylobacter, Food Safety, Turkeys, Chickens

Introduction

Campylobacter is one of the leading causes of foodborne illness (Batz et al, 2012). Traditional methods for controlling *Campylobacter* contamination have been focused within the processing plant, trying to prevent cross contamination through washing and evisceration (Berghaus et al, 2013). It has been suggested that if preharvest levels of *Campylobacter* could be controlled in the birds' intestinal tract, then contamination of processed birds will also be reduced. Research has shown that biosecurity and pest management measures may improve preharvest control of pathogens, but additional measures (i.e. dietary ingredients, vaccination) may be needed to have the optimal preharvest pathogen control (Dale et al, 2015). The objective of this research is to evaluate the effects of feeding Original XPC™, a *Saccharomyces cerevisiae* fermentation product, on cecal *Campylobacter coli* colonization and transmission following an oral challenge.

Materials and Methods

Two independent studies were recently conducted, in which broilers or turkeys were orally gavaged with approximately 10^6 CFU of gentamicin-resistant strains of *Campylobacter coli*. The broiler trial (Experiment 1) was conducted by Southern Poultry Research Group near Nicholson, GA and turkey trial (Experiment 2) was conducted at North Carolina State University near Raleigh, NC.

Experiment 1: Broiler Trial

Cobb chicks (n = 1200) were housed in 24 floor pens (50 birds/pen) and assigned to one of 3 feed treatments (8 replicates each). Control birds (T1) received industry standard diets with no antibiotics or coccidiostat. Treatments T2 and T3 received the control starter diet with the addition of XPC at 1.25 kg/tonne from 0-21 d. After 21 d, XPC was reduced to 0.62 kg/tonne for T2 birds, while T3 birds continued to receive feed with 1.25 kg/tonne of XPC. All birds and feed were weighed by pen at 0, 21, 35 and 42 d. All birds were vaccinated with Coccivac-B at 0 d.

At 14 d, 25-tagged birds per pen were challenged with *C. coli*. The remaining 25 birds per pen did not receive a tag or *C. coli* challenge, and were used to determine horizontal transmission (contact challenge between *C. coli* gavaged and non-gavaged birds). Fifteen birds per pen (5 tagged and 10 non-tagged) were sampled at 42 d and the ceca removed

for *C. coli* colonization evaluation. The MPN (most probable number) method was used to enumerate *C. coli* (Berghaus et al, 2013).

Experiment 2: Turkey Trial

Hybrid hen poults (n = 288) were assigned to one of 2 feed treatments (Control vs. XPC), with 12 replicate floor pens per treatment and 12 birds per pen. Four diet phases were fed, as either pelleted and crumbled or pelleted, during the 12-week study; 2 starter and 2 grower ratios. Original XPC was added to the XPC rations at 1.25 kg/tonne in the two starter feeds and 0.62 kg/tonne in the two grower feeds. Diets did not contain any antibiotic or coccidiostat. All birds and feed were weighed at 10 weeks and at the end of the study (12 weeks of age) and feed conversion ratio calculated.

At 10 weeks of age, 5 birds from each pen were wing banded and challenged with *C. coli*. The remaining 7 birds per pen were not banded nor challenged, and were used to determine horizontal transmission of *Campylobacter* from challenged birds. At 12 weeks of age, 10 birds per pen (5 banded and 5 non-banded) were euthanized and ceca were aseptically removed. Cecal contents were either directly plated onto CCDA+gentamicin to determine presence of *C. coli* or serially diluted in buffered peptone water, plated on CCDA+gentamicin, and incubated in a microaerophilic environment for 48 h at 42 C to determine numbers of *C. coli*.

Results

Body weight gains were not significantly different ($P > 0.05$) between treatments in both the broiler and turkey studies. Feed conversion was significantly improved ($P < 0.05$) in broilers by the addition of XPC at both inclusion levels on 35 d and 42 d, respectively (Table 1). Feed conversion was also significantly improved ($P < 0.05$) in turkeys, during the challenge (10-12 week) and overall (0-12 week; Table 1).

Cecal *C. coli* prevalence (percent positive) in contact challenged broilers was significantly reduced ($P < 0.05$) when 1.25 kg/tonne (T3) XPC was fed continuously and intermediate when 0.62 kg XPC/tonne (T2) was fed during grower/finisher period (Table 2). A similar response trend was observed in *C. coli* numbers (MPN; $P = 0.09$) in broilers when XPC was fed. In turkeys, cecal *C. coli* prevalence was significantly reduced ($P < 0.05$) in contact challenged and a trend for reduction ($P = 0.09$) in gavage (direct) challenged birds (Table 3). Cecal *C. coli* numbers were significantly ($P < 0.05$) reduced by one (1) log across all challenged turkeys (Table 3).

Table 1. Feed conversion ratio (FCR) in broilers and turkeys.

	Broilers FCR		Turkeys FCR	
Bird Age:	0-35 d	0-42 d	10-12 wk	0-12 wk
T1; Control	1.75[a]	1.93[a]	2.56[a]	1.95[a]
T2; XPC (1.25 and 0.62 kg/tonne)[1]	1.73[b]	1.90[b]	2.38[b]	1.90[b]
T3; XPC (1.25 and 1.25 kg/tonne)[1]	1.72[b]	1.90[b]	-	-
P value	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$

[ab] Values within a column with different superscripts are statistically different ($P < 0.05$).
[1] Starter and grower (turkey) or grower/finisher (broiler) dietary inclusion rates, respectively.

Table 2. Broiler cecal *Campylobacter coli* prevalence and numbers in contact challenged birds at 42 day of age.

Treatment	*C. coli* prevalence (% positive)	*C. coli* numbers (Most probable number)
T1; Control	17.5[a]	37
T2; XPC (1.25 and 0.62 kg/tonne)[1]	10.0[ab]	14
T3; XPC (1.25 and 1.25 kg/tonne)[1]	1.3[b]	0.9
P value	$P < 0.05$	$P = 0.09$

[ab] Values within a column with different superscripts are statistically different ($P < 0.05$).
[1] Starter and grower/finisher dietary inclusion rates, respectively.

Table 3. Turkey cecal *Campylobacter coli* prevalence in gavage and contact challenged birds and numbers in all birds at 12 week of age.

	C. coli prevalence (% positive)		*C. coli* numbers (log_{10} CFU)
Challenge method:	Gavaged	Contact	All birds
T1; Control	87	93[a]	4.51[a]
T2; XPC (1.25 and 0.62 kg/tonne)[1]	78	75[b]	3.49[b]
P value	$P = 0.09$	$P < 0.05$	$P < 0.05$

[ab] Values within a column with different superscripts are statistically different ($P < 0.05$).
[1] Starter and grower dietary inclusion rates, respectively.

Conclusions

Campylobacter prevalence was reduced in contact challenged broilers and turkeys when XPC was fed. *Campylobacter* numbers were also reduced in broilers and turkeys fed XPC. Improvements in feed conversion were observed in broilers and turkeys fed XPC.

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P-239 (ID 133)

THE INFLUENCE OF OZONE TREATMENT ON THE DEGRADATION OF DIFFERENT MYCOTOXINS IN CONTAMINATED MAIZE

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Abstract

Although commercial usage of ozone for grain treatments is not common, several results prove its efficiency against grain insects, moulds and different microbes. The scientific results of ozone on the different mycotoxin levels are contradictionary.

In the present study the grinded maize samples were contaminated with different purified mycotoxins, aflatoxin B1 (AFB1); ochratoxin A (OTA); T2-toxin, Deoxynivalenol (DON); fumonisin B1 (FB1) and zearalenon (F2). The mycotoxin levels were adjusted according to the concentrations that can be found under field conditions. Maize samples were treated with 50 ppm ozone for 48 hours. Mycotoxin concentrations were measured after 24 and 48 hours treatments.

After 48 hours the following reduction in the mycotoxin concentrations were detected: AFB1: 26.1%; DON: 24.3%; F2: 44.5%; FB1: 53.7%; OTA:12.6%; T2: 0,3%. Significant reduction was found after 24 hours in the concentrations of AFB1 (14.7%), DON (21.5%) and FB1 (44.7%). On the other hand the concentration of F2 has changed only during the second 24 hours of ozone treatment. Ozone seems to be less effective in breaking down T2 toxin..

Keywords: ozone, mycotoxins, maize

Introduction

Mycotoxins have several negative effects and decrease the production rate of farm animals. Beside that animals can excrete mycotoxins with the animal products (milk, eggs, liver) that mean also food safety concerns. It is the reason, that several tools are used to decrease the incidence of toxicosis. Among them a new direction is the ozone treatment of the contaminated products. Ozone is well known as a disinfectant, but its effect on the mycotoxin contaminated grains is not fully understood. The efficiency of ozone treatment is influenced by several factors, like its concentration, the air flow, the exposure time and the humidity (ISIKBER et al., 2014). According to the results of MENDEZ et al. (2003), the ozone degrades rapidly in the first few hours while it flowing through a grain column, but remains stable afterwards. Even 30 day long treatment failed to cause any difference in the fatty acid and amino acid profile of soybean, wheat or maize. The aim of the present study was to determine the effect of ozone on the degradation of different mycotoxins.

Materials and methods

Six mycotoxins, aflatoxin B1 (AFB1), zearalenon (F2), T-2 toxin (T2), fumonisin B1 (FB1), deoxynivalenol (DON) and ochratoxin A (OTA) have been purchased in purified form (Sigma Aldrich Fluka Analytical), solved in methanol and mixed with grinded maize samples. The toxin concentrations were adjusted close to the natural depressive levels *(Table 1.).* After evaporating the methanol, the samples were mixed and homogenized. The mycotoxin concentration of the samples was determined prior and after 24 and 48 hours of the ozone treatment. The grinded maize samples were analysed by HPLC after cleaning and separating the mycotoxins with toxin specific immune-affinity columns according to the validated methods of R-Biopharm Hungary Ltd.

Ozone treatments were carried out by an ExOzone (expanded capacity with a limited flashover high frequency cold charge emission with changed auxiliary electrode) type laboratory ozone generator. The actual treatment conditions (ozone concentration, temperature, humidity) were monitored with a TGM-100 type detector. The ozone concentration was adjusted according to the potential practical level.

Table 1. Adjusted mycotoxin concentrations of the maize samples

	Concentration (µg/kg)
AFB1	100
F2	2500
T2	2500
FB1	2,5
DON	3,0
OTA	500

Results

The average ozone concentration during the treatments was 50.3 ppm, the temperature 25.7°C and the relative humidity 54.9% *(Figure 1. and 2.).* After 24 hours no changes in the concentration of F2, T2 and OTA was measured. The following 24 hour long ozone treatment reduced the F2 concentration by about 44.5%. The decrease in OTA content was moderate, 12.6%. T2 concentration was not influenced by the ozone treatment.

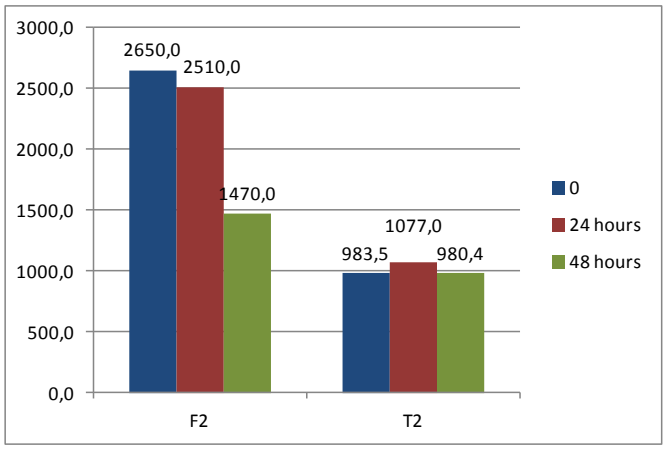

Figure 1. The effect of ozone treatment on the F2, T2 concentrations (ppb)

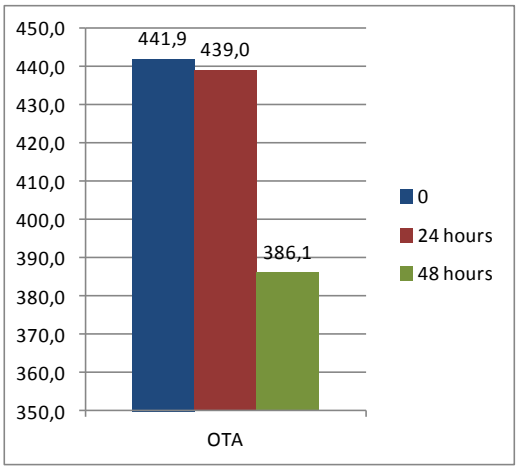

Figure 2. The effect of ozone treatment on the OTA concentrations (ppb)

Similarly to the T2 toxin the other trichothecene mycotoxin, DON showed also higher resistance against ozone, compared with FB1 or AFB1. In the case of FB1, its concentration decreased already after the first 24 hours by and only limited further decrease happened *(Figure 3.).* On the other hand gradual decrease was found in the AFB1 level *(Figure 4.).*

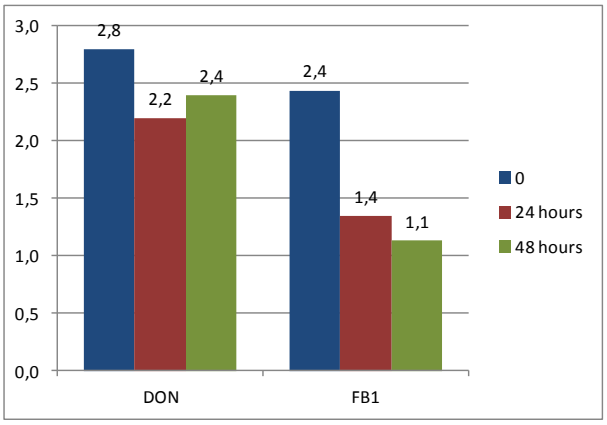

Figure 3. The effect of ozone treatment on the DON, FB1 concentrations (ppm)

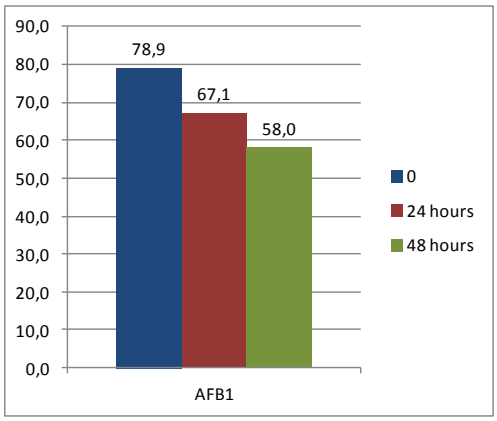

Figure 4. The effect of ozone treatment on the AFB1 concentrations (ppb)

Conclusions

From the results it can be concluded, that ozone treatment seems to be effective to decrease the mycotoxin content of the cereal grains. Its efficiency is however, depends on the type mycotoxins and the exposure time. Trichothecene mycotoxins (T2 and DON) seem to be more resistant against ozone than the other toxins, investigated in this trial. The reason for this could be the chemical structure of the different toxins. The trichothecene skeleton contains less ligand that can interact with ozone. Further research needed to understand the interactions of ozone with the mycotoxin molecules, the biological activity of the modified substances and the optimal treating conditions for the different mycotoxins.

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P-242 (ID 192)

EFFECT OF PARTICLE SIZE AND FEED PRESENTATION (MASH VS PELLETS) ON CECAL MORPHOLOGY AND *CAMPYLOBACTER JEJUNI* COLONIZATION OF BROILERS ORALLY INFECTED

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Abstract

An experiment was conducted within the EU-FP7 project CAMPYBRO for evaluating the effect of particle size (PS) of wheat (W) and feed presentation (FP) in morphology and *Campylobacter jejuni* colonization in the caeca of broilers orally infected. There were four treatments factorially arranged with two W screen sizes (2mm *vs* 5mm) and two FP (mash *vs* pellets). A total of 144 one-day-old Ross 308 broilers were divided into cages (3 birds/cage) and experimental treatments (12 cages/treatment). At 14 days of age, all broilers were orally gavaged with 100 µl of a solution containing 1 x 10^5 cfu/ml of ST-45 *C. jejuni* strain. On days 21, 35 and 42, caeca from 12 birds per treatment were collected and *Campylobacter* counts determined (ISO 10272). At 42d, the weight and the pH at caeca were taken. Data were analysed by GLM procedure of SPSS. Pelleting the diet decreased the relative importance of the caeca expressed as percentage of Gastrointestinal tract, body weight or empty body weight ($P<0.05$). There was an interaction between FP and PS for caeca weight: pelleting of fine size diets decreased the caeca weight and the opposite was observed for 5mm-diets ($P=0.048$). Neither the PS nor the FP caused a significant variation on *C. jejuni* counts or pH at the caeca of the birds. No interactions were observed between PS and FP. It is concluded that pelleting the diet decreased the relative weight of caeca but did not affect the pH or *C. jejuni* counts.

Keywords: *Campylobacter jejuni*, pelleting, particle size, broiler, gastrointestinal tract

Introduction

Campylobacter is the major cause of bacterial gastroenteritis in humans worldwide. In the EU, it is the most commonly reported gastrointestinal (GI) bacterial pathogen since 2005, with 214,779 human cases reported in 2013 (EFSA, 2015). *Campylobacter* preventive measures aim at reducing the probability of *Campylobacter* colonisation in birds (GHAREEB et al., 2013). For this purpose several approaches, as the use of bacteriophages (WAGENAAR et al., 2005), bacteriocins (LINE et al., 2008), organic acids (CHAVEERACH et al., 2004; SKÅNSENG et al., 2010) and their derivatives (HILMARSSON et al., 2006) or medium chain fatty acids (HERMANS et al., 2010) have been proposed over the past few years. However, few efforts have been done on modifying the upper GI tract in order to combat *C. jejuni* colonization in lower GI tract. HUANG et al. (2006) proposed that the gizzard may be a critical control point for reducing *Salmonella* contamination in growing broilers, and MOEN et al. (2012) have recently shown that a stimulated gizzard delays the horizontal spread of *C. jejuni* in broiler flocks. The aim of this work was to investigate the effect of particle size of wheat and feed presentation on *C. jejuni* colonization in broilers.

Material and Methods

A total of 144 Ross 308 broilers (50% male and 50% female) from 1 to 42 days of age were used and allocated to the experimental treatments. There were four treatments factorially arranged with two W screen sizes (2mm vs 5mm) and two FP (mash vs pellets). The trial was carried out in an Animal Biosafety Level 2 Unit for broilers. Broilers were housed in cages in groups of three. Mash (non-pelleted) feeds were fed *ad libitum* with no added coccidiostat, growth promoter or veterinary antibiotics. At 14 d of age, all broilers were orally gavaged with 100 µl of a solution containing 1 x 10^5 CFU/ml of ST-45 *C. jejuni* strain. On days 21, 35 and 42, ceca were collected from 12 birds per treatment and *Campylobacter* counts determined (ISO 10272). Detection limit was 2 logs, and animals below limit of detection were assigned 2 logs for statistical analysis. At 42d, the weight of the gastrointestinal tract (GIT), empty BW (EBW), the weight of caeca and its pH were taken. Data were analysed by GLM procedure as a factorial design with PS and FP as main

effects (SPSS v.19.0). Statistical significance is declared at P ≤ 0.05, with 0.05<P≤0.10 considered as a near-significant trend.

Results

Neither the PS of the cereal nor the FP caused a significant variation on *C. jejuni* counts in the caeca of broilers at any of the periods studied (Figure 1). Also, no interactions were detected between PS and FP (P < 0.05)

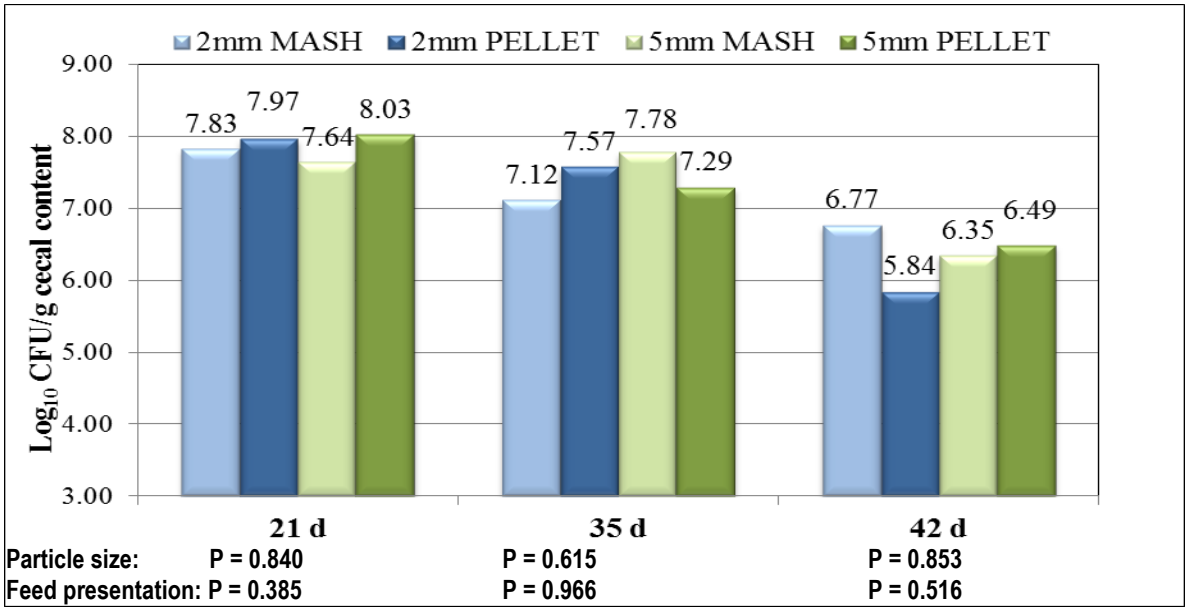

Figure 1. Effect of particle size of cereal (2 vs 5 mm) and feed presentation (mash vs pellet) on *Campylobacter* counts (log_{10} CFU/g) in the caeca of broilers at 21, 35 and 42 days of age.

Particle size did not affect any of the morphological parameters evaluated (Table 1). Pelleting the diet increased GIT weight (214.3 *vs* 178.4 g, for pellets *vs* mash; P = 0.001) and empty BW (2557 *vs* 1986 g, for pellets *vs* mash; P = 0.001). Pelleting also decreased the relative importance of the caeca expressed as percentage of GIT, BW or EBW (P<0.05). There was an interaction between PS and FP for caeca weight: pelleting 2mm diets decreased the caeca weight but the opposite was observed for 5mm-diets (1.17, 1.08, 1.08 and 1.23 g for 2mm mash, 2mm pellet, 5mm mash and 5mm pellet, respectively; P = 0.048). Dietary treatment did not affect pH at the caeca of the birds (P > 0.05).

Conclusions

It is concluded that pelleting the diet increased BW, GIT weight and empty BW of broilers and decreased the relative weight of caeca without affecting the pH or *C. jejuni* counts.

Acknowledgements

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Table 1. Effect of particle size of cereal (2 vs 5 mm) and feed presentation (mash vs pellet) on on caeca morphology and pH

	BW, g	GIT		Empty BW		Caeca				
		g	%BW	g	%BW	g	%GIT	%BW	%EBW	pH
Particle size										
2 mm	2440	191.7	7.91	2249	92.09	1.12	0.60	0.047	0.051	6.67
5 mm	2496	200.9	8.09	2295	91.91	1.15	0.59	0.047	0.051	6.75
Feed presentation										
Mash	2165	178.4	8.27	1986	91.73	1.12	0.64	0.052	0.057	6.78
Pellet	2772	214.3	7.74	2557	92.27	1.15	0.55	0.042	0.045	6.64
SEM (n=24)	59.9	7.13	0.237	56.3	0.237	0.043	0.027	0.002	0.002	0.070
Probability										
Particle size	0.512	0.367	0.607	0.559	0.607	0.591	0.906	0.897	0.884	0.400
Feed presentation	**0.001**	**0.001**	0.121	**0.001**	0.121	0.591	**0.017**	**0.001**	**0.001**	0.188
PS x FP	0.523	0.266	0.329	0.590	0.329	**0.048**	0.409	0.190	0.174	0.205

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P-243 (ID 289)

EFFECT OF PHYSICAL FEED QUALITY AND LOW ENERGY MASH DIET ON THE PERFORMANCE OF BROILER BREEDERS

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Abstract

Feed physical quality, fibre and energy content have been shown to influence eating-up time, gizzard function, feather pecking and plumage condition in laying hens. This experiment was conducted to study the effect of feed physical quality and of a low energy, high fibre diet on the performance and hatchability results of Ross 308 grandparent broiler breeders. Four treatments were given from 22 to 60 weeks of age in 4 replicates (147 females in each). Physical qualities of the diets for the first 3 treatments were crumble (C), coarse mash (CM) and coarse mash with 10% whole oats plus 5% whole wheat (CM15OW). The diets were formulated according to Aviagen recommendations (2013) (11.7 M/kg) The fourth diet was a mash with 20% whole oats and 10% whole wheat (M30OW), contained less energy (10.9 MJ/kg) and more digestible lysine (0.66%) than the other diets. All males (16 per replicate) were fed with CM15OW. Total hen week egg productions were 157, 165, 165.5 and 164.6; cumulative hatch of fertile eggs 80.89, 81.97, 83.17 and 82.43%, and 94, 100, 105 and 102 chicks per hen housed were obtained for C, CM, CM15OW and M30OW, respectively. Feeding breeders a coarse mash diet formulated according to recommendations gave better performance than a diet in crumble form. Whole cereal inclusion might have further improved hatchability and chick output. M30OW did not give additional benefits over CM15OW. Gizzard size was bigger on diets containing whole cereals.

Keywords: broiler breeder, nutrition, feed, physical quality

Introduction

Lifetime uniformity and bodyweight control support expression of the full genetic potential of the modern broiler breeder. In addition to fast feed distribution, slow feed clean-up time may help maintaining flock uniformity. In the trial of Tanaka et al. 1983 (ref. van Krimpen et al. 2005), hens spent more time on feed intake as the particle size of the diet decreased: 100 min per day for pellets, 154 min for crumbles and 234 min for mash. According to Röhe et al. (2014), feeding of coarsely ground mash diets have positive effects on the gastrointestinal function and may lead to improved bird health and welfare. If included at the correct particle size, fibre rich raw materials will provide sufficient structure to stimulate gizzard development which will regulate the flow of nutrients into the small intestine, optimising digestive processes and nutrient utilization (Hetland et al. 2005). This experiment was conducted to study the effect of feed physical quality and of a low energy, high fibre diet on the performance and hatchability results of modern broiler breeders.

Material and Methods

A total of 2352 grandparent Ross 308 female broiler breeders and 256 males were randomly allocated to 4 different experimental groups. Four dietary treatments were given from 22 to 60 weeks of age in 4 replicates (147 females and 16 males in each). Physical quality of the diets for the first 3 treatments were crumble (C), coarse mash (CM) and coarse mash with 10% whole oats plus 5% whole wheat (CM15OW). The diets were formulated according to Aviagen recommendations (2013) with energy content of 11.7 M/kg (WPSA). Digestible amino acids for raw materials were calculated using AminoDat4. The fourth diet was a mash with 20% whole oats and 10% whole wheat (M30OW), and contained less energy (10.9 MJ/kg) and more digestible lysine (0.66%) than the other diets. All males were fed CM15OW. Hammer mill (3000 rpm – 8 mm screen) was used for grinding the raw materials for treatments C and M30OW (excluding whole cereals). For CM corn, wheat and sunflower were roller milled (2.9 and 2.1 mm screen) and all other raw materials hammer milled (1050 rpm – 12 mm). Raw materials for CM15OW were grinded equally to treatment CM (excluding whole cereals). After peak feed the females were fed to equal pre-calculated ME intake and, thus, feed levels (g/d) were predefined according to the estimated ME level of the feed. Because of *Heterakis gallinarum* infection at 39 and 46 weeks of age the birds were treated with anthelminthic. After the second infection the birds lost weight and the feed levels had to be increased for all birds. Body weight (BW) and feed intake levels were recorded weekly until 35

weeks of age, fortnightly in weeks 35 – 45 and weekly again from 45 weeks onwards. Feathering was recorded at week 28, 38 and 48 and feed clean-up time at week 30, 40, 50 and 60 for all pens. Litter moisture and capping were measured at 30, 40, 50 and 60 weeks of age. Every other week eggs collected during one production week were set for hatching. At the end of the trial gizzard weight and thickness were measured of 16 birds per treatment.

Results

Body weight gain (BWG) was higher than expected for all treatments with differences in BWG between the treatments: hens were lightest on the low-energy diet and heaviest on the crumble diet. Egg production was close to the target during the first part of production but reduced as *Heterakis* problems appeared at weeks 39 and 46 (Figure 1). From 48 weeks onwards the production started to recover and outreached the target at 50 – 56 weeks of age. CM15OW and M30OW recovered better than C and CM. Total hen week egg productions were 157, 165, 165.5 and 164.6; cumulative hatch of fertile eggs 80.89, 81.97, 83.17 and 82.43%, and 94, 100, 105 and 102 chicks per hen housed were obtained for C, CM, CM15OW and M30OW, respectively. Gizzard size and thickness increased as the amount of coarse particles or whole cereals increased in the diets. Litter capping and moisture % were significantly lower for CM15OW and M30OW until 45 weeks but later on the differences between the treatments mostly disappeared. Feed clean-up time varied between 195 – 307 minutes. with a lot of variation and inconsistency between the treatments and weeks measured. The highest mortality rate was found on the crumble diet.

Higher than expected BWG indicates the energy content of the diet was underestimated when pre-calculating the feed allocations. The heaviest hens and the poorest egg laying rate were found on the crumbled diet. According to Leeson and Summers (2005), oversupply of nutrients results in increased growth and obesity in broiler breeders and leads to reduced egg production. Increasing gizzard size and thickness as the amount of coarse particles or whole cereals increased in the diets, is in accordance with the findings of Hetland et al. (2005). The egg production of the hens fed coarse particles and/or whole cereals recovered better after the *Heterakis*-problem than the birds fed the crumbled diet. Whether the well-developed gizzard plays a role is unclear. The differences in plumage condition were negligible and in practise no feather pecking appeared. Better cumulative hatchability with whole cereal treatments and lower mortality rates on diets containing coarse particles might be linked to better gut health and bird welfare but more research is needed on this field.

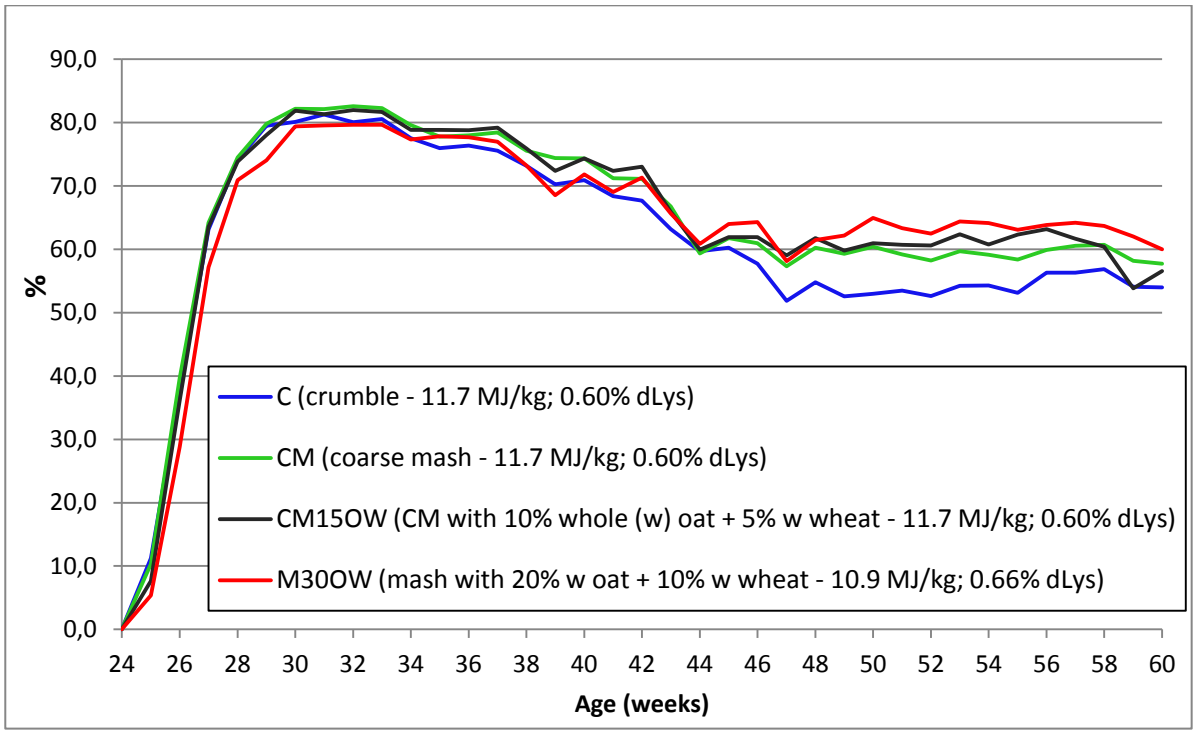

Figure 1. Hen week egg production %.

Conclusions

Feeding breeders a coarse mash diet formulated to current recommendations gives better breeder performance than a diet in crumbled form. Whole cereal inclusion may further improve the hatchability results and chick output.

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P-247 (ID 210)

DOES CAGE EFFECT REDUCTION THROUGH XYLANASE HAVE AN IMPORTANT EFFECT ON INTESTINAL MICROBIOTA?

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Abstract

Reduction of the cage effect (release of encapsulated nutrients) is one of the working mechanisms of xylanase, resulting in altered substrate for gut microbial fermentation. However, the true mechanism by which gut microbiota nutritionally are affected is not fully understood. Therefore, 720 1-d-old broiler chicks were randomly allocated to 24 pens. A corn-based diet was chosen to rule out the enzyme's effect on digesta viscosity. The corn was first frozen (5 days at -18°C) or not to separate the cage effect. Both diets were mixed with or without bacterial endo-1,4-β-xylanase. At day 34, caecum contents were collected for microbiota analysis (qPCR) and fresh digesta were taken for viscosity measurements. As expected, viscosity was not affected by enzyme or freezing. The bacterial xylanase significantly increased the numbers of Clostridium cluster IV (butyrate producers), total bacteria and the number of butyryl CoA-acetate CoA-transferase gene copies. Freezing corn resulted in increased total bacteria numbers and a tendency to increased Clostridium cluster IV. The results suggest the bacterial xylanase tested likely affected the gut microbial profile in 2 ways: 1) supply of fermentable arabinoxylan fragments and 2) changing digesta composition as substrate for the gut microbiota.

Keywords: cage effect, xylanase, frozen corn, gut microbiota.

Introduction

The use of xylanase is almost ubiquitous in poultry feeds. Nevertheless, the majority of research conducted with these enzymes has focused on determining their effects on animal performance and nutrient digestibility, and less on their effects on the intestinal microbiota.

Two major mechanisms have been proposed to explain the beneficial effects of xylanase on intestinal microbiota: 1) altering nutrient delivery to the host and gut microbiota 2) provision of fermentable oligosaccharides (Bedford and Cowieson, 2012). It is not clear, however the extent to which these mechanisms contribute to the beneficial effect of xylanase on gut microbiota. The aim of the present study was to assess how xylanase could affect intestinal microbiota in broilers.

Material and Methods

The experimental design was approved by the local Ethics Committee for Animal Experiments at the ILVO. A total of 720 male broiler chickens (Ross 308 Commercial strain) were purchased from a commercial hatchery (Belgabroed, Merksplas, Belgium) and were allocated at random in equal numbers of 24 floor pens (thirty chickens per pen) in an environmentally-controlled poultry house. Chickens received corn-soy based diets.

Half of the corn was frozen at -18 °C for 5 days, and milled after thawing. The experiment consisted of 4 dietary treatments 1) normal corn without bacterial endo-1,4-β-xylanase; 2) frozen corn without bacterial endo-1,4-β-xylanase ; 3) normal corn with bacterial endo-1,4-β-xylanase (Nutrase Xyla®, Nutrex, Lille, Belgium) and 4) frozen corn with bacterial endo-1,4-β-xylanase. At day 34, caecum contents were collected for microbiota analysis (qPCR) and fresh digesta were taken for viscosity. Caecum contents of the chickens were placed into a plastic jar and frozen at -80°C until analysis. To extract the DNA from the caecum content (100 mg), the CTAB method was used (Griffiths et al., 2000, Kowalchuk et al., 1998). The concentration of the DNA was measured using the Nano drop ND 1000 spectrophotometer (Nanodrop

Technologies, Wilmingtom, DE, USA) and diluted to a concentration of 50ng/µl to test the quality of the DNA. Primers used for quantification of the different bacterial groups and genes are mentioned in Table 1.

Table 1. Primer information for quantitative RT-PCR assay

Bacterial groups/ Functional gene	Primers (5'-3')	Reference
Enterobacteriaceae	F: CATTGACGTTACCCGCAGAAGAAGC R: CTCTACGAGACTCAAGCTTGC	Bartosch et al 2004
Clostridium Cluster I	F: TACCHRAGGAGGAAGCCA R: GTTCTTCCTAATCTCTACGCAT	Song et al 2004
Clostridium Cluster IV	F: GCACAAGCAGTGGAGT R: CTTCCTCCGTTTTGTCAA	Matsuki et al 2004
Clostridium Cluster XIV	F: GAWGAAGTATYTCGGTATGT R: CTACGCWCCCTTTACAC	Song et al 2004
Lactobacillus	F: GGAATCTTCCACAATGGACG R: CGCTTTACGCCCAATAAATCCGG	Abdulamir et al 2010
Total Bacteria	F: CGGYCCAGACTCCTACGGG R: TTACCGCGGCTGCTGGCA	Hopkins et al 2005
Butyryl-CoA:Acetate-CoA transferse	F: GCIGAICATTTCACITGGAAYWSITGGCAYATG R: CCTGCCTTTGCAATRTCIACRAANGC	Louis and Flint 2007

[1]F: Forward; [2]R: Reverse

Amplifications and detections were performed according to Eeckhaut et al. (2011). Viscosity measurements were done as described by (Bedford and classen 1992) The main effects of freezing, enzyme and their interaction were statistically analyzed by 2x2 factorial model.

Results and Discussion

Viscosity was not affected by xylanase addition or freezing. Xylanase addition significantly increased the number of gene copies of butyryl CoA:acetate CoA-transferase, the numbers of Clostridium cluster IV and of total bacteria in the caecum (Table 2). Freezing increased numbers of total bacteria and of Clostridium cluster IV, although for the latter it was only a trend. Numbers of Enterobacteriaceae, Clostridium cluster XIV and Lactobacillus were not affected by freezing or by xylanase addition. No interactions of freezing with xylanase were observed for any of the bacterial groups assessed or for the number of gene copies of butyryl CoA:acetate CoA-transferase. Releasing of dietary encapsulated nutrients will affect the substrate available for gut microbial fermentation. Xylanase tested affected the gut microbial profile most likely by the combination of 1) provision of fermentable arabinoxylan fragments (originating from the broken cell walls) and of 2) a change in digesta composition for gut microbiota fermentation (from the release of the entrapped nutrients).

Table 2. Effect of freezing of corn (F), xylanase addition (X) and their interaction (F*X) on colony forming units of bacterial species and enzyme (log CFU·g^{-1}) per 100 mg of caecal contents in broiler chickens

	Dietary treatment(n=6)							
	Without xylanase		With xylanase			P-value		
	Normal corn	Frozen corn	Normal corn	Frozen corn	SEM[1]	F	X	F*X
Butyryl CoA:acetate CoA-transferase	8.01	8.25	8.32	8.34	0.04	0.275	0.016	0.155
Clostridium cluster I	8.29	8.38	8.44	8.55	0.04	0.184	0.072	0.727
Clostridium cluster IV	9.39	9.57	9.74	10.03	0.06	0.056	0.001	0.742
Clostridium cluster XIV	8.42	8.36	8.38	8.54	0.03	0.381	0.326	0.105
Lactobacillus	8.03	8.29	8.09	8.10	0.06	0.266	0.541	0.282
Enterobactericeae	6.83	6.42	6.83	6.85	0.09	0.410	0.400	0.247
Total bacteria	10.55	10.74	10.75	11.04	0.04	0.012	0.007	0.299

[1]Standard Error of Mean.

Conclusions

The changes observed for the numbers of Clostridium cluster IV (where butyrate producers are present), total bacteria and butyryl CoA-acetate CoA-transferase gene copies indicate that xylanase supplementation was able to improve the intestinal microbiota and gut health in broilers fed with a corn–soy based diet. This could partly be attributed to cage effect reduction.

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P-248 (ID 155)

PERFORMANCE AND INCIDENCE OF TIBIAL DISCHONDROPLASIA IN BROILERS SUPPLEMENTED WITH 25-HYDROXY-CHOLECALCIFEROL IN DIET

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Abstract

Vitamin D3 and it metabolites could reduce the occurrence of Tibial Dyschondroplasia (TD) in broilers. Then, we evaluated the incidence of TD in broilers supplemented with vitamin D3 or 25-hydroxy-cholecalciferol (25OHD3) in diets. The experiment was conducted in the Federal University of Goias, Goiania, Brazil, with 405 Cobb 500 broilers. Treatments were: T1 – diets with 4.000 UI/kg vitamin D3; T2 – diets with 25OHD3; T3 – diets with 50% of each vitamin D3 source; with five replicates of 27 birds each. Birds and diets were weighed at 1st, 7th, 14th, 21st, 35th and 42th days of age to measure performance variables. Incidence of TD was measured weekly as the growing area of the bone with a digital caliper. The scores used were: 0 = normal growing area; 1 = +/- 3mm; 2 = +/- 6mm; 3 = above 6mm. At the same ages, a histopathological study was done and the scores used were: 0 = normal; 1 = pre-hypertrophic chondrocytes; 2 = changes in chondrocytes cytoplasm and nucleus and divided in three levels (discrete, moderate and severe). These data were submitted to Kruskall Wallis test and performance was analyzed as ANOVA with "t" test (5%). No effect was observed in performance. No effect was observed in macroscopic evaluation of lesion score, that was characterized as score 0 in all treatments. In microscopic evaluation, 20% of tibias presented TD lesions at 28 days of age in all treatments. No effects of dietetic vitamin D3 sources alone or combined were observed in performance and TD lesions.

Keywords: feed conversion, feed intake, leg problems, 25OHD3, weight gain

Introduction

Tibial dyschondroplasia (TD) is one of the most worrisome skeletal diseases, and a significant indicator in terms of animal welfare. To assist TD measurement, lesions can be monitored using various techniques. However, there is little data on the damage caused by TD, due to a scarcity of identification and quantification of these skeletal diseases in the industries. The high percentage of bone tissue deformities represents one of the major problems facing the poultry industry, especially on account of the intense genetic selection performed to increase the growth rate of animals.

In the animal production field, poultry breeding has seen the biggest genetic and technological advances. The rate of TD has increased significantly and is related to the pace of growth and the maximization of muscle mass in a short period of time. In recent years, vitamin D3 and its metabolites 1,25(OH)2D3 and 25(OH)D3 have been studied in the attempt to minimize problems caused by skeletal abnormalities. The purpose of this study was to evaluate the rate of tibial dyschondroplasia in chickens supplemented with 25-hydroxycholecalciferol in feed.

Material and Methods

The experiment was conducted in the experimental breeding house at the Animal Production Department of the Veterinary and Animal Science School (Escola de Veterinária e Zootecnia - EVZ) of the Federal University of Goiás – UFG in Goiânia, Goiás, Brazil. The experimental protocol used in this study was approved by the Research Ethics Committee of UFG, under protocol no. 014/12.

To conduct the experiment, 405 one-day-old male chicks of the Cobb 500® breed were housed, from a single matrix lot (45 weeks old). The eggs were incubated under the same conditions, in a multiple stage machine. After hatching, the one-day-old chicks were standardized by weight and housed in three different groups of 135 birds, with five repetitions of 27 birds per box, reaching 15 experimental units, in which an entirely random outline was adopted. The experiment consisted of three treatments, from two sources of vitamin D supplementation (vitamin D3 and 25(OH)D3) described below: Treatments: T1: Control = the control group received the experimental diet containing vitamin D3 at a dose of

4,000,000 U.I./kg; T2: 25(OH)D3 = received no vitamin D3 and its equivalence was replaced by including the metabolite 25(OH)D3 in the experimental diet; T3: D3 + 25(OH)D3 = consisted of the combination of two sources of vitamin D, where the proportion in feed was 50% from each source used.

The birds and diets were weight on the 1st, 7th, 14th, 21st, 35th and 42nd days of age, and these data were used to calculate weight gain, feed consumption, feed conversion and viability for study of the performance variables during breeding phases. Each week the number of birds was reduced due to weekly collections of one bird per box for necropsies and laboratory analyses. One bird with identification per box was removed weekly, weighed and sacrificed by cervical dislocation, proceeding to remove the legs (leg + thigh + foot). The macroscopic evaluations of the birds' tibia were performed at 7, 14, 21, 28 and 35 days of age.

The rate of TD was assessed weekly by macroscopic viewing of the growth plate of the proximal portion of the right tibia, cutting the bone lengthwise. A digital caliper was used to measure the thickness of the growth plate. The methodology adopted was that described by TAKITA (1998) and presented by GONZALES & MACARI (2000), which stipulates the following scores: Score 0 = normal growth plate; Score 1 = growth plate with +/- 3mm thickening; Score 2 = growth plate with +/- 6mm thickening; Score 3 = growth plate with thickening above 6mm. To verify possible TD alterations during macroscopic evaluations of the right tibia, a histopathological study of the growth plates of the proximal portion of the marked birds' tibia was performed at 1, 7, 14, 21, 28 and 35 days of age. The methodology adopted was that described by TAKITA (1998), which histologically characterizes TD severity according to the following scores: Score 0 = normal growth plate; Score 1 = growth plate with a wide and distinct band of pre-hypertrophic chondrocytes; Score 2 = growth plates presenting degenerative changes in the cytoplasm and nucleus of chondrocytes, which in this study was subdivided into three levels (discrete, moderate and severe).

The data from the macroscopic and histological evaluation of the tibia growth plate were subjected to non-parametric statistical analysis, using the Kruskall Wallis test of the R (2.14.2) software statistical program. For statistical evaluation of performance, the data were subjected to analysis of variance, ANOVA, using the 5% significance level.

Results and Discussion

There was no effect (p>0.05) for initial weight, final weight, average weight gain, average feed consumption, mortality and feed conversion (Table 1).

The macroscopic lesion scores from the proximal portion of the tibia bone, assessed at 7, 14, 21, 28 and 35 days old, presented no effect (p>0.05) from the different sources of vitamin D3 (Table 2). Upon macroscopically evaluating the growth plate thickening of the proximal portion of the tibia, it was observed that both treatments rated in the score (0) classification = normal TD growth plate.

Table 1 Performance of chickens at 42 days of age, supplemented with different sources and combinations of vitamin D in feed

	AIW, g[1]	AFW, g	AWG, g	AFC, g	FC	Mort, %
D_3	45.92	3005	3003	5517	1.8358	4.5
$25(OH)D_3$	46.14	2997	2994	5439	1.8148	6.3
$D_3 + 25(OH)D_3$	46.17	3050	3048	5626	1.8448	4.5
P	****	0.14	0.14	0.37	0.74	
C.V%	1.58	1.40	1.39	3.65	3.42	-

[1] Upon housing, average initial weight AIW; average final weight AFW; average weight gain AWG; average feed consumption AFC; feed conversion FC; mortality Mort. NS effect = not significant (p>0.05).

Table 2 Average of macroscopic lesion scores from the proximal portion of the tibia bone, at 7, 14, 21, 28 and 35 days old, fed with different sources and combinations of vitamin D3

Macroscopic lesion score	Treatments			P *
	D_3	$25(OH)D_3$	$D3 + 25(OH)D_3$	
7 days	0.2	0.0	0.6	0.1078
14 days	0.4	0.6	0.2	0.4594
21 days	0.6	0.6	0.4	0.7788
28 days	0.8	0.6	0.2	0.1737
35 days	0.2	0.0	0.2	0.5836

* Not significant difference for Kruskal-Wallis test (p>0.05).

When comparing the macroscopic results of lesion scores with the histopathological analyses, the non-existence of TD was confirmed. It was confirmed that there was no effect (p>0.05) from the different sources and combinations of vitamin D3 used in this experiment (Table 3). In the results, higher rates for TD were not found in either of the treatments (Table 4). Slight TD rates were noted, regardless of the treatment used, but without significant effect.

ATENCIO et al. (2005) concluded, in experiments conducted with reproducers, that the effect of the metabolite 25(OH)D3 in relation to vitamin D3 depends on the levels tested, and when compared to the sources of vitamin D3, the effect of the metabolite 25(OH)D3 was only higher when supplemented at low levels. According to BRITO (2008), the higher effectiveness of the metabolite 25(OH)D3 in reducing bone problems (reduction in rate) may be one of the explanations for improvement in the uniformity of lots reported in the field, and in the reduction in condemnations in slaughter. The results demonstrated that the combined use of the two sources, at the lower level of 25(OH)D3 supplementation in feed, generated similar values in rate of TD, when compared to the growing levels of 25(OH)D3 along, confirming the benefits of the more active metabolite as a source of vitamin D.

Table 3 Histological lesions score for tibial dyschondroplasia (TD) in the proximal portion of the growth plate, at 21, 28 and 35 days old, fed with different sources and combinations of vitamin D in feed

TD microscopic lesion score	Treatments			P *
	D_3	25(OH)D_3	D3 + 25(OH)D_3	
21 days	0.0	0.2	0.2	0.5836
28 days	0.0	0.0	0.2	0.3678
35 days	0.2	0.0	0.0	0.3678

* Not significant difference for Kruskal-Wallis test (p<0.05).

Table 4 Frequency and severity of tibial dyschondroplasia (TD) in the proximal portion growth plate, at 21, 28 and 35 days old, fed with different sources and combinations of vitamin D in feed

Age (days)	Treatments	TD (%)	Degree of lesion (Score)				
			0	1	2	3	4
21	D_3	0	5/5	0/5	0/5	0/5	0/5
	25(OH)D_3	20	4/5	1/5	0/5	0/5	0/5
	D_3 + 25(OH)D_3	20	4/5	1/5	0/5	0/5	0/5
28	D_3	0	5/5	0/5	0/5	0/5	0/5
	25(OH)D_3	0	5/5	0/5	0/5	0/5	0/5
	D_3 + 25(OH)D_3	20	4/5	1/5	0/5	0/5	0/5
35	D_3	20	4/5	1/5	0/5	0/5	0/5
	25(OH)D_3	0	5/5	0/5	0/5	0/5	0/5
	D_3 + 25(OH)D_3	0	5/5	0/5	0/5	0/5	0/5

Conclusions

The combined use of two sources of vitamin D supplementation (vitamin D3 and 25(OH)D3) did not alter the performance of chickens and did not aggravate tibial dyschondroplasia problems. It can thus contribute as a supplement source to minimize the effects of bone problem rates and condemnations in slaughter.

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P-249 (ID 189)

EMULSIFIER INFLUENCING FAT AND GROSS ENERGY DIGESTIBILITY OF BROILERS FED DIETS CONTAINING FAT POWDER

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Abstract

Dietary emulsifiers can be used to enhance fat digestibility, and thus increase the energy efficiency. This can be profitable for young birds having only a limited natural fat digestibility, and for lowering the energy content of the diets resulting in a lower feed cost. The objective of the current digestibility trial was to evaluate the effect of an emulsifier (0.01%) – with a hydrophilic-lipophilic balance (HLB) of around 17-18 – on the fat digestibility coefficient (DC, via total fecal collection method) of fast growing male broilers (age of 18-22 days) fed two types of diets. Diet A had a high free fatty acid (FFA) content (20.0%) and an unsaturated/saturated FA ratio (U/S) of 1.60; while diet B had a FFA of 10.0% and a U/S of 2.00. However, both diets had the same ME (12.27 MJ/kg) and crude fat (8.5%) content. Soy oil and two types of fat powder were used as lipid sources in both diets. The emulsifier was added on top of half of each diet. As expected, the dietary fat and gross energy (GE) DC were significantly lower when diet A (with and without emulsifier) was fed (67.0% vs 73.9%, and 71.9% vs 73.8%, resp.). The emulsifier improved dietary fat digestibility with 2.8% (trend) and 4.2% (significant) for diet A and B, resp. The energy efficiency (MEn) was improved with 214 kJ (trend) and 292 kJ (significant) for diet A and B, resp. It can be concluded that the energy content and thus the feed cost can be lowered when the emulsifier is added, even when a fat powder is used.

Keywords: emulsifier, fat powder, U/S ratio, free fatty acid, broiler

Introduction

Mixing an emulsifier into the diet reduces the size of the fat globules and increases the total surface available for enzymatic digestion (Jones *et al.*, 1992). As a result, dietary emulsifiers can be used to enhance fat digestibility, and thus increase the energy efficiency. This can be profitable for young birds having only a limited natural fat digestibility (Al-Marzooqi and Leeson, 1999), and for lowering the energy content of the diets resulting in a lower feed cost.

The objective of the trial was to evaluate the effect of the emulsifier tested on the digestibility of feed containing fat powder.

Material and Methods

Day-old male broilers (Ross 308) were housed on deep litter under conventional conditions for lighting, heating and ventilation. They were fed *ad libitum* the same standard starter with animal fat as main fat source.

From day 13 to 22, the birds were brought to the digestibility unit and placed in the balance cages. The balance cages consist of a wire bottom (with below the bottom a plastic tray for excreta collection), a feed trough in front, and a drinking cup at the back side. Groups of 5 birds (at 13 days of age) were assigned randomly to each of the 24 digestibility cages and received from than off their experimental diet. At day 18 an additional selection was done in order to have 4 comparable and healthy birds. During the main period (18-22 days of age) the birds were fed at a nearly *ad libitum* level, while water was freely available. The balance trial was further carried out according to the EU-reference method (Bourdillon *et al.,* 1990).

The experiment consisted of four treatments, each with six repetitions:

- T1: grower diet A
- T2: T-1 + Emulsifier (0.01% ; on top)
- T3: grower diet B
- T4: T-3 + Emulsifier (0.01% ; on top)

The grower diets used for the balance trial were wheat-soy-corn based. The two grower diets differed in FFA content and U/S ratio (Table 1). This was achieved by using different concentrations of soy oil and two types of fat powder as lipid sources in both diets.

Table 1. Calculated nutrient content of the grower diets

	FFA (%)	U/S ratio	Crude fat (%)	ME (MJ/kg)
Grower diet A	20.0	1.60	8.5	12.27
Grower diet B	10.0	2.00	8.5	12.27

The two grower diets were prepared and divided in two equal parts. Using a small mixer, the emulsifier was added to produce the four experimental diets. The dietary levels of all nutrients were in agreement with the requirements of broilers following the AVIAGEN requirements.

Samples of feeds and freeze-dried excreta were analysed in the ILVO accredited laboratory for gross energy (GE; adiabatic calorimetry), nitrogen (macro-Kjeldahl; protein = N x 6.25) and fat (ether extract). The MEn-contents of the experimental diets were calculated from their respective "freeze-dried" excreta/feed (E/F) ratios as well as their corresponding GE-contents, followed by a correction for N-retention to zero, by using an energy equivalent of 34.36 kJ/g N-retained. The retention of fat was calculated from the E/F-ratio as well as the corresponding fat contents in the feed and the excreta.

All digestibility parameters were analysed by a linear mixed model with diet type and addition of the emulsifier as fixed factors (Statistica 12.5). Data that differ significantly have a P-value below 0.05.

Results

The analysed dietary crude fat of the grower diets A and B was somewhat higher than the calculated content, and amounted to 9.24% and 8.92%, respectively.

No significant effect on feed intake was found. However, the birds treated with the emulsifier produced a significantly higher (17%) amount of excreta (fresh).

The E/F ratio was significantly influenced by the diet and the addition of the emulsifier: diet B had lower ratios compared to diet A and the addition of the emulsifier lowered the ratio in comparison to when no emulsifier was added (Table 2). As expected, diet B had a significantly higher fat and GE digestibility compared to diet A (with and without emulsifier) (67.0% vs 73.9%, and 71.9% vs 73.8%, resp.), because diets with a low U/S ratio and a high FFA content result in lower fat digestibility. Adding the emulsifier also significantly improved the fat and GE digestibility. Roy *et al.* (2010) also found that the use of a dietary emulsifier proved to be a tool to improve the utilization of fats in birds. Dietary fat digestibility was improved by the emulsifier with 2.8% (trend) and 4.2% (significant) for diet A and B, resp. The energy efficiency (MEn) was improved with 214 kJ (trend) and 292 kJ (significant) for diet A and B, resp.

Table 2. Excreta/feed (E/F) ratio, digestibility data and energy content of the diets.

	Treatment (mean ± SD*)				P	
	T1	T2	T3	T4		
Grower diet	A (FFA:20.0%; US:1.60)	A (FFA:20.0%; US:1.60)	B (FFA:10.0%; US:2.00)	B (FFA:10.0%; US:2.00)	Diet effect	Emulsifier effect
Emulsifier	-	+	-	+		
E/F ratio	0.301 ±0.011	0.292 ±0.005	0.288 ±0.010	0.273 ±0.009	<0.001	<0.01
Fat digestibility (%)	65.5 ±3.0	68.3 ±1.6	71.9 ±1.4	76.0 ±1.3	<0.001	<0.001
GE digestibility (%)	71.3 ±1.2	72.4 ±0.6	73.1 ±1.1	74.6 ±0.8	<0.001	<0.01
ME (kJ/kg DM)	14,166 ±239	14,381 ±127	14,375 ±215	14,672 ±167	<0.01	<0.01
N retention (kJ/kg)	830 ±14	830 ±10	803 ±19	808 ±17	<0.001	NS§
MEn (kJ/kg DM)	13,326 ±226	13,540 ±118	13,562 ±198	13,853 ±154	<0.01	<0.01

*SD: Standard Deviation, §NS: not significant

Conclusions

It can be concluded that the energy content and thus the feed cost can be lowered when the emulsifier is added, even when a fat powder is used and at different U/S ratios and FFA contents.

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THE EFFECT OF INCREASING METABOLIZABLE ENERGY, LEVEL AND DURATION OF FEED RESTRICTION ON PERFORMANCE AND BLOOD PARAMETERS IN BROILER CHICKENS

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Abstract

The effect of increasing metabolizable energy (ME), level and duration of feed restriction on performance and serum metabolites was studied in broiler chickens fed pellet diets. A total of 480 Ross 308 male broiler chickens were randomly allocated to 12 treatments, each one consisted of 4 replicates, and 10 birds per replicate in 3×2×2 factorial arrangement. Experimental treatments consisted two levels of metabolizable energy (normal, and +100kcal/kg), two levels of feed restriction (90% and 85% restricted feed than recommended of Ross 308 management guide), and three periods of feed restriction duration including 14, 21 and 28 days. Results indicated that BW significantly increased in broilers fed high-ME diets and 90% feed restriction (P<0.01), except that, high-ME diets decreased BW at 7 days of age. FCR in High-ME diets significantly increased at 7 days of age, but decreased at 14 and 21 days of age (P<0.05). Broilers with 90% feed restriction and high-ME diets had lowest glucose concentration at 21 days, but interestingly, had highest glucose concentration at 35 days of age (P<0.01). Increasing ME level, elevated serum TG concentration at 42d of age (P<0.01). But increasing level and duration of feed restriction decreased TG level (P<0.05). In conclusion, energy level, duration and level of feed restriction in pellet diets affected performance and economical outcome of broiler farms, and need more researches to prepare relevant and practical program.

Keywords: Metabolizable energy, Pellet, Feed restriction, Broiler

Introduction

Feeding strategies including pelleting and increasing metabolizable energy in broiler chickens resulted in higher growth performance and improving feed conversion ratio, while, increasing occurrence of metabolic disorders, fat deposition and mortality. One possible nutritional approach to reduce feed cost, improvement FCR and decreasing the occurrence of metabolic disorders and mortality is to restrict feed intake of the birds. Improvement in FCR and decrease in metabolic disorders noted with the use of feed restriction programs is due to reduced overall maintenance requirements and basal metabolic rate in feed-restricted birds linked with a smaller body weight during early growth (Rincon and Lesson, 2002). Therefore, the main objective of this study was to determine the suitable levels and timing of feed restriction regimes and level of metabolizable energy in pellet diet on productivity and blood parameters in broiler chickens.

Material and Methods

The research protocol was approved by the Razi university animal care and use committee. A total of 480 one-d-old Ross 308 male broilers were placed in 48 individual cages and 12 treatments, arranged factorially including: 2 levels of ME (normal, and normal+100 kcal.kg^{-1}), 2 levels of restriction intensity (90% and 85% restricted feed than recommended of Ross 308 management guide), and 3 periods of restriction duration (8-21, 8-28 and 8-35d of age). Each treatment replicated 4 times. Body weight and feed intake were recorded weekly. At 21, 28, 35 and 42d of age one chick per each replicate were randomly selected and bled for GLU, TG and T_3 serum analysis. Data analyzed using GLM procedure of SAS software (2011).

Results

No interaction between ME content of the diet and level and duration of feed restriction (FR) were observed for BW and FCR (Table 1). Increasing ME level of diet significantly improved BW and FCR from 8 to 21 and 1 to 42d of age (P<0.01), but interestingly, except for first week that decreased BW and elevated FCR (P<0.05). level of FR had no significant effect on BW and FCR at 8 to 21 and 22 to 42d of age but from 1 to 42d of age, high-restriction level (85%) decreased BW (P<0.01). The present study indicates that chickens subjected to 85% FR for complete recovery of BW needed more time rather than 90% FR (Lippenz et al., 2000). Applying feed restriction during ages 8-35 days significantly affected performance and decreased BW (P<0.01), also increased FCR (P<0.05) at 22-42 and 1-42 days of age.

Table 1. Influence of metabolizable energy, level and duration of feed restriction on performance of broilers

variable	FCR				BW (g)			
	1-7 d	8-21d	22-42d	1-42d	1-7 d	8-21d	22-42d	1-42d
ME (kcal/kg)	*	**	NS	**	*	**	NS	**
Norm	1.03±0.05[b]	1.53± 0.04[a]	1.85±0.05	1.73±0.04[a]	145.4±7.1[a]	565.4± 24.0[b]	1797.4±66.1	2508.3±75.3[b]
High	1.07±0.05[a]	1.40±0.04[b]	1.82±0.08	1.68±0.06[b]	141.0±6.1[b]	618.7±26.5[a]	1827.1±103.4	2586.2±119.0[a]
Level of FR (%)	NS	NS	NS	NS	NS	NS	NS	**
90	1.05±0.05	1.46±0.07	1.84±0.08	1.70±0.06	143.9±7.5	611.8±30.7	1826.6±91.1	2582.3±106.1[a]
85	1.06±0.05	1.47±0.08	1.83±0.06	1.70±0.05	142.5±6.3	571.7±30.7	1797.9±82.5	2512.1±95.9[b]
Duration of FR (age)	NS	NS	*	*	NS	NS	**	**
8 -21	1.04±0.03	1.46±0.07	1.80±0.07[b]	1.68±0.05[b]	143.9±4.6	592.7±35.6	1862.7±67.6[a]	2599.3±84.8[a]
8 -28	1.05±0.05	1.46±0.08	1.84±0.08[ab]	1.71±0.07[ab]	142.6±6.6	595.4±39.4	1837.3±89.0[a]	2575.3±114.6[a]
8 -35	1.05±0.07	1.48±0.08	1.86±0.05[a]	1.72±0.04[a]	143.1±9.2	587.2±36.5	1736.8±43.0[b]	2467.1±66.1[b]
SEM	0.01	0.01	0.01	0.01	0.10	5.3	12.6	15.3

[a–b] Means within columns with different superscripts are significantly different ($P < 0.05$).
No interaction were observed for BW and FCR. *significant at P ≤0.05. **significant at P ≤0.01. NS: not significant.

Table 2 presents mean glucoce (GLU), triglyceride (TG) and triidothyronine (T_3) concentrations in serum of broilers. Results showed that no significant difference was detected for the concentration of T_3 and TG between treatments at 21, 28 and 35d of age and also, concentration of T3 and GLU at 42d of age. Serum concentrations of TG was significantly higher in norm-ME group rather than high-ME group at 42d of age (P<0.01). Also, interaction between level of FR and duration of FR were observed for TG content at 42d of age (P<0.05). Content of TG in birds that received 90% FR was more than 85% FR at 14 and 28d of FR, but at 21d of FR was least in 90% FR. In total, TG content in birds that received FR regimens with 85% for 28 days was lower content. GLU concentration at 28d of age was higher in treatments with high-ME value (P<0.01). As shown in Table 3, interaction between ME of diet and level of FR were observed for GLU content at 21d and 35d of age (P<0.01). Increasing of ME in treatments with 90% FR decreased GLU content at 21d of age. In contrast at 35d of age, increase in ME enhanced GLU content in broilers with 85% or 90% FR (Table 4). In this experiment, elevating ME level increased TG content at 42d of age while did not influenced GLU and T_3 content. Also, in treatments with 14 and 28d of FR duration, GLU content was increased with increase in IFR (85%). Presumably, increase in ME enhanced growth rate and decreased adiponectine hormone secretion, stimulation of fatty acids oxidation, reduction content of TG and improved GLU metabolism (Zhu et al., 2004; Maddineni et al., 2005).

Conclusions

According to the results, elevating energy content in the first week of age, reduced BW, while improved BW and FCR during 2 to 6 w of age. It seems, higher ME content and applying feed restriction in 90% of ross recomendation during 8-28 days (3 week) provide better productivity of birds fed pellet diet. It is recommended that in future trials, measured the occurrence of metabolic disorders that common in pellet diet especially in areas with high altitude.

Table 2. Effect of dietary metabolizable energy, level and duration of feed restriction on serum metabolites in broiler chickens

Variable	D 21			D 28			D 35			D 42		
	TG (mg/dl)	**GLU** (mg/dl)	**T_3** (ng/ml)	TG (mg/dl)	**GLU** (mg/dl)	**T_3** (ng/ml)	TG (mg/dl)	**GLU** (mg/dl)	**T_3** (ng/ml)	TG (mg/dl)	**GLU** (mg/dl)	**T_3** (ng/ml)
ME (kcal/kg)	NS	NS	NS	NS	**	NS	NS	**	NS	**	NS	NS
Norm	124.2±43.6	226.1±27.0	1.55±0.05	220.2±59.9	201.1±45.1[b]	1.57±0.15	187.0±99.1	212.5±32.1	1.52±0.10	209.1±61.7[a]	226.2±37/5	1.51±0.06
High	131.5±89.8	220.6±30. 0	1.52±0.15	182.1±13.7	250.2±18.6[a]	1.54±0.08	165.1±18.0	263.2±13.9	1.55±0.11	137.7±62.6[b]	236.8±22.8	1.54±0.09
Level of FR (%)	NS	NS	NS	NS	NS	NS	NS	**	NS	*	NS	NS
90	145.4±87.2	220.5±30.0	1.55±0.05	242.4±24.5	212.3±37.1	1.58±0.15	132.3±49.4	254.8±17.2	1.52±0.11	152.3±66.9	227.7±34.5	1.51±0.08
85	107.2±47.7	226.1±27.0	1.52±0.15	258.0±6.8	239.1±44.3	1.53±0.08	107.2±47.7	226.1±27.0	1.48±0.06	192.2±71.2	235.4±27.6	1.53±0.07
Duration of FR (age)										NS	NS	NS
8-21	-	-	-	-	-	-	-	-	-	163.6±81.3	227.4±29.6	1.55±0.09
8-28	-	-	-	-	-	-	-	-	-	162.9±75.6	238.2±26.4	1.51±0.08
8-35	-	-	-	-	-	-	-	-	-	192.8±53.4	229.3±37.4	1.51±0.05
SEM	17.1738	6.923	0.0311	23.2465	10.4656	0.033	17.6419	8.853	0.0302	10.402	4.5873	0.0128
P-Values												
E×I	NS	**	NS	NS	NS	NS	NS	**	NS	NS	NS	NS
E×D										NS	NS	NS
I×D										*	NS	NS
E×I×D										NS	NS	NS

[a–b] Means within columns with different superscripts are significantly different ($P < 0.05$).
* significant at P ≤0.05. ** significant at P ≤0.01. NS: not significant.

Table 3. Interaction of ME, level and duration of FR on TG content (mg/dl) at 42d of age

Level of FR (%)	Duration (age)		
	8-21d	**8-28d**	**8-35**
90	210.1±69.8[a]	162.5±75.1[ab]	182.5±64.7[a]
85	164.7±92.4[ab]	201.7±43.8[a]	115.6±47.9[b]

[a–b]Means within rows and columns with different superscripts are significantly different ($P < 0.05$).

Table 4. Interaction of ME and level of FR on GLU content (mg/dl) at 21d and 35d of age

ME	D 21		D 35	
	Level of FR (%)			
(Kcal/kg)	90	85	90	85
Norm	241.1±1.7[a]	211.0±33.0[ab]	240.3±6.7[b]	184.8±17.7[c]
High	199.9±31.0[b]	241.2±1.6[a]	269.4±8.8[a]	256.9±16.3[ab]

[a–c] Means within rows and columns with different superscripts are significantly different ($P < 0.05$).

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SIMULATION AND EXPERIMENTAL VALIDATION TO PREDICT FEED INTAKE IN PRE-LAYING PHASE

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Abstract

The objective of this study was to make a simulation using mathematical model to predict feed intake (FI) taking into account the growth of reproductive organs in pre-laying phase and evaluate the models. The FI was calculated based on the deposition of protein and lipid into three compartments (body, feathers, reproductive organs and liver). Two trials were conducted. Ninety-six Isa Brown pullets from 16 to 28 weeks were used in the first trial. Twice a week four of these birds were slaughtered. The weight of the birds, liver, ovary and oviduct were measured and samples were taken to analyze dry matter, gross energy and crude protein. Seventy-six Isa Brown pullets from 16 to 24 weeks were used in the second trial. The FI was measured every day for each hen until the first egg was laid. Gompertz function was fitted to the data of the first trial and the parameters were used to estimate protein (DP) and lipid deposition (DL). The energy for maintenance (MH) was calculated based on actual protein content and protein weight at maturity. The model of effective energy requirement is EER=MH+50DP+56DL. FI was calculated by dividing the EER and effective energy content in the feed. The output of the simulation of feed intake for Isa-Brown and Hy-Line were evaluated by regressing residual value with the predicted value centered. The simulation of feed intake overall overestimated 0.41g/day and 2.65 g/day for Hy-Line and Isa-brown, respectively. On the other hand, significant linear bias was observed for Hy-Line ($P<0.001$), in contrast Isa-Brown ($P>0.05$). The assessment of the result indicates the simulation models for predict feed intake was more accurate and less precision for Hy-Line than Isa-Brown.

Keywords: evaluation, pullets, nutrition model

Introduction

In poultry production, feed intake is largely influenced by environment, genetic potential and economic circumstances leading poultry nutritionists to develop an optimum feeding strategy under these conditions. Considering these circumstances in poultry production is essential for accurately predicting feed intake, which could lead to a reduction of feed costs. In this context, models seem to be a strategic tool for poultry industry and, despite the fact that several models to predict and simulate feed intake for poultry have been used.

The accuracy of model for predicting feed intake depends on successful description of the bird's growth potential. In this context, Silva (2014) developed a model to predict daily amino acid intake prior the birds reach the sexual maturity taking into account the development of the reproductive organs (ovary and oviduct). However, the effect of the development of the reproductive organs and liver on the feed intake has not been described and experimental evaluation is necessary to compare the simulated data with the real data to determine the accuracy and the precision of the models. Thus, the objective of this study was to make a simulation using a mathematical model to predict feed intake taking into account the growth of reproductive organs in pre-laying phase and evaluate the result of the simulation model.

Material and Methods

Two experiments were conducted at the Laboratory of Poultry Science, Faculty of Agriculture and Veterinary Sciences (FCAV), Universidade Estadual Paulista (UNESP), Jaboticabal, São Paulo, Brazil. The first experiment was conducted to describe the growth of the reproductive organs and estimate the protein and lipid deposition into three compartments (body, reproductive organs and liver), based on that predict desire feed intake in pre-laying phase. The second experiment was conducted to evaluate the output of simulation model (desire feed intake).

Ninety six Isa-Brown pullets were slaughter from 15 to 28 weeks in the first experiment. Twice a week four birds were slaughtered. The birds selected for slaughter were kept in a fasting period for 24 hours and then slaughter by CO_2 asphyxiation. The feathers, body free of feathers, liver, ovary and oviduct were weight. These samples were taken and identified to analyze dry matter, gross energy, crude protein (N × 6.25) and ash.

The protein content of each compartment (liver, ovary and oviduct) was fitted by Gompertz equation to estimate the parameter. The derivative of the Gompertz function described by Emmans (1997) was used to estimate the deposition of protein (DP, g/day) by the following equation $DP = b \times P_t \times \ln\left(\frac{P_m}{P_t}\right)$ and the deposition of lipid (DL, g/day) was estimated DL= $DP \times LP_m \times b_1 \times (Pt/Pm)^{b1-1}$, where b_1 value is estimated by $b_1 = 1.46 \times {LP_m}^{0.23}$. The effective energy requirement (EER, KJ/day) of the birds was calculated by the following equation: $EER = EEM + 50\left(\frac{DP}{1000}\right) + 56\left(\frac{DL}{1000}\right)$, where *EEM* is the effective energy for maintenance (KJ/day) and is calculated as $EEM = M_E \times P_t \times (P_m)^{-0.27}/1000$ and M_E is 1.63 MJ per maintenance. The value of energy for maintenance requirement only considered the energy requirement for maintenance of the body. For these reason, energy for maintenance was calculated based on protein body deposition and protein maturity of body. The parameters to describe the body protein for Isa-Brown and Hy-Line were obtained from Silva (2014). Who calculated the Gompertz parameter of different strains of pullets. Desired food intake (DFI, g) is estimated as follows equation: $DFI = EER/EEC$, where EEC is the effective energy content in the feed (KJ/g) and this value estimated by $EEF = 1.17ME - 4.29CP - 2.44$ and ME is the metabolizable energy content in the feed is expressed as KJ/g and crude protein content (CP) in the feed is expressed as g/g.

The models described above were integrated in the Vensim simulation software (Ventana Systems, 2003). The simulation was defined from 105 to 137 days of age, as a result of the development of each compartment during this period. In order to evaluate the simulation model, the results of DFI from two different pullets strain were compared with the data compiled from the second experiment. Seventy six of Isa-Brown and seventy six of Hy-Line pullets, from 15 to 24 weeks of age, were used. All the birds were allocated in individual cage. The feed intake was measured every day and was register the day, which the first egg was laid. This arrangement allowed to monitor the change of feed intake before and after 14 days from the first egg for each. The predicted result of feed intake (DFI) by the model was compared with the observed value of feed intake fourteen days before the first egg in the experiment. The output of the simulation model of feed intake was evaluated by regressing residual (observed minus predicted) values with the predicted values centered (DFI-mean of DFI) using PROC GLM procedure of the statistical software SAS (Statistical Analysis System, version 9.0)

Results

The weight of body, liver, ovary and oviduct increased in function of the age but a different rate. The liver, ovary and oviduct have greater development in pre-laying phase which is in agreetment with the literature (Bowmaker and Gous, 1989). The Gompertz equation was used to describe the growth of the reproductive organs and liver. Oviduct reaches maturity earlier than the other organs because presented higher W_m (44.2 g) and b (0.11/d) values than liver (W_m=35.6 g, b = 0.06/d) and ovary (W_m=33.6 g, b = 0.07/d). Predicted value of protein weight in the liver (W_m=5.58 g, b=0.09/d) increased slightly in the experimnetal period (105 to137 days of age) because the maximal growth rate for liver happened at 93 days thus the daily deposition of protein decreased over the pre-laying phase. However, the predicted values of protein weight in the ovary (W_m=4.81 g, b = 0.18/d) and oviduct (W_m=6.96 g, b = 0.12/d) had a higher gain over this period because the daily deposition of protein increased in pre-laying phase. Additionally, the values obtained for maturity rate of protein in the ovary was higher than oviduct, for this reason protein in ovary reaches maturity earlier than the oviduct. In contrast, the lipid deposition was estimated as desired lipid growth to quantify the relationship between protein and lipid (Emmans, 1981). This concept has the advantage of controlling the lipid growth based on the voluntary feed intake. Predicted value of lipid weight in the liver (W_m=1.04 g, b = 0.06/d) increased in this period in contrast to protein growth. Growth of liver in this period is mainly due to the fat stored in this compartment, probably to support yolk output throughout the laying period (Bowmaker and Gous, 1989). The predicted value of lipid weight in the ovary (W_m=6.68 g, b = 0.34/d) also increased and was probably due to the follicle development. Similarly, lipid weight in the oviduct (W_m=0.81 g, b = 0.09/d) increased but in a lower rate than in the ovary.

The effective energy requirement was calculated take into account all the parameters to explain above. Additionally, the effective energy content in the feed was 10.74 KJ/g. The maximum predicted value of feed intake in this period was 69.64 and 79.5 g/day for Isa-Brown and Hy-Line, respectively. This value is 0.5 grams less than that suggested by the Isa Brown guidelines at 17 weeks of age and 7 grams more than that suggested by the Hy-Line guidelines at the same age (Isa Brown, 2011; Hy-Line, 2013). Considering the nutritional specification for pre-laying diets from the guidelines, the effective energy content was 10.27 KJ/g (EM = 2750 kcal/kg; CP=17%) and the EER calculated by the simulation model, the desire feed intake was 79.39 g/day, which is in agreement with Isa-Brown guidelines (Isa Brown, 2011) at 17 week of age. In the same way, the effective energy content was 11.09 KJ/g (EM = 2911 kcal/kg; CP=17%) and the EER

calculated by the simulation model, the desire feed intake was 63.57 g/day, which is in agreement with Hy-Line guidelines (Hy-Line, 2011) at 17 week of age. This result indicates that the difference between the predicted feed intake by simulation and the feed intake suggested by guidelines due to the energy feed content value.

Conclusions

The simulation of feed intake overall overestimated 0.41(P>0.05) and 2.65 g/day (P<0.001) for Hy-Line and Isa-brown, respectively. On the other hand, significant linear bias was observed for Hy-Line (P<0.001), in contrast Isa-Brown (P>0.05). The assessment of the result indicates the simulation models for predict feed intake was more accurate and less precision for Hy-Line than Isa-Brown.

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THE DEVELOPMENT OF LACTOBACILLI IN THE BROILER GASTROINTESTINAL TRACT

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Abstract

A feeding trial with maize based feed (MBF) and maize based feed supplemented with 30% crimped kernel maize silage (CKMS) was conducted to investigate the age-related microbial community compositions in the gastrointestinal (GI) tract of broilers. Contents from crop, gizzard, ileum and ceca were collected on day 8, 15, 22, 25, 29 and 36. Microbial diversity was analysed by 454 pyrosequencing of the16S rRNA gene. Since no significant difference was observed between MBF and CKMS, the data from both treatments were pooled. In all segments and at all ages, the majority of the sequences belonged to the phylum Firmicutes. Of these, the Lactobacillaceae were most abundant in all segments except in the ceca. Within the genus *Lactobacillus,* the abundance of *L. johnsonii* was generally highest in all intestinal segments and at all bird ages. From day 15 to day 22, a remarkable increase of *L. salivarius* was observed in the contents of crop (7%-33%), gizzard (4%-12% and ileum (1%-26%). The abundance of *L. salivarius* remained high throughout the entire growth period. The *L. salivarius* de-conjugates bile acids and is suspected to depress broiler growth via decreased fat digestion. Decreased numbers of *L. salivarius* have been observed earlier when antibiotic growth promoters were used. Problems related to reduced broiler growth and GI disorders occurred in the growth period between days 21-28 after the withdrawal of these compounds, which might be associated with the steep increase of *L. salivarius* at this time point.

Keywords: broiler, maize silage, fermented feed, gut microflora, Lactobacilli, dysbacteriosis

Introduction

Antibiotic growth promoters (AGPs) were successfully used as a feed additive to modulate the gastrointestinal (GI) microflora for maintaining gut health and improved broiler growth. After the discontinuation of AGPs in broilers, gastrointestinal (GI) health problems related to dysbacteriosis have increased in the European Union (EU). Dysbacteriosis is typically observed between 20 to 30 days of age resulting in wet litter, non-specific enteritis, decreased nutrient digestibility, and poor broiler performance (TEIRLYNCK et al., 2011). Thus, among other dietary interventions to address these GI problems, fermented feed has gained increasing attention in animal nutrition. Fermented feed has a low pH (3.5-4.5) and contains numerous lactic acid bacteria (10^8-10^9 cfu/g feed) and has been shown to improve the gut health and performance (CANIBE et al., 2007). Considering the lactobacilli as a dominant bacterial population in GI tract, the study of the succession of *Lactobacillus* species might be relevant to understand these GI disorders in broilers. In this respect, age-related development of lactobacilli was studied in the upper and lower GI tract of broilers fed with either a maize based diet or a diet supplemented with crimped kernel maize silage (CKMS).

Material and Methods

The CKMS was prepared by crimping and ensiling maize kernels for approximately 8 weeks with the addition of a commercial organic acids mixture (Kemira AIV Pro®, Kemira, Finland). A total of 240 day-old male broiler chicks (Ross 308) were divided into 2 dietary groups with 4 replicates, and distributed randomly into 8 pens (30 birds/pen). A starter feed containing 22.5% crude protein and 11.4 MJ metabolizable energy per kg was provided from 1-7 days, and was the same for both groups. From day 8 onwards, Group 1 received a grower diet containing 58% maize (maize based feed, MBF), and Group 2 received a maize based grower feed supplemented with 30% CKMS (CKMS-30), replacing maize on dry matter basis. Additionally, 10% whole wheat was added in both diets to increase the gizzard function. Both diets were formulated as iso-caloric (11.58 MJ/kg) and iso-nitrogenous (CP 19.78%) without addition of enzymes and coccidiostat. Feed and water were provided ad libitum during the feeding trial (1-36 days). Lactic acid bacteria (LAB) in the feed were counted on MRS agar.

Four birds were randomly selected from each pen and killed by cervical dislocation at six time points (8, 15, 22, 25, 29 and 36 days). The contents from crop, gizzard, ileum and ceca were rapidly collected and stored at -80^{0}C until analysis. DNA was extracted using QIAGEN DNA Stool Mini Kit. Sequencing of the samples was carried through pyrosequencing of the 16S rRNA gene using Roche 454 FLX sequencing method. Analysis of the sequencing results were carried out using QIIME and UPARSE pyrosequencing pipeline packages with default settings. Statistical analysis on the relative abundance of lactobacilli was done using the GLM procedure of SAS® with following general model: $Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ij}$, where Y_{ij} was the observed dependent variable, μ was the overall mean, α_i was the effect of treatments (MBF and CKMS-30), β_j was the effect of days, $(\alpha\beta)_{ij}$ was the interaction, and ε_{ij} was the random error. Results are given as least square means (LSM) with a pooled standard error (SE). Probability values <0.05 were considered as significant.

Results

The LAB count was higher in CKMS-30 (6.89 log cfu/g) compared to MBF (3.60 log cfu/g). Pyrosequencing data analysis showed Firmicutes to be the most dominant phylum in all age groups representing up to 98% of the total sequences. The family Lactobacillaceae represented most of the Firmicutes in all intestinal segments (58-98%) except in the ceca where Lachnospiraceae (20-39%) were dominant. Since no significant difference was observed in the relative abundance of microbiota between the two dietary treatments in any of the segments, a pooled data set was used in the further data analysis. The genus *Lactobacillus* represented 99-100% of Lactobacillaceae in all four GI segments. Apart from the ceca, the relative abundance of *Lactobacillus* was highest and quite stable in the crop (94-98%), gizzard (58-86%) and ileum (61-73%) during the entire broiler production period (Table 1). In the ceca, *Lactobacillus* started to decrease from 17.5% to 7.8% at day 15, and remained low (2-3%) during the remaining growth period (P<0.001). Variations in *Lactobacillus* species were determined in all intestinal segments as broilers grew older. Among the four dominant species of *Lactobacillus*, *L. johnsonii* was abundant in all age groups (Table 1). The relative abundance of *L. reuteri* and *L. crispatus* was higher during the early growth period (up to d 15), but *L. salivarius* dominated in the final growth phase (from d 22) in all GI segments. From d 15 to d 22, a remarkable increase of *L. salivarius* was observed in the contents of crop (7%-33%, P<0.001), gizzard (4%-12%, P=0.003) and ileum (1%-26%, P<0.001).

Conclusions

There was no influence of the diets on the lactobacilli population although the CKMS-30 diet showed high lactobacilli counts (6.89 log cfu/g) compared to MBF (3.60 log cfu/g). Keeping the high numbers of lactobacilli in crop contents (10^9 cfu/g) in mind, the contribution of CKMS containing about 10^7 cfu/g diet (primarily *L. plantarum*) added at dietary levels of 30% was below 1% in crop content. Therefore, the modulatory action of *Lactobacillus* provided as probiotic feed additives or fermented feed will possibly be more effective if used in higher concentration exceeding 10^9 cfu/g. A clear temporal variation in the relative abundance of *Lactobacillus* species was observed in all GI segments. The *L. salivarius* is known to de-conjugation bile acids, leading to poor lipid digestion and depressed broiler growth (GUBAN et al., 2006; RAMASAMY et al., 2010). The rise in the abundance of *L. salivarius* coincides within the period between 20-30 days where typically GI problems occurred in broilers related to dysbacteriosis. A significant decrease of *L. salivarius* was reported in the GI tract of broilers receiving feed supplemented with antimicrobials (salinomycin, bacitracin, monensin) compared to the broilers fed a non-supplemented control diet (ENGBERG et. al., 2000; GUBAN et al., 2006). Therefore, the substantial increase in the abundance of *L. salivarius* in the ileum after d 15 could be a possible factor contributing to the increased frequency of GI problems related to dysbacteriosis in broilers after the discontinuation of AGPs.

Table 1: Relative abundance of *Lactobacillus* in crop, gizzard, ileum and ceca on different time points

		Days							
Segment	Item	8	15	22	25	29	36	SE	*P-value*
Crop	*Lactobacillus*	97.43	96.74	96.10	97.58	96.30	94.21	1.17	0.399
	L. johnsonii	47.66	56.39	51.57	46.32	50.47	40.42	6.89	0.692
	L. reuteri	19.79[a]	23.81[a]	8.67[b]	16.95[ab]	8.34[b]	10.94[b]	3.29	0.007
	L. crispatus	23.27[a]	4.19[b]	1.81[b]	0.52[b]	1.48[b]	2.78[b]	5.39	0.041
	L. salivarius	2.19[b]	6.99[b]	32.59[a]	32.74[a]	33.56[a]	37.9[a]	5.62	<0.001
Gizzard	*Lactobacillus*	64.17	86.49	63.75	62.27	60.94	58.42	6.94	0.075
	L. johnsonii	36.01[b]	55.44[a]	33.05[b]	31.06[b]	32.36[b]	32.91[b]	6.06	0.058
	L. reuteri	6.95[ab]	11.77[a]	4.11[b]	6.20[b]	3.22[b]	5.95[b]	1.96	0.059
	L. crispatus	6.49	3.60	1.65	0.90	1.59	3.21	1.71	0.239
	L. salivarius	0.26[c]	3.74b[c]	12.25[a]	14.50[a]	10.70[ab]	6.81[bc]	2.61	0.003
Ileum	*Lactobacillus*	60.30	65.93	72.64	73.29	67.34	67.69	7.19	0.856
	L. johnsonii	34.77	45.06	39.57	48.82	34.62	26.46	6.09	0.145
	L. reuteri	9.96[a]	9.96[a]	3.57[b]	4.97[b]	3.15[b]	1.57[b]	1.34	<0.001
	L. crispatus	15.59	7.15	2.97	2.24	2.19	3.71	3.55	0.079
	L. salivarius	0.94[c]	0.87[c]	26.45[ab]	17.14[b]	27.31[ab]	35.81[a]	4.68	<0.001
Ceca	*Lactobacillus*	17.47[a]	7.79[b]	3.14[bc]	3.05[bc]	2.18[c]	3.27[bc]	1.25	<0.001
	L. johnsonii	7.04[a]	4.04[b]	1.74[c]	1.41[c]	0.77[c]	1.09[c]	0.71	<0.001
	L. reuteri	6.72[a]	2.61[b]	0.17[c]	0.10[c]	0.15[c]	0.26[c]	0.790	<0.001
	L. crispatus	3.58[a]	0.81[b]	0.06[b]	0.01[b]	0.00[b]	0.10[b]	0.58	<0.001
	L. salivarius	0.11[b]	0.31[b]	1.17[a]	1.49[a]	1.25[a]	1.82[a]	0.27	<0.001

[a-c] LSMEANS within the same row with different superscripts differ significantly (P < 0.05)

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HISTOMORPHOMETRIC MEASURES OF INTESTINAL SEGMENTS AND BIOMETRY OF THE DIGESTIVE SYSTEM AND IMMUNE SYSTEM OF BROILER CHICKS HATCHED FROM DIFFERENT EGG WEIGHTS AND BREEDER AGES

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Abstract

The purpose of this study was to evaluate the intestinal development of embryos and of broiler chicks supplemented with different levels of digestible lysine in pre-starter diet. A total of 740 eggs from breeders at 36 weeks of age and different egg weights (ranging from 56 to 65g and from 66 to 72g) were weighed, identified and incubated. After 18 days of incubation, 4 eggs per treatment were broken, the embryo removed and fragments of the duodenum and jejunum were collected. Fragments were fixed in 10% formalin for preparation of histological slides, and stained by HE method. Images were digitized, and histomorphometric measures were recorded by Image J software in a Carl Zeiss microscope. The same procedures were performed at hatch, and at 7 and 14 days of age. Statistical analyses were performed using Anova and Tukey test (5%) to compare averages. Chicks from heavy eggs presented higher values of villus height of jejunum at hatch, which could result in an increased nutrient absorption. However, at 7 and 14 days of age this difference was not observed, showing the use of increasing levels of digestible lysine in pre-starter diets did not result in any significant effect. Pre-starter diets supplemented with digestible lysine did not show the same efficacy to accelerate intestinal cells development as they do to muscle tissues.

Keywords: amino acid, crypt, diet, nutrition, villus

Introduction

The high productivity observed in poultry production depends, among several factors, on the nutrients obtainment by the body. Therefore, specific diets for each life period, genetic line or environmental conditions have been researched and employed. However, the intestinal mucosa must present adequate structural morphological and physiological characteristics to properly digest and absorb such diets (MAIORKA, 2004).

The small intestine mucosa is a functional element that can be characterized as a permeable layer for nutrients absorption and a barrier against pernicious compounds (VAN LEEUWEN, 2002).

At hatch, broiler's gastrointestinal tract is anatomically complete, but its functional capacity for digestion is not completely mature. The gastrointestinal tract needs morphological and physiological adaptations, which are stimulated by the presence of the feed, justifying the importance of quality and composition of the diet in the first week of life of broiler chicks (MAIORKA et al., 2000b).

In this experiment, we aimed to evaluate the development of intestines, digestive and immune organs of broiler chicks hatched from different egg weights and breeder ages supplemented with different levels of digestible lysine in pre-starter diets.

Material and Methods

We used 740 eggs of heavy Cobb 500 genetic line breeders at 36 weeks of age and two different egg weight range (56 to 65 grams and 66 to 72 grams). Of these, 40 eggs were separated, identified and weighed (20 light eggs, 56-65g, and 20 heavy eggs, 66 a 72g) for physical evaluation.

Eggs separated for physical evaluation were immersed in saline solution to determine specific gravity. Eggs were then dried and cracked to measure yolk and albumen height and diameter with a digital caliper. Yolk and albumen were individually weighed in a 0.01 grams precision scale and pH was measured with a potentiometer. Eggshells were washed and maintained in a forced air movement oven at 65 ^{o}C during 72 hours. Eggshell thickness were determined with digital caliper.

The remaining eggs were individually identified, weighed and allotted uniformly in the incubators. At the beginning of the hatching period, temperature and moisture were maintained constant at 37.8 °C and between 60 and 65%, respectively. At the final period, temperature was reduced to 37.5 °C and moisture increased to 70%. After 14 days of hatching period, candling was carried out in 24 eggs from each incubator in order to verify egg fertility. At 18 days of the incubation period, eggs were individually involved in air-permeable tissue according to each treatment.

On the 18th day of incubation, four eggs of each incubator, two from each experimental treatment (light or heavy eggs), totaling 12 eggs, were removed from the incubator and cooled for 6 hours. After that, each egg was identified and broken in a Petri's plate. The intestines were weighed and fragments of duodenum and jejunum were collected to prepare the histological slides.

At hatch, all chicks were weighed and sexed by the wings, and 60 of them were randomly distributed into groups according to the digestible lysine level in four heated batteries. At birth, seven and fourteen days of age, one chick per replicate was euthanized and fragments of duodenum and jejunum were collected to prepare the histological slides. The slides received pigments according to the methodology of Luna (1968) using Hematoxilin-Eosin method, fixed in a formalin 10% solution. Slides images were digitized in a light field optical microscope to the computer and captured by Image J program. Statistical analysis was performed by the procedure ANOVA of SAEG and Tukey test (5% probability) was applied for average comparison.

Results and Discussion

The results of villus height and crypt depth of duodenum and jejunum for 18-day old embryos, and for chicks at birth, seven and fourteen days of life, from egg with different weights at the beginning of incubation, are displayed in Tables 1 and 2. No statistical difference was observed for villus height in duodenum and crypth depth in duodenum and jejunum for the egg weights analyzed. Different results were observed by Ferreira (2010), who verified difference for villus height and crypt depth for duodenum and jejunum but did not verify any effect of the digestible sulfur amino acids analyzed for chicks hatched from different egg weights before incubation. In our experiment, digestible lysine levels did not affect the results.

These results allowed us to conclude the increment of digestible lysine levels in pre-starter diet did not act effectively to improve the development of intestinal structures cells as it does in muscle tissue growing. Basic function of lysine is an intense participation in body protein deposition (Bertechini, 2012) and this amino acid tends to affect poultry body growing, specially muscles (Tesseraud et al., 1999).

It is possible to conclude that in this experiment egg weight and digestible lysine supplementation did not affect intestinal organ development of chicks.

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Table 01. Vilus height (µm) and crypt depth (µm) of broiler embryos at 18 days of incubation and neonatal chicks hatched from eggs with different weights

		Villus height (µm)		Crypt depth (µm)	
		Duodenum	Jejunum	Duodenum	Jejunum
		Broiler embryos at 18 days of incubation			
	56-65g	276.66	170.44 b	96.37	74.81
Egg	66-72g	299.56	175.02 a	85.74	81.24
weight	P	ns	0.022	Ns	ns
	CV (%)	13.04	13.87	16.59	21.27
		Neonatal chicks			
	56-65g	601.98	435.09 a	131.61	106.62
Egg	66-72g	690.72	353.59 b	134.91	123.21
weight	P	ns	0.030	ns	ns
	CV (%)	25.60	19.62	17.56	40.16

[a,b] Averages followed by distinct letters in the same column differs by Tukey test (P<0,05); ns = non-significant, CV = coefficient of variation

Table 02. Vilus height (µm) and crypt depth (µm) of broiler at 7 and 14 days of age hatched from eggs with different weights, supplemented with increasing levels of digestible lysine

	Villus height (µm)		Crypt depth (µm)		Villus height (µm)		Crypt depth (µm)	
	Duodenum	Jejunum	Duodenum	Jejunum	Duodenum	Jejunum	Duodenum	Jejunum
	7 days				14 days			
Egg weight								
56-65g	1178	695	427	290	1177.0	687	687	304
66-72g	1160	559	345	284	1246.9	698	698	269
Digestible Lysine Levels (%)								
1.1	1173	523	411	365	1246	567	392	274
1.2	1168	553	327	220	1148	623	372	240
1.3	1125	551	449	298	1098	799	374	390
1.4	1214	578	368	276	1354	761	338	260
P								
Age	Ns	ns	ns	ns	Ns	ns	Ns	Ns
Lysine	Ns	ns	ns	ns	Ns	ns	Ns	Ns
Age* Lys	Ns	ns	ns	ns	ns	ns	Ns	Ns
CV (%)	13.2	41.2	13.7	28.4	14.5	16.8	11.3	30.8

[a,b] Averages followed by distinct letters in the same column differ by Tukey test (P<0,05); ns = non-significant, CV = coefficient of variation

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EFFECTS OF SLAUGHTER WEIGHT AND SEX ON CARCASS YIELD, FAT AND FATTY ACID COMPOSITION OF MEAT IN BROILERS

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Abstract

The aim of this research was to investigate the effects of slaughter weight and sex on carcass yield, fat and fatty acid composition of broiler meat. A total of 360 broilers were grown separately as female and male. At age of 42 day, broilers were classified by live weight as light and heavy. For measurements, a total of 30 broilers each group (slaughter weight/sex) were slaughtered. Meat samples of breast and thigh were analysed for fat and fatty acids. Slaughter weight was found to be highest as 3214.6 g in heavy-male broilers. Carcass yield was higher in light broilers than heavy broilers, whereas it was higher in females than males. Significant interactions were observed for breast and thigh meat, were highest as 808.3 g and 907.2 g, respectively in heavy-males. Fat content of breast was affected by both slaughter weight and sex, whereas fat content of thigh was affected by only slaughter weight. Saturated fatty acid content of breast was higher in males (33.8%) than females (33.4%). The highest content of MUFA and PUFA was observed in female-light broilers (31.6% and 33.5%, respectively). The highest MUFA content of 27.5% was found in female-light broilers, whereas PUFA content was found to be higher in light-males (34.5%) and heavy-females (34.5%). These results shows that slaughter weight at the same age and also sex affected carcass yield and also fat and fatty acid contents of breast and thigh meat in broilers.

Keywords: broiler, slaughter weight, sex, fat, fatty acid

Introduction

Nowadays, consumers and the broiler industry have shown a demand for desirable carcass conformation scores, sensory characteristics and nutritional value, besides high slaughter yield (Holchman et al., 2003). Poultry meat quality is affected by some factors like slaughter age, sex, handling and slaughter conditions. Breast and thigh muscles of chicken represent the highest proportion of whole carcass and differ in their chemical composition and sensory quality (Oluyemi ve Robert, 2000). So the aim of the current study was to determine the effects of slaughter weight and sex on carcass yield, fat and fatty acid composition of breast and thigh of broiler carcass.

Material and Methods

A total of 360 chicks (180 males/females) were obtained from a commercial Ross 308 broiler breeder flock at 52 weeks of age. The chicks were placed in 12 floor pens (n=3 pens per treatment group). The chicks were weighed using a balance at ±0.1 g precision on the 1st day of the growing period and received a starter diet (22.5% CP and ME 12.8 MJ/kg; days 1-14), a grower diet (22.0% CP and ME 13.3 MJ/kg; 15-28), and a finisher diet (21.0% CP and ME 13.5 MJ/kg; days 29-42. Feed and water were offered ad libitum during the growing period.

On slaughter day, broilers were classified as light and heavy. Before slaughter, feed was withdrawn 12 h before slaughter at 42 d of age. A total of 30 broilers from each group were individually weighed to determine slaughter weight and then were slaughtered. The carcass yield was calculated as a percentage of slaughter weight. Then the carcasses were divided into pieces as wing, breast, neck and back, thigh and drumstick (Raseta and Dakic, 1984). During slaughter process, samples of breast and thigh pieces were collected and stored at −20°C for further fatty acid analysis. Fatty acid analyses were performed with method described by Cherian et al. (2002). The fatty acid contents of the breast and thigh meat were given as percentages of total fatty acids.

A factorial arrangement of treatments was used in the study. Data were subjected to analysis of one-way variance analyses (SAS, 1989), by ANOVA procedures. Analyses for percentage data were conducted after a square root of arc sine transformation of the data. Significant differences among treatment means were determined by the Tukey's test.

Results

The effects of slaughter weight and sex on slaughter yield are presented in Table 1. Slaughter and carcass weight was found as the highest in heavy male broilers (P<0.05). On the other hand, the carcass yield was similar between the treatment groups.

Table 1: The effects of slaughter weight and sex on slaughter yield

Groups	Slaughter yield		
	Slaughter weight	Carcass weight	Carcass yield
	Slaughter weight (SW)		
Light (L)	2598.3 ± 59.9 [b]	1961.9 ± 42.8 [b]	75.6 ± 0.72 [a]
Heavy (H)	2948.3 ± 59.9 [a]	2132.0 ± 42.8 [a]	72.3 ± 0.72 [b]
	Sex (S)		
Female (F)	2576.5 ± 59.9 [b]	1922.8 ± 42.8 [b]	74.7 ± 0.72 [a]
Male (M)	2970.0 ± 59.9 [a]	2171.3 ± 42.8 [a]	73.2 ± 0.72 [b]
	Slaughter weight x Sex (SW x S)		
L x F	2471.1 ± 84.7 [b]	1898.8 ± 60.5 [b]	76.9 ± 1.01
L x M	2725.4 ± 84.7 [c]	2025.1 ± 60.5 [b]	74.3 ± 1.01
H x F	2681.9 ± 84.7 [bc]	1946.7 ± 60.5 [b]	72.6 ± 1.01
H x M	3214.6 ± 84.7 [a]	2317.4 ± 60.5 [a]	72.1 ± 1.01
	P-Values		
SW	<0.01	<0.01	<0.01
Sex	<0.01	<0.01	0.044
SW x S	0.024	0.006	NS

a,b Means ±SEM in column that possess diferent superscripts differ significantly (P<0.01;P < 0.05). NS: P > 0.05

Table 2. The effects of slaughter weight and sex on carcass pieces weight

Groups	Carcass pieces			
	Wing (g)	Breast (g)	Thigh + Drumstick (g)	Neck + Back (g)
	Slaugher weight (SW)			
Light	198.1 ± 3.97 [b]	685.1 ± 18.8 [b]	771.9 ± 18.78 [b]	306.8 ± 7.37 [b]
Heavy	215.8 ± 3.97 [a]	763.4 ± 18.8 [a]	823.8 ± 18.78 [a]	329.0 ± 7.37 [a]
	Sex (S)			
Female	191.7 ± 3.97 [b]	701.3 ± 18.8 [b]	736.2 ± 18.78 [b]	293.5 ± 7.37 [b]
Male	222.2 ± 3.97 [a]	747.3 ± 18.8 [a]	859.4 ± 18.78 [a]	342.4 ± 7.37 [a]
	SW x S			
L x F	189.8 ± 5.62 [c]	684.0 ± 26.5 [b]	732.1 ± 26.56 [c]	292.9 ± 10.43 [c]
L x M	206.5 ± 5.62 [b]	686.3 ± 26.5 [b]	811.6 ± 26.56 [b]	320.8 ± 10.43 [b]
H x F	193.7 ± 5.62 [bc]	718.5 ± 26.5 [b]	740.3 ± 26.56 [c]	294.1 ± 10.43 [bc]
H x M	238.0 ± 5.62 [a]	808.3 ± 26.5 [a]	907.2 ± 26.56 [a]	363.9 ± 10.43 [a]
	P-Values			
SW	<0.01	<0.01	0.008	0.004
Sex	<0.01	0.017	<0.01	<0.01
SW x S	0.001	0.023	0.024	0.006

a,b Means ±SEM in column that possess diferent superscripts differ significantly (P<0.01;P < 0.05). NS: P > 0.05

The weight of carcass pieces by slaughter weight and sex are presented in Table 2. The weights of carcass pieces including wing, breast, thigh and drumstick were found higher in heavy male broilers (P<0.05).

The effects of chick weight and sex on saturated and unsaturated fatty acid content of breast and thigh are presented in Table 3. Whereas the fat content of breast were higher in light broilers and female broilers, it was higher in only heavy broilers. Saturated fatty acids of thigh were higher by 35.6% and 35.4%, respectively in light broilers and also in male broilers. The highest contents of MUFA and PUFA were observed in light female broilers. Whereas, MUFA content of thigh was the highest in light female, PUFA content was higher in light male and heavy female broilers (P<0.01).

Table 3. The effects of chick weight and sex on saturated and unsaturated fatty acid content of breast and thigh (%)

Groups	Breast				Thigh			
	Fat content	Saturated	MUFA	PUFA	Fat content	Saturated	MUFA	PUFA
Slaughter Weight (SW)								
Light	1.17 ± 0.08 [a]	33.6 ± 0.16	28.8 ± 0.27 [a]	32.9 ± 0.17 [a]	1.36 ± 0.10 [b]	35.6 ± 0.32 [a]	25.6 ± 0.26 [a]	33.0 ± 1.18
Heavy	0.95 ± 0.08 [b]	33.7 ± 0.16	26.8 ± 0.27 [b]	31.7 ± 0.17 [b]	1.66 ± 0.10 [a]	34.1 ± 0.32 [b]	23.0 ± 0.26 [b]	31.8 ± 1.18
Sex (S)								
Female (F)	1.35 ± 0.08 [a]	33.4 ± 0.16 [b]	29.8 ± 0.27 [a]	32.5 ± 0.17 [a]	1.43 ± 0.10	34.3 ± 0.32 [b]	25.3 ± 0.26 [a]	33.5 ± 1.18
Male (M)	0.77 ± 0.08 [b]	33.8 ± 0.16 [a]	25.9 ± 0.27 [b]	32.1 ± 0.17 [b]	1.59 ± 0.10	35.4 ± 0.32 [a]	23.3 ± 0.26 [b]	31.3 ± 1.18
SW x S								
L x F	1.52 ± 0.11	33.3 ± 0.22	31.6 ± 0.39 [a]	33.5 ± 0.22 [a]	1.44 ± 0.15 [b]	33.4 ± 0.46	27.5 ±0.37 [a]	31.5 ± 1.67 [ab]
L x M	0.81 ± 0.11	33.8 ± 0.22	26.1 ± 0.39 [c]	32.3 ± 0.22 [b]	1.28 ± 0.15 [b]	34.9 ± 0.46	23.6 ±0.37 [b]	34.5 ± 1.67 [a]
H x F	1.17 ± 0.11	33.5 ± 0.22	28.0 ± 0.39 [b]	31.5 ± 0.22 [c]	1.41 ± 0.15 [b]	35.3 ± 0.46	23.1 ±0.37 [b]	34.5 ± 1.67 [a]
H x M	0.72 ± 0.11	33.8 ± 0.22	25.7 ± 0.39 [c]	31.9 ± 0.22 [bc]	1.91 ± 0.15 [a]	36.0 ± 0.46	22.9 ±0.37 [b]	28.1 ± 1.67 [b]
P- Values								
SW	0.011	NS	<0.01	<0.01	0.006	<0.01	<0.01	NS
S	<0.01	0.015	<0.01	0.019	NS	0.002	<0.01	NS
SW x S	NS	NS	<0.01	<0.01	0.003	NS	<0.01	<0.01

a,b Means ±SEM in column that possess diferent superscripts differ significantly (P<0.01;P < 0.05). NS: P > 0.05

Conclusions

In conclusion, slaughter weight and sex affected carcass yield, fat and fatty acid composition of breast and thigh. Consumers prefer to buy whole carcass or carcass pieces due to nutritional value, especially fat and fatty acid content and also sensory quality. At this point, determining the effecting factors of chemical composition of broiler meat could be beneficial to manipulate broiler feeding practices.

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P-262 (ID 82)

ELEVATED HATCHER TEMPERATURE AFFECTS ONE DAY OLD CHICK QUALITY AND GROWTH PERFORMANCE DURING EARLY POST-HATCH PERIOD

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Abstract

The aim of the study was to determine the effects of elevated hatcher temperature on one day old chick quality and broiler growth performance during early post-hatch period. The hatcher temperatures were applied as follows: control (36.8-37.0˚C) and elevated hatcher temperature (38.8-39.0˚C) during hatching period. One day old chick quality was measured by different scoring systems including Tona score, Pasgar score, chick body weight, yolk free body weight and chick length. The quality of chicks from control group was found to be higher with Tona and Pasgar scores (P<0.01). One day old chick weight and yolk free body weight were found to be higher in chicks as compared to control group as 44.3 g and 40.7 g, respectively (P<0.01). Chick length was found to be similar between groups. During early post-hatch period, live weight was higher in chicks from control group than chicks from elevated hatcher temperature group (P<0.01). Relative growth was found to be higher by 373.0% in chicks from control group between days 1 and 7, whereas it was found higher with a value of 172.3% in chicks from elevated hatcher temperatures between days 7-14 (P<0.01). In conclusion, elevated hatcher temperature affected one-day old chick quality including chick weight and yolk free chick weight, and broiler growth performance during early term of post-hatch period.

Keywords: elevated hatcher temperature, chick quality, Tona, Pasgar, growth rate

Introduction

The one-day old chick quality is an important issue for hatchery success and also profitability of broiler producers (Decuypere and Bruggemen 2007). Some various quality scoring systems have been developed considering different quantitative and qualitative parameters such as Tona score and Pasgar score (Boerjan 2002, Tona et al. 2003). There are four quantitative methods for scoring chick quality; Tona or Pasgar score, the one-day old chick weight, yolk free chick weight and chick length (Meijerhof 2009).

Incubation temperature is one of the most important factors to assure optimum chick quality and also for post-hatch broiler performance (Lourens 2003, Willemsen et al. 2008, Ipek et al. 2014). Fluctuations in setter or hatcher temperatures have negative impacts on chick quality and it causes major economic losses because of the negative effects on post-hatch performance and slaughter yield (Wilson 1991, Shafey 2004). So the aim of the current study was to determine the effects of elevated hatcher temperatures on one day old chick quality and growth performance during early post-hatch period.

Material and Methods

The total of 1800 eggs was obtained from a commercial Ross 308 broiler breeder parent stock at 42 weeks of age. All eggs were weighed (55-65 g) and then incubated in same incubator at 37.5°C and a 55-60% RH during the first 18 days of incubation. On 18th day of incubation, the eggs placed into hatching baskets (560 eggs per treatment group) and then the eggs were transferred to two hatcher cabinets (640 capacity egg hatcher) for these temperature: control (36.8-37.0 ˚C), and elevated hatcher temperature (38.8-39.0 ˚C).

After completing hatching process, the chick quality was measured with Tona score, Pasgar score, chick hatching weight, yolk free chick weight and chick length. A total of 90 chicks per group were randomly chosen for Tona and Pasgar methods (Boerjan, 2002; Tona et al., 2003). Then these chicks were measured for chick weight and length (Hill 2001). A total of 40 chicks were randomly killed by cervical dislocation to determine yolk free chick weight.

A total of 50 chicks each two groups were placed rearing pens. Feed and water were offered ad libitum during the rearing period. The live weight values were monitored on a weekly basis and relative growth was calculated with formula reported by Willemsen et al. (2008).

The effects of different hatcher temperatures on chick quality and growth performance were analyzed by 1-way ANOVA using the general linear model procedure (SAS). Analyses for percentage data were conducted after square root of arc sine transformation of the data. Significant differences among means were determined by a Duncan's multiple range test.

Results

The effects of different hatcher temperatures on chick quality are presented in Table 1. Tona and Pasgar scores were higher by 90.4 and 8.9, respectively in the control group (P<0.01). Similarly, the chick weight and yolk free chick weight were higher in the control group (P<0.01). The chick length was similar between the treatment groups.

Table 1. Chick quality scores with different scoring methods

Groups	Scoring Methods				
	Tona Score[1]	Pasgar Score[1]	CW[1]	YFC[2]	Chick Length[1]
	n= 90	n=90	n=90	n=40	n=90
Control	90.4 ± 6.03	8.9 ± 1.05	44.3 ± 2.78	39.3 ± 2.27	20.0 ± 0.58
Elevated	68.9 ± 16.66	8.3 ± 1.10	40.7 ± 1.48	35.3 ± 1.00	20.1 ± 0.65
Probabilities	<0.01	<0.01	<0.01	<0.01	0.356

[1]: n=90 chicks/treatment [2]: n=40 chicks/treatment CW: Chick weight YFCW: Yolk free chick weight

The live weights of chicks from different hatcher temperatures during the growing period are presented in Table 2. On 1st day of growing period, live weights of chicks were higher in the control group than other (P<0.01). From 1st day to 14th day of the growing period, the chicks of the control group was consistently heavier by 208.5 and 543.8 on day 7 and 14, respectively, compared to the chicks of the elevated hatcher temperature group (P<0.01).

Table 2. Live weight of chicks from different hatcher temperatures during the growing period

Groups	Live weight (g)		
	1.day	7.day	14.day
Control	44.2 ± 0.44	208.5 ± 8.80	543.8 ± 70.34
Elevated	40.5 ± 0.24	165.6 ± 4.69	449.6 ± 11.43
Probabilities	<0.01	<0.01	<0.01

n=50 chicks/treatment

The relative growth of the chicks from different hatcher temperatures during the growing period are presented in Table 3. Between day 1 and 7 of growing period, the relative growth was the highest in the control group by 373.0%, compared to elevated hatcher temperature group by 307.9%, respectively (P<0.01). Between day 7 and 14, the higher relative growth rate was found in elevated hatcher temperature group by 172.3% than the control (P<0.01).

Table 3. Relative growth (%) of chicks from different hatcher temperatures with during the growing period (n=50 chicks)

Groups	Relative growth (%)	
	1-7	7-14
Control	373.0 ± 32.8	160.9 ± 34.4
Elevated	307.9 ± 26.3	172.3 ± 17.54
Probabilities	<0.01	<0.01

Conclusions

In conclusion, the one-day old chick quality can be measured by different scoring methods to predict broiler performance. The first term of profitability in broiler production depends on hatchery management and good quality chick production. During incubation, especially incubation temperature affects seriously embryonic development during incubation, chick quality at hatch and post-hatch broiler performance. In large scale broiler production, growth rate, feed conversion rate, total mortality and slaughter yield are too important parameters. Because of first week broiler growth performance and mortality are related with chick quality, determining the chick quality with a high accuracy and objectivity is very important issue. At this point, the effects of incubation conditions on chick quality could be known well and considered in scoring of chick quality.

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P-263 (ID 240)

COCCIDIOSIS PREVENTION STRATEGIES AND PRACTICES IN THE AMERICAS

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Abstract

The study objective was to evaluate the most commonly used strategies and practices in coccidiosis control in the Americas. Seventy five interviews with participants from Argentina, Brazil, Canada, Colombia, Mexico and Peru were carried out in the period March-April 2014. Nearly half of all broiler production in the region was represented.

In respect to the number of rotations, 84% of the respondents have used two or more different anticoccidial programs in the last 12 months, and only 16% have not rotated at all. The study indicated considerable understanding and agreement with the basic coccidiosis prevention postulates: (i) not to use a certain drug for too long (77% strongly agree); (ii) to give products sufficient rest after each period of use (69% strongly agree); (iii) to rotate between products of different classes (67% strongly agree) and (iv) to use a synthetic drug in a clean-up program at least once per year (63% strongly agree). The majority of respondents agreed that to avoid resistance development, the drugs should be rotated before having signs of inefficacy, demonstrating a high level of judicious drug use.

Only 9.4% of the respondents were not satisfied by the current control of coccidiosis in their respective operations, while 48% were “completely satisfied” and 42.7% “somewhat satisfied”. In countries with low rotation rate, satisfaction of coccidiosis control was the least, indicating correlation between the use of rotation and efficacy of prevention.

Introduction

Coccidiosis is one of the greatest threats to flock health and profitability of the poultry industry, costing an estimated $3 billion globally each year (Lillehoj, 2006). The disease can manifest itself in a clinical or a subclinical form. While the clinical form is relatively easy to diagnose and most of the time efficaciously treated, subclinical disease often remains hidden. Nevertheless, subclinical coccidiosis comprises up to 80% of the costs related to coccidiosis (Williams, 1998). To combat this costly disease, poultry producers and veterinarians have long reached for in-feed anticoccidial drugs and, more recently, vaccines. In-feed anticoccidials are by far the most vastly used prevention tool for coccidiosis control in poultry, under modern production practices. In its commission report 233/2008, the European Commission recognizes their use as “essential”. Unfortunately if these products are used injudiciously, resistance of field *Eimeria* strains ultimately develops and anticoccidial efficacy declines (Mathis *et al*., 1984; Chapman, 1986; 1997 and 2007). Moreover, the latest anticoccidial drug came in commercial use in the 1990s and no new products are anticipated to enter this segment in the near term, making it critically important for producers and veterinarians to use products in a way that maximizes performance while preserving long term efficacy.

In this respect the basic postulates in anticoccidial prevention are (i) not to use same drug for too long, as it is generally acknowledged that overuse of anticoccidial products will eventually lead to the development of resistance (Mathis *et al*., 1984; Chapman, 1986; 1997 and 2007); (ii) to give a rest period to all products that share a similar resistance mechanism, so that the *Eimeria* population is not exposed to the same/similar drug, which is beneficial for restoration of sensitivity (Chapman and McFarland, 2003); (iii) to avoid the use of drugs with similar structure and mode of action avoiding cross-resistance (McDougald *et al*., 1987; Bedrnik *et al*., 1989; Chapman and Hacker, 1994; Chapman, 2007; Marien et al., 2007), by rotating between products of different classes; and lastly (iv) to use a synthetic drug at least once yearly to reduce infection pressure (DeGussem, 2005).

The objective of the study was to evaluate the most commonly used strategies and practices in coccidiosis control in the Americas. To gain a deeper understanding and insights regarding current use of anticoccidials and vaccines; to identify common programs used, including rotational practices; to explore the efficacy perceptions among users and evaluate the correlation between satisfaction level and the prevention strategies employed.

Materials and Methods

Poultry producers, nutritionists and veterinarians across North and South America were interviewed. Seventy five interviews with participants from Argentina, Brazil, Canada, Colombia, Mexico and Peru were carried out during the period of March and April, 2014. Nearly half of all broiler production in the region was represented by the study participants (Fig.1). The following areas where explored:

- Satisfaction with current control of coccidiosis
- Types of programs used to control coccidiosis
- Number of rotations in the past 12 months
- Industry stand on basic coccidiosis control strategy's postulates
    - To change anticoccidial control tools to maximize program performance while preserving long term efficacy
    - To rest the anticoccidial drugs after period of use to allow them restoring efficacy
    - Rotation should be carried out among drugs of different classes in order to provide effective coccidiosis control
    - Once a year synthetic anticoccidial should be used as a chemical clean-up in order to reduce the infection pressure on the farm
- Anticcodial drugs should be rotated at given intervals even if they still seem to be working in order to avoid resistance development, thus increasing the level of subclinical coccidiosis or having clinical outbreaks, but most important to preserve the anticoccidial drugs viable for future use (avoid 'burning' the products).
- Significance of the coccidiosis challenge on the field.

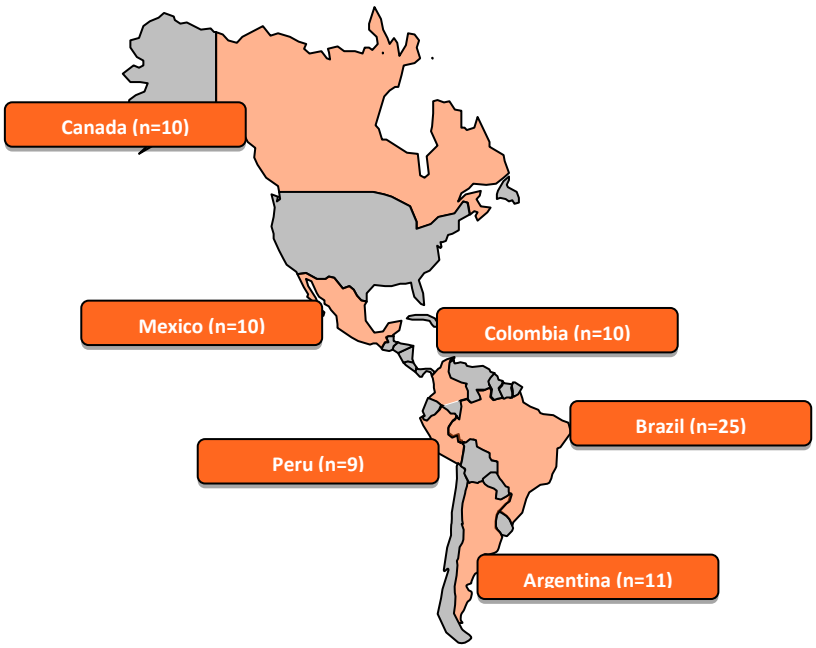

Fig 1. Sample representativeness

Results

Only 9.4% of the respondents were not satisfied by the current control of coccidiosis in their respective operations, while 48.0% are "completely satisfied" and 42.7% "somewhat satisfied". Significantly more respondents in Argentina and Mexico are "completely satisfied" with current control of coccidiosis, as compared to respondents in all other countries. Respondents in Colombia and Peru were least satisfied (Fig. 2).

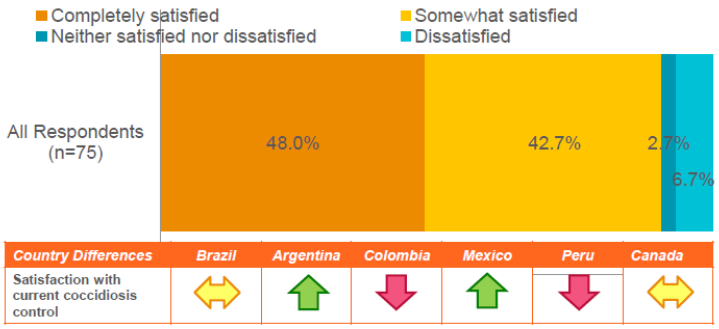

Fig. 2 Satisfaction with current coccidiosis control program. Arrows for the different countries indicate higher than average, average or below average satisfaction level.

In respect to the number of different programs used, 84% of the respondents replied they have used two or more different anticoccidial programs in the last 12 months, and only 16% have not rotated at all. Based on the number of broilers produced in a weekly basis, broiler operations used an average of 2.5 different protocols for coccidiosis over the past year. Incidence of rotation is highest in Argentina and Canada and lowest in Colombia and Peru (Fig. 3).

Nearly two-thirds of protocols (prevention programs) involve a combination product (ionophore + synthetic) or a synthetic coccidiostat in the starter feed followed by an ionophore in the grower/finisher feed, while 22% of the protocols involved a synthetic product (either on its own or in combination product) during the whole grow-out cycle (Fig. 4).

In respect to the basic coccidiosis control postulates, 77.3% of the respondents "strongly agree" with the need to rotate between different coccidiosis control products in order to maximize program performance while preserving long term efficacy. 66.7% are aware of the cross-resistance between products with similar chemical structure and mode of action, so agree that rotation should be carried out between products of different classes. 69.3% of the respondents "strongly agree" that "resting" the anticoccidial drugs is needed to allow them restoring their efficacy and 62.7% "strongly agree" with the importance of at least an annual chemical clean-up (Fig. 5).

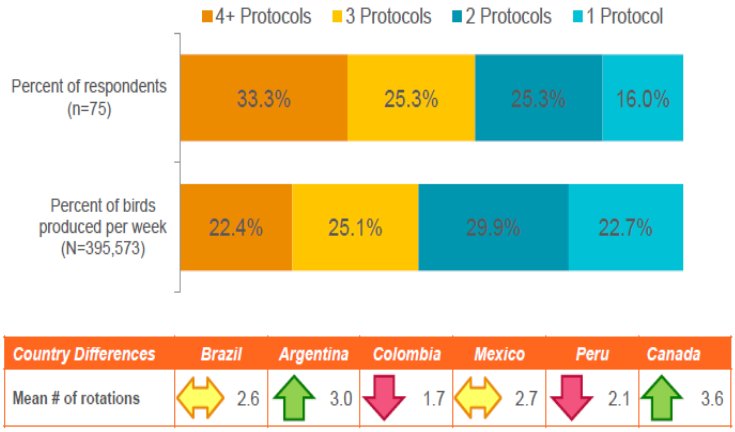

Fig. 3 Number of rotations in the last 12 months Arrows for the different countries indicate more than average, average or below average frequency of rotation.

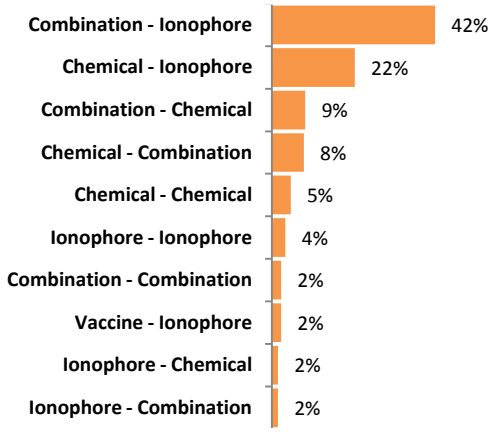

Fig.4 Types of coccidiosis prevention programs used in the last 12 months

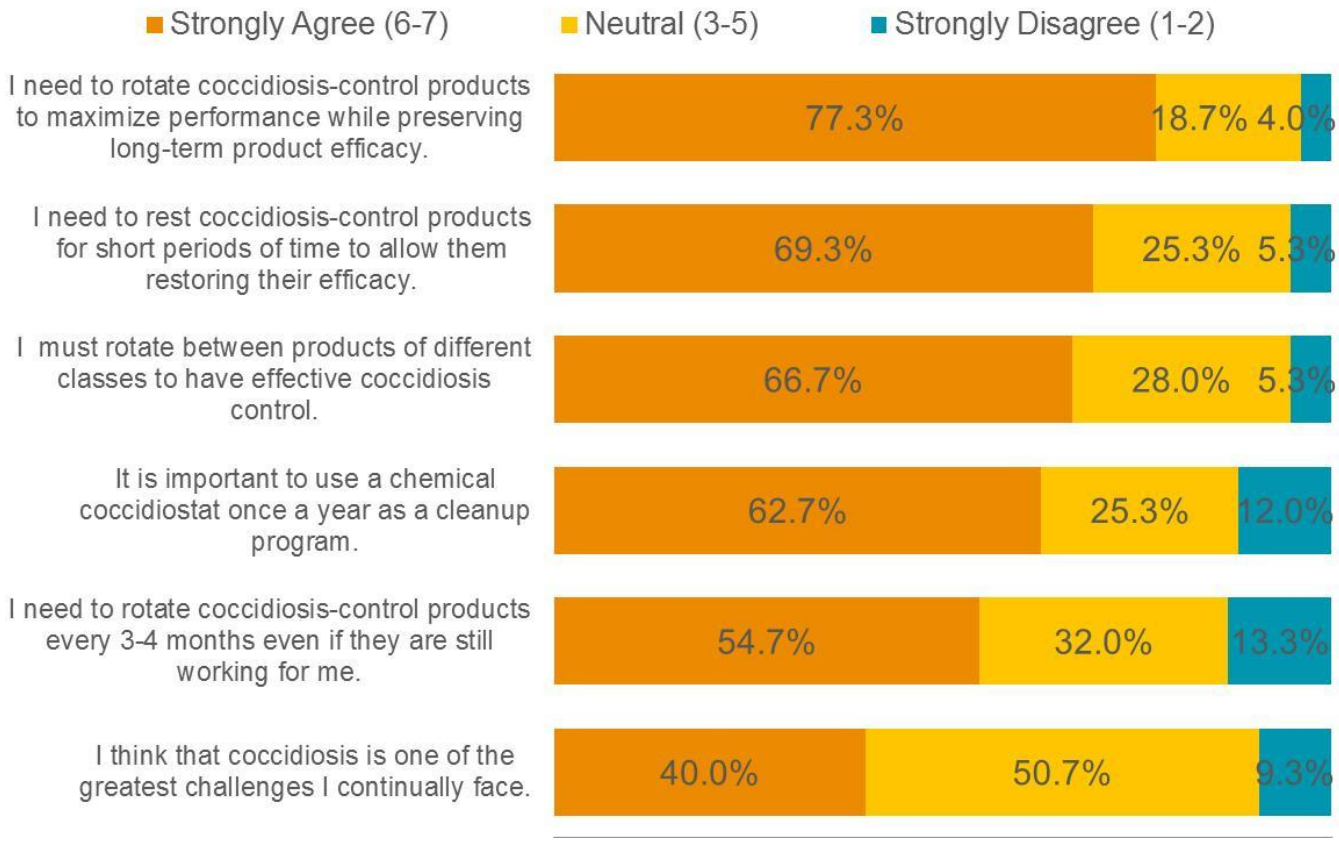

Fig.5 Agreement with the basic coccidiosis control postulates.

Discussion and Conclusions

The study data indicated that there was considerable understanding and agreement with the basic coccidiosis prevention postulates. The majority of respondents consented with the basic anticoccidial prevention postulates: (i) not to use same drug for too long, as it is generally acknowledged that overuse of anticoccidial products will eventually lead to the development of resistance (Mathis *et al*., 1984; Chapman, 1986; 1997 and 2007); (ii) to give a rest period to all products

that share a similar resistance mechanism, so that the *Eimeria* population is not exposed to the same/similar drug, which is beneficial for restoration of sensitivity (Chapman and McFarland, 2003); (iii) to avoid the use of drugs with similar structure and mode of action avoiding cross-resistance (McDougald *et al*., 1987; Bedrnik *et al*., 1989; Chapman and Hacker, 1994; Chapman, 2007; Marien et al., 2007), by rotating between products of different classes; and lastly (iv) the use of a synthetic drug at least once yearly to reduce infection pressure (DeGussem, 2005). Moreover, the majority of respondents agreed that the drugs should be rotated before having signs of inefficacy in order to avoid resistance development. This demonstrates a high level of judicious drug use. Only 9.4% of the respondents were not satisfied by the current control of coccidiosis in their respective operations, while 48% were "completely satisfied" and 42.7% "somewhat satisfied". In countries with low rotation rate, satisfaction of coccidiosis control was the least, indicating correlation between the use of rotation and efficacy of prevention

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P-264 (ID 149)

EFFECT OF DIFFERENT DIETARY LEVELS OF ENERGY AND PROTEIN ON PERFORMANCE OF JAPANESE QUAIL DURING GROWER PHASE

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Abstract

This research was conducted to investigate the effect of different levels of metabolisable energy and crude protein on performance, carcass characteristics and blood parameters of Japanese quail during grower phase (14-28 d of age). Nine hundreds 14-d-old male birds were used to examine 3 levels of energy (2900, 3050 and 3200 Kcal of ME/Kg of the diet) and 3 levels of protein (21.6, 24 and 26.4% CP) by a 3*3 factorial arrangement in a completely randomized design with 5 replicates of 20 birds. Feed intake, weight gain and feed conversion ratio (FCR) were measured during whole period. At the end of the experiment, 2 birds from each replicate were selected to measure the blood parameters (TG, total cholesterol, LDL and uric acid) and carcass characteristics. Respectively, the lowest and highest levels of energy and protein enhanced (P<0.05) daily feed intake. Daily weight gain was not affected by the treatments (P>0.05), however, FCR was improved by the diets of 3050 and 3200 Kcal ME/Kg compared with that of 2900 Kcal ME/Kg. Energy and protein interaction was significant (P<0.05) for the relative weight of liver; the treatment with 3200 Kcal ME/Kg and 24% CP showed the highest value. Among blood parameters, uric acid was decreased (P<0.05) by the diets of 3050 and 3200 Kcal ME/Kg. Based on performance results, 3050 Kcal ME/Kg of the diet can be proposed for Japanese quail nutrition during the grower phase.

Keywords: crude protein, grower phase, Japanese quail, metabolisable energy, performance

Introduction

Energy and protein are primary nutritional requirements of all classes of animals and types of production, including growing quails. These requirements must be met before requirements for other nutrients are addressed. Although few works have been conducted to investigate the effect of metabolisable energy in Japanese quail nutrition (Kaur et al., 2008), there is a lack of information on energy levels more than 3100 Kcal/kg, alone or in combination with different levels of crude protein. In addition, the literature is silent on the effect of different levels of energy and protein in different phase of growth period, separately. Therefore, this research was conducted to investigate the effect of different levels of metabolisable energy and crude protein on growth performance, carcass characteristics and blood parameters of Japanese quail (*Coturnix coturnix Japonica*) during grower phase (14 to 28 d of age).

Material and Methods

Nine hundreds 14-d-old male birds were used to examine 3 levels of energy (2900, 3050 and 3200 Kcal of ME/Kg of the diet) and 3 levels of protein (21.6, 24 and 26.4% CP) by a 3*3 factorial arrangement in a completely randomized design with 5 replicates of 20 birds (table 1). All the groups were subjected to similar management practices (brooding, lighting, feeding and watering) throughout the experiment except the diets offered. Lighting cycle was 23L:1D and brooding temperature gradually decreased from 37°C during the initial 3 days to 22- 24°C by 28 d of age. Water and experimental diets were supplied *ad libitum*. All the replicates had similar mean body weight (64±3 g) at d 14. All the birds received a regular diet (2900 Kcal/Kg ME and 24% CP) before the experiment (i. e. from 1 to 14 d of age). Daily feed intake, daily weight gain and feed conversion ratio (FCR) were measured during whole period. At the end of the experiment, 2 birds from each replicate were selected to measure the blood parameters (triglyceride, total cholesterol, LDL and uric acid) and relative weight of carcass and liver. All data were analyzed using GLM procedure of SAS. The treatment means were compared using Duncan test.

Table 1. Composition of experimental diets

	Experimental diets								
ME levels (Kcal/Kg)	2900	2900	2900	3050	3050	3050	3200	3200	3200
Crude protein levels (%)	21.6	24	26.4	21.6	24	26.4	21.6	24	26.4
Diet ingredients (%)									
Corn	53.9	49	24.95	51.9	47	42.61	50	44	39.5
Soybean meal (44% CP)	25	29.7	33	24.6	29.6	31.3	22.6	27	30
Corn gluten meal (60% CP)	7	8.5	11	7.7	9	12.5	9.6	11.2	13.7
Wheat bran	3.58	2.22	0.64	2.71	1.24	0.63	2.12	1.65	0.7
Wheat	5	5	5	5	5	5	5	5	5
Vegetable oil	0.8	1	0.9	3.3	3.5	3.3	5.7	6.3	6.3
Dicalcium phosphate	1.65	1.61	1.57	1.65	1.61	1.57	1.65	1.61	1.57
Calcium carbonate	1.05	1.05	1.12	1.05	1.05	1.12	1.05	1.05	1.12
Common salt	0.2	0.2	0.18	0.2	0.2	0.18	0.2	0.2	0.18
Sodium bicarbonate	0.42	0.43	0.46	0.42	0.43	0.46	0.42	0.43	0.46
Potassium bicarbonate	0.3	0.14	0	0.35	0.2	0.2	0.49	0.33	0.2
L-threonine	0.21	0.23	0.23	0.21	0.23	0.23	0.21	0.23	0.23
L-lysine hydrochloride	0.39	0.42	0.45	0.41	0.44	0.5	0.46	0.5	0.54
Mineral premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Calculated analysis									
ME (Kcal/Kg)	2914	2911	2915	3054	3048	3048	3203	3204	3203
Crude protein (%)	21.62	24	26.4	21.6	23.97	26.42	21.65	24	26.35
Lysine (%)	1.23	1.37	1.49	1.23	1.38	1.49	1.23	1.37	1.5
Methionine+Cystine (%)	0.74	0.81	0.89	0.74	0.81	0.9	0.75	0.82	0.91

[1]Each Kg of vitamin premix contained; vitamin A, 3500000 IU (retinyl acetate); cholecalciferol, 1000000 IU; vitamin E, 9000 IU (dl-a-tocopheryl acetate); menadione, 1000 mg; thiamine, 900 mg; riboflavin, 3300 mg; niacin, 5000 mg; pantothenic acid, 15000 mg; pyridoxine, 150 mg; folic acid, 500 mg; vitamin B_{12}, 7.5 mg; choline, 500 mg; biotin, 50 mg. Each Kg of mineral premix contained; manganese, 50000 mg; zinc, 50000 mg; iron 25000 mg; copper 50000 mg; iodine, 500 mg; selenium, 100 mg; cobalt 90 mg.

Results

Respectively, the lowest and highest levels of energy and protein enhanced daily feed intake (table 2). In poultry, it has been shown that feed intake increase in response to low levels of energy and decrease due to high crude protein or amino acid imbalance of the diets. A higher feed intake with decrease in dietary energy concentration was to compensate energy intake. Daily weight gain was not affected by the treatments, however, FCR was improved by the diets of 3050 and 3200 Kcal ME/Kg compared with that of 2900 Kcal ME/Kg. The results of growth performance indicate Japanese quail may need more concentration of energy than that suggested by the National Research Council (2900 Kcal ME/Kg). There was no interaction between energy and protein for performance parameters. Energy and protein interaction was significant for the relative weight of liver; the treatment with 3200 Kcal ME/Kg and 24% CP showed the highest value. There was no significant difference among treatments for relative weight of carcass. Among blood parameters, uric acid was decreased by the diets of 3050 and 3200 Kcal ME/Kg. The reduction of blood uric acid may be due to the improved protein utilization at high levels of energy. This result can explain the better performance of Japanese quail given diets with 3050 or 3200 Kcal ME/Kg. Main effects and also the interaction of energy and protein were not signifi-

cant for other blood parameters and these results were contrary to our expectation, because high concentration of energy usually enhance the lipogenesis and increase the blood lipid parameters in animals.

Table 2. Effect of different levels of ME and CP on growth performance[1] (14 to 28 d of age) carcass characteristics (g/100 g body weight, on d 28) and blood parameters (mg/dl, on d 28) of Japanese quail during grower phase

Treatments											
			DWG (g)	DFI (g)	FCR	Carcass	Liver	TG	TC	LDL	Uric acid
ME (Kcal/Kg)	2900		6.29	18.07[a]	2.88[a]	57.88	2.14	94.46	187.80	82.13	2.80[a]
	3050		6.29	16.84[b]	2.69[b]	57.50	2.15	90.86	177.20	71.08	1.57[b]
	3200		6.17	15.80[c]	2.58[b]	56.77	2.14	89.86	179.33	69.54	1.26[b]
SEM			0.144	0.225	0.059	0.439	0.059	4.862	8.649	4.567	0.222
CP (%)		21.6	6.18	16.45[b]	2.68	57.95	2.06	87.93	195.67	81.41	1.58
		24	6.11	16.98[ab]	2.79	56.84	2.13	88.80	173.80	70.71	2.10
		26.4	6.66	17.29[a]	2.69	57.37	2.24	98.46	174.87	70.64	1.94
SEM			0.144	0.225	0.059	0.439	0.059	4.862	8.649	4.567	0.222
ME * CP	2900	21.6	6.41	18.23	2.58	58.18	1.93[b]	87.60	208.60	94.36	2.54
	2900	24	5.83	15.73	2.72	58.57	2.24[ab]	85.80	188.00	75.86	1.36
	2900	26.4	6.32	15.38	2.46	57.09	2.01[ab]	90.40	190.40	74.02	0.86
	3050	21.6	6.07	18.05	2.98	56.84	2.23[ab]	88.60	167.40	72.24	3.42
	3050	24	6.47	17.20	2.66	57.31	1.93[b]	90.40	170.60	70.30	1.64
	3050	26.4	5.80	15.67	2.72	56.36	2.17[ab]	87.40	183.40	69.60	1.26
	3200	21.6	6.41	17.94	2.82	58.62	2.24[ab]	107.20	187.40	79.80	2.44
	3200	24	6.59	17.59	2.68	56.63	2.29[a]	96.40	173.00	67.10	1.72
	3200	26.4	6.40	16.34	2.57	56.86	2.17[ab]	91.80	164.20	65.02	1.68
SEM			0.250	0.389	0.102	0.760	0.103	8.421	14.981	7.911	0.385
							P values				
ME			0.78	0.01	0.01	0.20	0.98	0.78	0.66	0.11	0.01
CP			0.19	0.03	0.36	0.21	0.12	0.24	0.14	0.17	0.25
ME * CP			0.18	0.08	0.60	0.03	0.47	0.82	0.70	0.80	0.44

Means within columns with no common superscript differ significantly (P<0.05).

[1]Daily Weight Gain (DWG), Daily Feed Intake (DFI) and Feed Conversion Ratio (FCR).

Conclusions

Based on performance results, 3050 Kcal ME/Kg of the diet can be proposed for Japanese quail nutrition during the grower phase. In addition, low level of crude protein did not affect the performance adversely and can be considered in formulation of quail diets.

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P-266 (ID 6)

STARCH AND PROTEIN INTESTINAL UPTAKES IN TANDEM INFLUENCE WEIGHT GAIN OF BROILER CHICKENS

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Abstract

Phosphorus-adequate, maize-based diets, without and with 500 FTU/kg *Buttiauxella* phytase, were offered to broilers to assess the effects of phytase on starch and amino acid digestive dynamics at 40 days post-hatch. Starch and protein (sum of 16 amino acids) disappearance rates from four small intestinal segments and starch:protein disappearance rate ratios were calculated. The multiple linear regression equation ($r = 0.936$; $P < 0.001$) indicated that increasing protein disappearance rates from the three anterior small intestinal segments advantages weight gains; whereas, increasing starch disappearance are disadvantageous. There was a negative, linear relationship ($r = -0.766$; $P < 0.005$) between starch:protein disappearance rate ratios at the proximal ileum with 40-day weight gains. Phytase supplementation effectively condensed starch:protein disappearance rates in the three distal small intestinal segments ($P < 0.02$ - < 0.001). The branched chained amino acids were the most indicative of the sixteen amino acids. Starch:isoleucine ($r = -0.832$; $P < 0.001$) starch:valine ($r = -0.810$, $P < 0.001$) and starch:leucine ($r = -0.752$, $P < 0.005$) disappearance rate ratios were negatively correlated to weight gains. It is evident from the present study that intestinal uptakes of starch and protein in tandem have a marked influence on weight gains that were favourably manipulated by phytase with the suggestion that branch-chained amino acids may play a a pivotal role.

Keywords: digestive dynamics, branch-chain amino acids, phytase, extra-phosphoric effects

Introduction

The probability is that phytate has anti-nutritive properties in respect of energy utilisation in poultry and that phytase generates reciprocal 'extra-phosphoric' energy responses (RAVINDRAN and SELLE, 2010). As starch is the dominant energy source, it follows that phytase responses at least partially stem from improvements in starch utilisation. As ileal starch digestibility is inherently high, it may prove instructive to determine apparent starch digestibility coefficients in anterior small intestinal segments, where starch digestion is less complete, in order to detect responses to phytase and its effects on starch digestive dynamics. Similarly with amino acids, while the capacity of phytase to improve ileal amino acid digestibility is established which is not the case with amino acid digestive dynamics. Increasing evidence suggests that starch and protein utilisation should not be considered separately but in tandem (LIU and SELLE, 2015). Therefore this investigation considers the effects of phytase on both starch and protein digestion dynamics in relation to weight gains of broilers.

Materials and Methods

The feeding study consisted of P-adequate, maize-based diets without and with 500 FTU/kg *Buttiauxella* phytase; each dietary treatment was offered to eight replicate cages with six birds per cage. Diets were steam-pelleted at a conditioning temperature of 84°C and fed as crumbles. At day 40, all birds were weighed and euthanised to take digesta samples from four small intestinal segments [proximal jejunum (PJ), distal jejunum (DJ), proximal ileum (PI), distal ileum (DI)] which were pooled for each cage. Starch concentrations in diets and digesta were determined by a procedure based on dimethyl sulfoxide, α-amylase and amyloglucosidase (MAHASUKHONTHACHAT et al., 2010). Amino acid concentrations of diets and digesta samples were determined following 24 hour liquid hydrolysis at 110ºC in 6M HCl and then 16 amino acids are analysed using the Walters AccQTag Ultra chemistry on a Waters Acquity UPLC. The apparent digestibility coefficients of starch and amino acids in four small intestinal sites were calculated from the following equation where acid insoluble ash (AIA) was the dietary marker:

$$\text{Apparent digestibility coefficient} = \frac{[(\text{nutrient/AIA})_{diet} - (\text{nutrient/AIA})_{digesta}]}{(\text{nutrient/AIA})_{diet}}$$

Starch and protein disappearance rates (g/bird/day) from four small intestinal segments were calculated from the following equation:

$$\text{Disappearance rate} = \text{feed intake}_{(g/bird/day)} \times \text{dietary nutrient}_{(g/kg)} \times \text{digestibility coefficient.}$$

Protein concentrations in diets were based on the sum of the 16 amino acids assessed and digestibility coefficients were calculated from the average values of the 16 amino acids quantified. Experimental data was analysed as a one-way ANOVA of dietary treatments using the IBM® SPSS® Statistics 20 program (IBM Corporation. Somers, NY USA).

Results

Inclusion of 500 FTU/kg *Buttiauxella* phytase in P-adequate, maize-based broiler diets increased weight gains by 11.8% (2937 versus 2627 g/bird; P < 0.001**)** at 40 days post-hatch. Starch to protein disappearance rate ratios in the distal jejunum, proximal ileum and distal ileum were significantly condensed following phytase supplementation as shown in Table 1. In these three segments the average ratio was narrowed by 7.36% from 2.99 to 2.77:1

Table 1. Effects of exogenous phytase on starch:protein disappearance rate ratios in four small intestinal segments in broilers offered maize-based diets at 40 days post-hatch.

Treatment	Starch:protein disappearance rate ratios			
	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum
0	2.93	3.22	2.90	2.85
500 FTU/kg	2.44	2.78	2.79	2.73
SEM	0.2090	0.1041	0.0129	0.0289
Significance (P =)	0.132	0.013	< 0.001	0.015

The was a negative linear relationship (r = -0.766; P < 0.005) between starch:protein disappearance rate ratios at the proximal ileum with weight gains. Relationships between weight gain and starch:amino acid disappearance rate ratios were deduced where the branched chained amino acids were the most closely correlated. Starch:isoleucine (r = -0.832; P < 0.001) starch:valine (r = -0.810, P < 0.001) and starch:leucine (r = -0.752, P < 0.005) disappearance rate ratios were negatively correlated to weight gains as shown in Figure 1.

Conclusions

The negative, linear relationship (r = -0.766; P < 0.005) between starch:protein disappearance rate ratios at the proximal ileum with 40-day weight gains is an important outcome as it demonstrates the interactive impact of glucose and amino acid intestinal uptakes on broiler performance. The multiple linear regression equation (r = 0.936; P < 0.001)

$$y_{\text{weight gain (g/bird)}} = 857.53 + 109.12 * \text{protein}_{(g/bird/day)} - 13.34 * \text{starch}_{(g/bird/day)}.$$

clearly demonstrates that it is advantageous to accelerate protein digestion but retard starch digestion.

As phytase condensed starch:protein disappearance rate ratios to significant extents in the three caudal small intestinal segments it positively impacted on starch and protein digestion in tandem. In this respect the impact of phytase on branched-chain amino acids may be pivotal. The antagonism between branched-chain amino acids is rcognised and this probably stems from their systemic catabolism in broilers (CALVERT et al., 1982). Also, it appears that among essential amino acids, branched-chain amino acids are especially suscpetible to catabolism in the gut mucosa in pigs (CHEN et al., 2007). The effects of phytase on the digestive dynamics of branch-chained amino acids in respect of their catabolism in the gut mucosa appears to merit further attention. In conclusion, this study has demonstrated that phytase possesses 'extra-phosphoric' effects in respect of the digestive dynamics of starch/glucose and protein/amino acids via the manipulation of their disappearance rate ratios. Narrower starch:protein disappearance rate ratios benefit weight gains. It appears that the impact of phytase on intestinal uptakes of amino acids may be more pronounced than on glucose which would should favour narrower strach:protein disappearance rate ratios and benefit broiler performance.

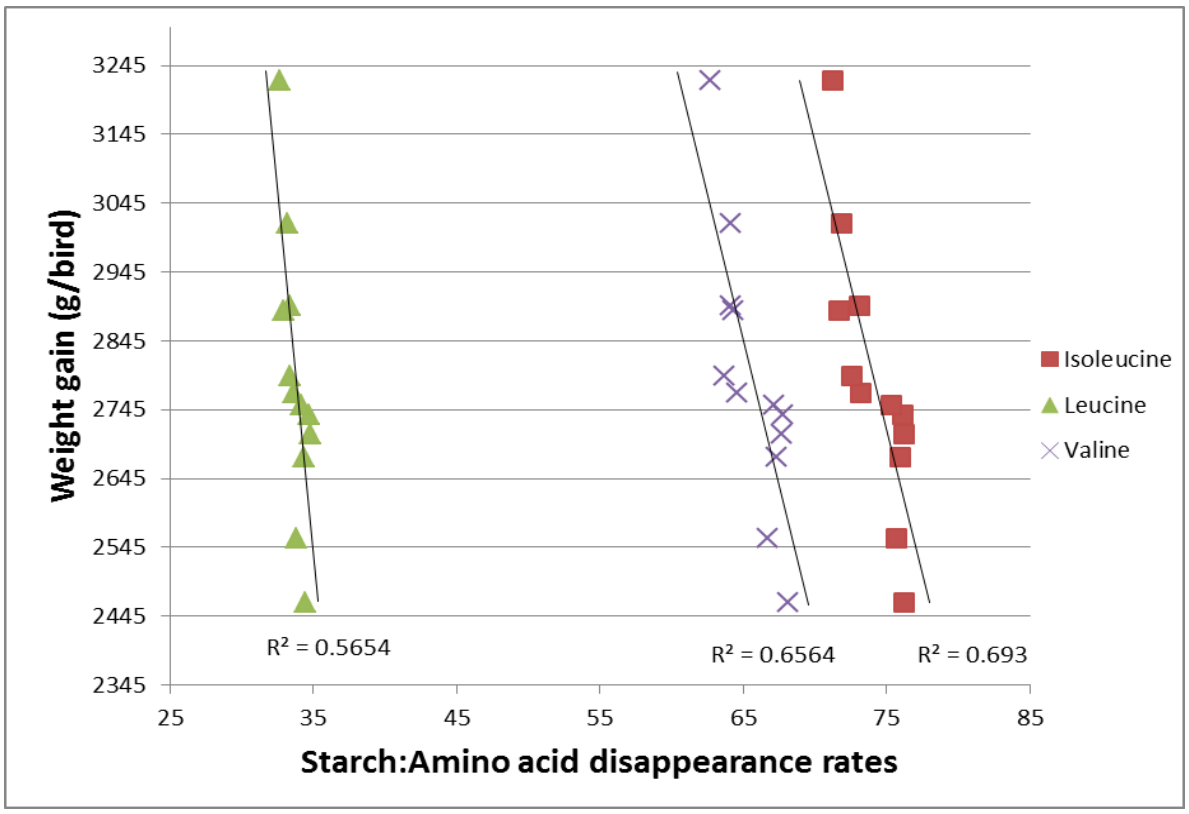

Figure 1. Linear relationship between starch:isoleucine (r = -0.832; P < 0.001), starch:leucine (r = -0.752; P = 0.005) and starch:valine (r = -0.810; P < 0.001) disappearance rate ratios and weight gains at 40 days post-hatch.

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P-267 (ID 158)

THE EFFECT OF GENOTYPE, HOUSING SYSTEM AND CALCIUM ON OVIPOSITION TIME AND EGG SHELL QUALITY

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Abstract

The aim of the study was to evaluate the effect of housing system (cages vs. litter), genotype (commercial hybrid Lohmann White and pure breed Czech Hen) and calcium content in feed mixture (3.0 % vs. 3.5 %) on time of oviposition and egg shell quality. Oviposition time was evaluated during laying cycle at 6.00, 10.00 and 14.00 h. Eggs for quality assessment were collected in a four week interval, all eggs laid from each cage or litter pen at each collection time. The significant interaction of genotype, housing and Ca (P=0.044) was observed in oviposition time. The highest number of eggs was laid by Czech Hen on litter and 3.5 % Ca at 6.00 h (21.28 %) and the lowest (0.85 %) by the same genotype in cage on 3.0 % Ca at 10.00 h. Also egg weight was affected by the significant interaction of genotype, housing and Ca (P=0.001); however, there was not interaction of genotype, housing, Ca and oviposition in egg weight. The heaviest eggs were in Lohmann White in cages with 3.0 % Ca (62.1 g). Egg shell quality was affected by genotype and time of oviposition. The significant interaction of genotype, oviposition and Ca in egg shell strength (P=0.003) revealed the strongest shells in Lohmann White in cages with 3.5 % Ca at 6.00 h. Results of the study show of interactions of genotype, housing and Ca in oviposition time as well as in egg shell quality.

Keywords: laying hens, oviposition, strain, cage, litter, calcium, egg shell

Introduction

Oviposition time plays a key role in determining egg shell quality because the amount of deposited shell is a linear function of the time spent in the shell gland. Eggs being normally laid between 7.30 and 16 h (Campo et al., 2007). Egg shell characteristics are better in the afternoon eggs (TŮMOVÁ et al., 2009, 2013). Time of oviposition and egg shell quality are affected by genotype (CAMPO et al., 2007, TŮMOVÁ et al., 2009, 2013), housing system (TŮMOVÁ et al., 2009, 2013). There are a few data of the effect of Ca on oviposition and egg shell quality (NOVO et al., 1997). The aim of the study was to evaluate the effect of housing system (cages vs. litter), genotype (commercial hybrid Lohmann White and pure breed Czech Hen) and calcium content in feed mixture (3.0 % vs. 3.5 %) on time of oviposition and egg shell quality.

Material and methods

The experiment with 126 laying hens of Lohmann LSL (Lohmann) and pure breed Czech Hen (CH) were housed in conventional cages Eurovent (72 hens, 550 cm^2 / hen, 3 hens in a cage, 12 cages for genotype) and in six littered pens (60 hens, 7 $hens.m^{-2}$, 10 hens / pen and 3 pens for each genotype) was carried out from 20 to 60 weeks of age. Laying hens in both housing systems were fed commercial type of feed mixtures which differed only in Ca content, 3.5% respectively 3.0%. The daily photoperiod consisted of 15 h light and 9 h darkness. Oviposition was evaluated according to a collection time which was at 6.00, 10.00 and 14.00 during the whole experiment. Eggs for quality assessment were collected in a four week interval, two days in row, all eggs laid from each cage or litter and there were analysed in total 1496 eggs. Eggs were weighed, and the shell strength was determined by the shell-breaking method using a QC-SPA device (TSS York, UK). Egg shell thickness was evaluated by QCT shell thickness micrometer (TSS York, UK). Egg shell proportion was calculated from egg shell weight, which was determined after drying, and egg weight.

Time of oviposition and egg shell quality data were evaluated by four-way (oviposition, housing, genotype, and Ca level interaction) analysis of variance using the GLM procedure of SAS (SAS Institute Inc, Cary, Nc, 2003).

Results and discussion

The results of oviposition (Table 1) show the significant interaction of all factors evaluated in the present study (P=0.044). The highest number of eggs was laid by Czech Hen on litter and 3.5 % Ca at 6.00 h (21.28 %) and the lowest (0.85 %) by the same genotype in cage on 3.0 % Ca at 10.00 h. Results corresponds with CAMPO et al. (2007), TŮMOVÁ et al. (2009, 2013) that different genotypes vary in their laying pattern, litter housing delayed oviposition time (TŮMOVÁ et al., 2009, 2013). Higher dietary calcium had a minor effect on oviposition in Lohmann hens which agrees with NOVO et al. (1997) but decreased a number of laid eggs at 14.00 h in Czech Hen.

Egg weight (P=0.001) and egg shell strength (P=0.036) were affected by the significant interaction of genotype, housing and Ca (Table 2). Results show that egg weight and egg shell strength can be affected by interaction of different factors which is in agreement with our previous data (TŮMOVÁ et al., 2009, 2013). Lohmann hens produced eggs with higher egg shell percentage (P=0.001) and thickness (P=0.001) which is a result of long-term selection for these characteristics, and corresponds with our previous results (TŮMOVÁ et al., 2013).

Acknowledgement

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Table 1 Mean number of eggs from two genotypes housed in cages and on litter and fed two Ca levels (%)

Time of collection	Housing system	Dietary Ca level (%)	Lohmann	Czech Hen	Significance
6.00	Cage	3.0	2.69	14.89	
		3.5	2.29	7.66	
	Litter	3.0	2.61	7.66	
		3.5	4.98	21.28	
10.00	Cage	3.0	14.86	0.85	
		3.5	12.96	2.13	0.044
	Litter	3.0	9.49	3.40	
		3.5	18.97	2.55	
14.00	Cage	3.0	10.04	11.49	
		3.5	9.41	8.08	
	Litter	3.0	3.95	8.94	
		3.5	7.75	11.06	

Table 2 Mean egg weight and egg shell measurements of eggs collected at three times during a day from two genotypes housed in cages and on litter and fed two Ca levels

Time of collection	Genotype	Housing system	Dietary Ca level (%)	Egg weight (g)	Egg shell percentage (%)	Egg shell thickness (mm)	Egg shell strength (g/cm^2)
6.00	Lohmann	Cage	3.0	61.3^{b}	11.9	0.34	3964
			3.5	61.6^{b}	12.3	0.36	4603
		Litter	3.0	59.2^{d}	11.9	0.34	3944
			3.5	62.0^{ab}	12.2	0.36	4259
	CH	Cage	3.0	47.4^{g}	11.4	0.32	4500
			3.5	47.6^{g}	10.6	0.29	3620
		Litter	3.0	49.9^{e}	12.0	0.35	4501
			3.5	46.7^{g}	11.4	0.32	4053
10.00	Lohmann	Cage	3.0	61.8^{b}	12.3	0.35	4526
			3.5	60.2^{c}	12.6	0.36	4502
		Litter	3.0	58.5^{d}	12.1	0.34	4294
			3.5	60.8^{c}	12.3	0.36	4541
	CH	Cage	3.0	51.3^{e}	13.5	0.38	5432
			3.5	57.1^{d}	11.6	0.33	3051
		Litter	3.0	48.2^{f}	11.8	0.33	4131
			3.5	49.9^{e}	11.8	0.34	4347
14.00	Lohmann	Cage	3.0	62.1^{ab}	12.0	0.35	4169
			3.5	60.6^{c}	12.3	0.35	4153
		Litter	3.0	57.8^{d}	12.0	0.35	4399
			3.5	59.8^{d}	12.2	0.36	4330
	CH	Cage	3.0	46.9^{g}	11.8	0.31	4385
			3.5	54.0^{d}	10.9	0.30	3871
		Litter	3.0	52.8^{e}	11.8	0.32	4090
			3.5	50.8^{f}	11.3	0.31	4281
Significance: Genotype				0.001	0.001	0.001	0.002
Housing				0.001	NS	0.002	NS
Ca				NS	0.011	NS	NS
Genotype*housing*Ca				0.001	NS	NS	0.036

P-268 (ID 118)

THE EFFECT OF EARLY FEED RESTRICTION, GENOTYPE AND SEX ON PERFORMANCE AND CARCASS COMPOSITION OF BROILER CHICKENS

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Abstract

The purpose of this study was to evaluate the effect of intensity of early feed restriction along with genotype and sex on performance and carcass composition in two genotypes and both sexes of broiler chicken. In three-factorial experiment (2x2x3) a total of 4860 one day old broiler chickens were divided into 12 groups (genotypes Ross 308 and Cobb 500; males and females; groups ADL, R1 and R2). ADL chickens were fed *ad libitum* over the whole experiment, whereas the R1 a R2 chickens had the feed restriction (R1 80% of ADL group, R2 65% of ADL group) between 8th and 14th day of age. Feed restriction reduced live weight at 35 days of age (P=0.001), feed intake (P=0.001) and mortality (P=0.021; 8.55 % Ross ♂ ADL and 2.25 % Cobb ♂ R2). Genotype affected live weight at 35 days of age (P=0.004) in favour of Cobb hybrid. Regarding the sex, higher live weight at 35 days of age (P=0.001) and feed intake (P=0.001) were found in males. There was a minor effect of evaluated factors on carcass composition. It can be concluded that feed restriction significantly decreased mortality without negative effect on growth, feed conversion ratio and carcass composition.

Keywords: chicken, feed restriction, genotype, performance, carcass composition

Introduction

Modern genotypes of broiler chicken are due to genetic selection able to reach required slaughter weight in shorter period of time. On the other hand, this increased growth rate brings also several negative responses such as greater susceptibility to skeletal and metabolic disorders or increased fat deposition (LIPPENS et al., 2000).

One of the possibilities, how to avoid rapid growth rate and negatives associated with it, is an application of early feed restriction. Although early feed restriction reduces growth performance, compensatory growth in realimentation period allows to reach required live weight. Slower growth rate in early stages of life is beneficial in terms of feed efficiency and breeding cost (ZHAN et al., 2007). Regarding the fat deposition, results obtained from feed restriction programs to reduce the carcass fat content in broiler chickens have been inconsistent. Such inconsistency may relate to different feeding strategies applied, which may affect the bird's response to feed restriction (URDANETA-RINCON and LEESON, 2002). BENYI et al. (2009) reported that another factor that influences the reaction of broiler chickens to feed restriction is the genetic makeup of chickens. Genetic potencial not only affects the growth intensity and body composition of the bird because of its effects on nutrient requirement, but also affects compensatory growth of broilers subjected to feed restriction.

The aim of this study is to evaluate the effect of early feed restriction, genotype and sex on growth performance and carcass composition of broiler chickens.

Material and Methods

A total of 4860 one day old broiler chickens were weighed and divided into 12 groups (405 chickens per group) according to genotype (Ross 308 and Cobb 500), sex and feeding regime (ADL, R1 and R2). ADL chickens were fed *ad libitum* over the entire experiment, whereas the R1 and R2 groups had the feed restriction between 8th and 14th day of age. The R1 group received 80 % of ADL group's feed intake, whereas the R2 received 65 % of ADL group's feed intake. Before and after the restriction period both restricted groups were fed *ad libitum* until the end of the experiment.

Chickens were housed in pens on litter (135 chickens per pen) and fed complete feed mixtures, BR1 (crude protein content 23.39 %) to 14 days of age, BR2 (crude protein content 20.89 %) to 28 days of age and BR3 (crude protein content 19.98 %) until the end of experiment which ran until 35 days of age. Weighing was conducted weekly and feed consumption was recorded daily. At the end of the fattening period 8 birds of each group were slaughtered on average weight of the group. After the slaughtering carcass composition was evaluated according to standard method.

The results were processed in SAS software (SAS Institute Inc., Cary, NC, 2003) by multiple analysis of variance with interaction of genotype, sex and group.

Results and discussion

Results of growth performance are presented in Table 2. No significant interactions between genotype, sex and group were recorded. Feed restriction significantly reduced live weight at 35 days of age (P=0.001), weight gain (P=0.017), feed intake (P=0.001) and mortality (P=0.021). With increasing intensity of feed restriction measurements more decreased. Genotype affected significantly live weight at 35 days of age (P=0.004) as well in favour of Cobb hybrid. Regarding the sex, higher live weight at 35 days of age (P=0.004), weight gain (P=0.017) and feed intake (P=0.001) were in males. These results are in agreement with LIPPENS et al. (2000) and ZHAN et al. (2007).

Table 2: Growth performance

Genotype	Sex	Group	LW 1 (g)	LW 35 (g)	WG 1 – 35 (g/d)	FI 1 – 35 (g/d)	FCR 1 – 35	Mortality 1 – 35 (%)
Ross	♂	ADL	43.87	2098	58.32	105.63	1.96	8.55
		R1	43.80	2026	56.67	99.09	1.87	6.30
		R2	43.85	1965	54.76	97.04	1.88	4.95
	♀	ADL	43.21	1920	53.44	90.98	1.81	5.85
		R1	43.41	1855	51.75	88.48	1.82	7.20
		R2	43.31	1836	51.14	89.25	1.84	2.70
Cobb	♂	ADL	46.72	2107	58.91	100.33	1.83	6.75
		R1	46.17	2019	56.51	97.17	1.83	4.50
		R2	46.22	1991	55.76	96.05	1.81	2.25
	♀	ADL	46.07	1966	54.91	92.79	1.80	4.50
		R1	45.87	1893	52.73	89.71	1.80	4.50
		R2	45.97	1847	51.48	87.61	1.80	3.60
Genotype			**0.001**	**0.004**	0.405	0.175	0.271	0.073
Sex			**0.001**	**0.001**	**0.001**	**0.001**	0.322	0.335
Group			0.281	**0.001**	**0.017**	**0.001**	0.925	**0.021**
Genotype x Sex x Group			0.788	0.201	0.893	0.176	0.887	0.551

LW 1 – live weight at 1 day of age, LW 35 – live weight at 35 days of age, WG 1 – 35 – weight gain 1 – 35 days of age, FI 1 – 35 – feed intake 1 – 35 days of age, FCR 1 – 35 – feed conversion ratio 1 – 35 days of age

Table 3 shows results of selected carcass parameters. There were no significant interactions between genotype, sex and group as well as group and genotype did not affect evaluated parameters. Significant effect of sex was found in abdominal fat share from carcass (P=0.001) with higher values in females and thig muscle share from carcass that was higher in males (P=0.022). Our results are similar to those of LIPPENS et al. (2000). On the contrary, ZHANG et al. (2007) reported significantly lower dressing percentage in restricted chickens.

Table 3: Selected carcass parameters

Genotype	Sex	Group	Dressing percentage (%)	Abdominal fat / carcass (%)	Breast muscle / carcass (%)	Thigh muscle / carcass (%)	Meat / carcass (%)
Ross	♂	ADL	71.13	1.83	25.02	21.31	46.33
		R1	78.11	1.96	26.48	20.53	47.01
		R2	72.29	2.02	25.26	21.35	46.62
	♀	ADL	73.15	2.19	26.55	20.29	46.84
		R1	73.55	2.13	24.73	19.01	43.74
		R2	74.10	2.30	25.88	20.72	46.60
Cobb	♂	ADL	73.28	1.22	26.43	21.02	47.46
		R1	73.18	1.53	25.94	22.33	48.28
		R2	74.07	1.92	25.35	21.11	46.45
	♀	ADL	74.08	2.15	27.07	20.74	47.82
		R1	74.27	2.12	25.27	20.98	46.26
		R2	74.83	2.35	27.88	20.08	47.96
Genotype			0.516	0.138	0.288	0.227	0.179
Sex			0.304	**0.001**	0.445	**0.022**	0.575
Group			0.107	0.137	0.674	0.964	0.744
Genotype x Sex x Group			0.990	0.790	0.161	0.859	0.915

Conclusions

Based on the obtained results it can be concluded that feed restriction significantly decreased mortality without negative effect on growth performance, feed conversion ratio and carcass composition.

Acknowledgement

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P-270 (ID 153)

EFFECTS OF DIFFERENT FEEDING STRATEGIES DURING REAR AND LAY ON EGG WEIGHT, AGE OF SEXUAL MATURITY AND PEAK PRODUCTION IN BROILER BREEDER FEMALES

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Summary

An experiment was conducted to investigate the effects of different feeding strategies during rear and lay on egg weight (EW), age of sexual maturity (ASM) and peak production (% and age) of broiler breeder females. A 2 × 3 × 2 factorial design was used with 2 crude protein (CP) levels (high = CPh; low = CPl) during rearing; high, standard and low dietary energy levels (3,000 = MEh1; 2,800 = MEs1; 2,600 = MEl1) during the first phase of lay; and standard and high dietary energy levels (2,800 = MEs2; 3,000 = MEh2) during the second phase of lay. A total of 2,880 one-day old Ross 308 breeder female chicks were placed in 36 pens and followed until 60 wk of age. EW was recorded on a daily basis. Weekly and average EW per phase was calculated. ASM was defined as age at 50% production. Peak production was determined as a 3-wk rolling average. No effects of rearing treatments were found. Dietary energy level during the first phase of lay did not affect EW during that period. However, a linear decrease in dietary energy level during the first phase of lay resulted in a linear decrease in EW during the second phase of lay. Birds fed the MEl1 diet showed a delayed ASM of 1.2 and 1.6 d, while age at peak production was advanced by 11.1 and 5.8 d compared to the MEh1 and MEs1 birds, respectively. Feeding birds the MEh2 diet decreased EW during the second phase of lay. It was concluded that different feeding strategies during rear and lay can affect EW, ASM and early production traits.

Keywords: broiler breeder, rearing, laying, feeding strategies, production traits

Introduction

Modern broiler strains show a 4 times higher daily growth while feed conversion ratio is less than half compared to strains in use 60 year ago (ZUIDHOF et al., 2014). This selection on increased growth efficiency has not only affected the progeny but also the parent stock (broiler breeders). The main goal in broiler breeder production is to provide fertilized eggs to produce maximum number of healthy and robust day-old broiler chicks. Besides a wide range of different methods, feed also play an important role in optimizing reproduction. For example, egg weight is affected by daily dietary protein and amino acids intake (LOPEZ and LEESON, 1995). More specific, a higher egg weight is caused by a higher daily intake of sulphur amino acids (effect on albumen and yolk) and/or higher daily intake of linoleic acid (effect on yolk). Daily protein intake during initial lay is important as a stimulus for development of the reproductive tract (LILBURN and MYERS-MILLER, 1990). Therefore, the objective of the study was to determine the effects of different dietary protein levels during rear and different dietary energy levels during lay on egg weight, age of sexual maturity and peak production in Ross 308 broiler breeder females.

Materials and methods

The effects of different dietary protein levels during rearing and different dietary energy levels during lay on body composition and reproduction were studied using a 2 × 3 × 2 factorial design with 2 CP levels (CPh = high protein; or CPl = low protein) during rearing between 2 and 22 wk of age; high, standard and low dietary energy levels during the first phase of lay between 22 and 45 wk of age (3,000, MEh1; 2,800, MEs1; and 2,600, MEl1, kcal/kg AME_n, respectively); and standard and high dietary energy levels during the second phase of lay between 45 and 60 wk of age (2,800, MEs2; and 3,000, MEh2, kcal/kg AME_n, respectively). A flock of 3,000 Ross 308 female broiler breeder day-old chicks was allotted to 36 floor pens (4.5 × 2.5 m) in two identical climate controlled rooms. The experiment started at d 14 with 80 pullets per pen. Due to mortality, sex errors and culling (removing the smallest), the number of pullets was gradually reduced to 70 at 22 wk of age. Males were reared elsewhere and introduced into the pens at 22 wk of age. Egg weights of all hatching eggs (settable and small) were recorded on a daily basis. Weekly and average egg weight per phase were calculated. Age of sexual maturity (ASM) was defined as age at 50% production and was determined by a linear interpolation of the week (in days) where birds passed 50% rate of lay. Peak egg production was determined as a 3-wk rolling average.

Results and discussion

No effects of rearing diets were found on egg weight, ASM, and peak production (Table 1). Dietary energy level during the first phase of lay did not affect egg weight during that period. However, a carryover effect on increased egg weight (+0.6 g) during the second phase of lay was found when birds were fed the MEh1 diet during the first phase of lay (low daily CP/AA intake) compared to the MEl1 birds (high daily CP/AA intake). A comparable effect was observed by SUN and COON (2005) when birds were fed a 5.5% higher dietary energy level. They found no effect on egg weight between 24 to 30 wk of age, however, egg weight between 30 and 65 wk of age was increased when birds received an isonitrogenous diet with a higher energy level during lay. No effect on egg weight between 23 and 48 wk of age was observed by JOSEPH et al. (2002) when birds received an 8% decreased daily CP intake. However, in an earlier experiment they observed a decreased effect on egg weight during initial lay (24 to 29 wk of age) when birds receive an approximately 10% lower daily CP intake (JOSEPH et al., 2000).

Table 1. Egg weight, ASM, peak egg production, and age at peak egg production as affected by dietary protein level (CP) during rearing (2 to 22 wk of age), dietary energy level (ME1) during the first phase of lay (22 to 45 wk of age), and dietary energy level (ME2) during the second phase of lay (45 to 60 wk of age) of Ross 308 broiler breeders[1]

Source	Egg weight phase 1 (g)	Egg weight phase 2 (g)	Egg weight laying period (g)	ASM (d)	Peak egg production (%)[4]	Age at peak egg production (d)
Protein rearing						
CPh	60.2	69.0	63.7	181.3	90.3	217.7
CPl	60.2	68.8	63.7	180.8	89.2	221.7
SEM	0.1	0.1	0.1	0.3	0.4	2.4
Energy lay 1						
MEh1	60.3	69.2[a]	63.9	180.8[b]	89.8	225.2[a]
MEs1	60.3	68.9[ab]	63.8	180.4[b]	89.6	219.9[b]
MEl1	60.1	68.6[b]	63.5	182.0[a]	89.9	214.1[c]
SEM	0.1	0.1	0.1	0.4	0.5	2.9
Energy lay 2						
MEs2	-	69.1[a]	63.8	-	-	-
MEh2	-	68.7[b]	63.7	-	-	-
SEM	-	0.1	0.1	-	-	-
P-value						
CP	0.68	0.35	0.84	0.24	0.094	0.25
ME1	0.41	0.012	0.099	0.019	0.91	0.037
ME2	-	0.024	0.40	-	-	-
CP x ME1	0.70	0.47	0.50	0.10	0.38	0.006
CP x ME2	-	0.68	0.60	-	-	-
ME1 x ME2	-	0.37	0.11	-	-	-

[a-c]Means within a column and within a source without a common superscript differ significantly ($P < 0.05$).

Birds fed the MEl1 diet showed a small delayed sexual maturity of, on average, 1.4 d compared to those fed the MEh1 and MEs1 diets (Table 1). Contrary to our findings, PISHNAMAZI et al. (2011) found that providing a low energy diet (2,800 vs. 2,900 kcal/kg ME) advanced sexual maturity with 2 d, while SUN and COON (2005) did not find an effect on sexual maturity when different dietary energy levels were provided. Thereafter the increase in rate of lay in the current

study differed between the treatment groups. An opposite effect of 11.1 and 5.8 d on age at peak production was found for the MEl1 birds compared to the MEh1 and MEs1 birds, respectively (Table 1). This may be due to the fact that birds fed the MEl1 diet received a 7.3 or 14.6% higher daily CP intake compared to the MEh1 and MEs1 diet, respectively. A high daily CP intake during initial lay could be a stimulus for development of the reproductive tract, resulting in an earlier peak egg production (LILBURN and MYERS-MILLER, 1990). Moreover, PISHNAMAZI et al. (2011) stated that a lower protein intake (19.7 vs. 20.4 g/d) during initial lay may hamper ovary development and, as a consequence, egg production.

Feeding birds the MEh2 diet decreased egg weight during the second phase of lay which was affected by a lower daily intake of sulphur amino acids and linoleic acid. In contrast to the current study, SUN and COON (2005) found an increased egg weight when birds received a high energy diet. The difference between the current study and the study of SUN and COON (2005) is that in the latter study, not only energy intake was increased, but also CP intake.

Conclusions

A low protein diet during rear did not affect egg weight, age of sexual maturity and peak production. A low energy diet (high daily protein intake) during the first phase of lay decreased egg weight during the second phase of lay, delayed ASM and advanced age at peak egg production. A high energy diet (low daily protein intake) during the second phase of lay decreased egg weight.

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THE EFFECT OF INCREASING METABOLIZABLE ENERGY, LEVEL AND DURATION OF FEED RESTRICTION ON PERFORMANCE AND INTESTINAL MORPHOLOGY IN BROILER CHICKENS

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Abstract

The effect of increasing metabolizable energy (ME), level and duration of feed restriction on performance, intestinal morphology and gastrointestinal pH was studied in broiler chickens fed pellet diets. A total of 480 Ross 308 male broiler chickens were allocated to 12 treatments, arranged factorially, with two levels of metabolizable energy (normal, and +100kcal/kg), two levels of feed restriction (90% and 85% restricted feed than recommended of Ross 308 management guide), and three periods of feed restriction duration including 14, 21 and 28 days. Each treatment was replicated 4 times. Results showed that BW was increased significantly in broilers fed on high-ME diets and 10% feed restriction (P<0.01). High-ME diets increased FCR at 7 days and reduced FCR at 14 and 21 days of age (P<0.05). Relative weight of ileum, duodenum, jejunum and cecum in birds fed high-ME diets with 85% feed restriction during 8-28 days were significantly increased (P<0.05). The depth of crypt in jejunum and ileum and villi/crypt index in jejunum increased with increasing ME level and decreased in 90% feed restriction at 42 of age (P<0.05). Increasing duration of feed restriction, elevated digesta pH of the crop at 42 days of age (P<0.01). Also, digesta PH of ileum at 42d of age was significantly decreased in 90% and 28d of feed restriction (P<0.01). In conclusion, Energy level and feed restriction affected weight gain, relative weight of intestinal parts, also pH of digesta in broiler chickens.

Keywords: Metabolizable energy, pellet, feed restriction, intestinal morphology

Introduction

Feeding strategies including pelleting and increasing metabolizable energy in broiler chickens resulted in higher growth performance and improving feed conversion ratio, while, increasing occurrence of metabolic disorders, fat deposition and mortality. One possible nutritional approach to reduce feed cost, improvement FCR and decreases the occurrence of metabolic disorders and mortality is to restrict feed intake of the birds. Improvement in FCR and decrease in metabolic disorders noted with the use of feed restriction programs is due to reduced overall maintenance requirements and basal metabolic rate in feed-restricted birds linked with a smaller body weight during early growth (Rincon and Lesson, 2002).The GIT of chickens responds very quickly and dramatically to changes in nutrient status and feed intake (Thompson and Applegate, 2006). Therefore, the main objective of this study was to determine the suitable level and timing of feed restriction (FR) regimes and level of metabolizable energy (ME) content on productivity and gastro intestinal tracts characteristics in broiler chickens.

Material and Methods

The research protocol was approved by the Razi university animal care and use committee. A total of 480 one-d-old Ross 308 male broilers were placed in 48 individual cages and 12 treatments, arranged factorially including: 2 levels of ME (normal, and normal+100 kcal.kg^{-1}), 2 levels of restriction level (90% and 85% restricted feed than recommended of Ross 308 management guide), and 3 periods of restriction duration (8-21, 8-28 and 8-35d of age). Each treatment replicated 4 times. Body weight and feed intake were recorded weekly. At 42d of age one chick per each replicate was randomly selected and dissected and tissue samples for intestinal morphology were collected (Barri et al. 2011). Measurement of pH were done based on Walk et al. (2012) procedure. Data analyzed using GLM procedure of SAS software (2011).

Results

No interaction between ME content of the diet and FR was observed for BW and FCR (Table 1). Increasing ME level of diet significantly improved BW and FCR from 8 to 21 and 1 to 42d of age (P<0.01), but interestingly, except for first week that decreased BW and elevated FCR (P<0.05). level of FR had no significant effect on BW and FCR at 8 to 21 and 22 to 42d of age but from 1 to 42d of age, high-restriction level (85%) decreased BW (P<0.01). Chickens subjected to 85% FR, needed to more time for complete recovery of BW rather than 90% FR (Lippenz et al., 2000). Applying feed

restriction during ages 8-35 days significantly affected performance and decreased BW (P<0.01), also increased FCR (P<0.05) at 22-42 and 1-42 days of age.

There was no significant difference between broilers fed high-ME diets or FR regimen in regard to villus hight (VH), and villus to crypt rate (VCR) within ileum (Table 2). An interaction between dietary ME and level of FR was observed for crypt dept (CD) of jejunum (P<0.05). Birds fed norm-ME diet and 90% FR had higher CD of jejunum that had significant difference with 85% and norm-ME bird groups (Table 4). Results of increase in ME and decrease in level of FR (90%) showed higher VH of jejunum and CD of ileum (P<0.05). Also, larger VCR in jejunum observed in broiler with high-ME that was 5.85 (P<0.01).

Influence of FR level (*P*<0.05) and interaction of level of FR × duration of FR (*P*<0.01) was significant for digesta pH of ileum (Table 2). In treatment with 28d of FR, ileum digesta pH in 90% FR groups was lowest content (Table 3). Gizzard digesta pH was not affected by ME, level and duration of FR. Minimum digesta pH of crop observed in birds recieved 14d restriction (8-21 days of age) that had significant difference with 21 and 28 days of restriction (*P*<0.01). Lower pH of the contents in ileum in treatment with norm-ME during 28d of restriction was seen, may be indicating higher population of lactic acid bacteria and better fermentation of food.

Conclusions

According to the results, elevating energy content in the first week of age, reduced BW, while improved BW and FCR during 2 to 6 w of age. Elevating dietary ME content increased crypt depth of ileum and villus height and villi/crypt rate in jejunum. Maximum villus height of jejunum and crypt depth observed in treatment with lower level of restriction. Also, pH of the crop was lowest in birds received 14 d of restriction. It seems, higher ME content and applying feed restriction in 90% of ross recomendation during 8-28 days (3 week) provide better productivity of birds fed pellet diet.

Table 1. Influence of metabolizable energy, levely and duration of feed restriction on performance of broilers

variable	FCR				BW (g)			
	1-7 d	8-21d	22-42d	1-42d	1-7 d	8-21d	22-42d	1-42d
ME (kcal/kg)	*	**	NS	**	*	**	NS	**
Norm	1.03±0.05^{b}	1.53±0.04^{a}	1.85±0.05	1.73±0.04^{a}	145.4±7.1^{a}	565.4±24.0^{b}	1797.4±66.1	2508.3±75.3^{b}
High	1.07±0.05^{a}	1.40±0.04^{b}	1.82±0.08	1.68±0.06^{b}	141.0±6.1^{b}	618.7±26.5^{a}	1827.1±103.4	2586.2±119.0^{a}
Level of FR (%)	NS	NS	NS	NS	NS	NS	NS	**
90	1.05±0.05	1.46±0.07	1.84±0.08	1.70±0.06	143.9±7.5	611.8±30.7	1826.6±91.1	2582.3±106.1^{a}
85	1.06±0.05	1.47±0.08	1.83±0.06	1.70±0.05	142.5±6.3	571.7±30.7	1797.9±82.5	2512.1±95.9^{b}
Duration of FR (age)	NS	NS	*	*	NS	NS	**	**
8 -21	1.04±0.03	1.46±0.07	1.80±0.07^{b}	1.68±0.05^{b}	143.9±4.6	592.7±35.6	1862.7±67.6^{a}	2599.3±84.8^{a}
8 -28	1.05±0.05	1.46±0.08	1.84±0.08^{ab}	1.71±0.07^{ab}	142.6±6.6	595.4±39.4	1837.3±89.0^{a}	2575.3±114.6^{a}
8 -35	1.05±0.07	1.48±0.08	1.86±0.05^{a}	1.72±0.04^{a}	143.1±9.2	587.2±36.5	1736.8±43.0^{b}	2467.1±66.1^{b}
SEM	0.01	0.01	0.01	0.01	0.10	5.3	12.6	15.3

$^{a–b}$ Means within columns with different superscripts are significantly different (*P* < 0.05).

No interaction between were observed for BW and FCR. *significant at P ≤0.05. **significant at P ≤0.01. NS: not significant.

Table 2. Influence of ME content, level and duration of feed restriction on jejunal and ileal morphology and digesta pH in intestine of broilers at d 42

			pH	Intestine Morphology					
				Jejunum			Ileum		
variable	Crop	Gizzard	Ileum	VH(µm)	CD(µm)	VCR	VH(µm)	CD(µm)	VCR
ME (kcal/kg)	NS	NS	NS	**	NS	*	NS	*	NS
Norm	5.16±0.96	4.31±0.33	5.06±0.46	121.2±23.7[b]	23.5±3.1	5.32±1.24[b]	103.9±15.0	23.4±2.0[b]	4.45±0.66
High	5.17±0.73	4.12±0.42	5.13±0.59	139.1±18.8[a]	23.8±2.0	5.85±0.63[a]	108.5±19.1	24.7±2.3[a]	4.36±0.59
Level of FR (%)	NS	NS	*	*	NS	NS	NS	*	NS
90	5.14±0.81	4.12±0.43	4.94±0.49	135.8±19.4[a]	24.1±2.9	5.70±1.01	108.4±19.0	25.0±2.3[a]	4.35±0.56
85	5.19±0.89	4.31±0.32	5.28±0.51	124.5±25.3[b]	23.2±2.3	5.46±1.02	103.5±14.7	23.3±1.8[b]	4.47±0.70
Duration of FR (age)	**	NS	NS	NS	NS	NS	NS	NS	NS
8 -21	4.10±0.47[b]	4.31±0.31	5.08±0.44	128.5±21.7	22.4±1.5	5.76±0.96	107.1±16.8	23.6±2.3	4.55±0.66
8 -28	5.67±0.42[a]	4.06±0.43	5.18±0.39	130.7±27.1	24.6±2.7	5.46±1.21	105.7±15.1	24.6±2.1	4.34±0.58
8 -35	5.66±0.37[a]	4.28±0.38	5.05±0.70	130.9±21.3	23.7±2.9	5.55±0.88	105.5±20.7	24.4±2.6	4.33±0.66
SEM	0.12	0.06	0.08	3.47	0.39	0.15	2.61	0.35	0.10
P-Values									
E×I	NS	NS	NS	NS	*	NS	NS	NS	NS
E×D	NS	NS	NS	NS	NS	NS	NS	NS	NS
I×D	NS	NS	**	NS	NS	NS	NS	NS	NS
E×I×D	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a–b] Means within columns with different superscripts are significantly different ($P < 0.05$).

VH: villus hight. CD: crypt dept. VCR: villus to crypt rate.

*: significant at P ≤0.05. **: significant at P ≤0.01. NS: not significant.

Table 3. Interaction of intensity FR and duration FR on pH of ileum at 42d of age

Level of FR (%)	Duration of FR (age)		
	8-21d	8-28d	8-35d
90	5.10±0.55[a]	5.14±0.42[a]	4.57±0.30[b]
85	5.06±0.35[a]	5.24±0.39[a]	5.52±0.37[a]

[a–b]Means within rows and columns with different superscripts are significantly different ($P < 0.05$).

Table 4. Interaction of ME level and intensity FR on jejunum CD (µm) of broilers at 42d of age

ME (Kcal/kg)	Level of FR (%)	
	90	85
Norm	24.76±3.46[a]	22.21±2.10[b]
High	23.40±1.79[ab]	24.11±2.17[ab]

[a–b]Means within rows and columns with different superscripts are significantly different ($P < 0.05$).

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