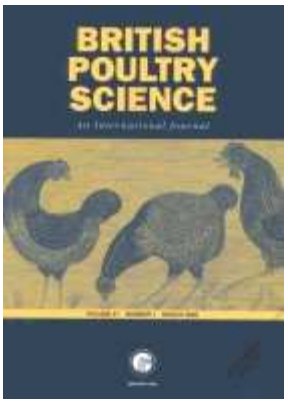
Effect of trypsin inhibitor activity in soybean on the growth performance, protein digestibility and incidence of sub-clinical necrotic enteritis in broiler chicken flocks

M. W. Chamari Devika Palliyeguru



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Effect of trypsin inhibitor activity in soya bean on growth performance, protein digestibility and incidence of sub-clinical necrotic enteritis in broiler chicken flocks

M.W.C.D. PALLIYEGURU, S.P. ROSE AND A.M. MACKENZIE

National Institute of Poultry Husbandry, Harper Adams University College, Edgmond, Newport, Shropshire TF10 8NB, England

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Correspondence to: Mrs. M.W.C.D. Palliyeguru, National Institute of Poultry Husbandry, Harper Adams University College, Edgmond, Newport, Shropshire TF10 8NB, UK.
E-mail: mpalliyeguru@harper-adams.ac.uk

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Abstract. 1. The effect of three different levels of dietary trypsin inhibitor activity (achieved by varying the amount of non-toasted full fat soya bean in replacement for toasted full fat soya bean) on the incidence of spontaneously-occurring sub-clinical necrotic enteritis (NE) in broiler chickens was compared. A fourth dietary treatment compared the effect of a diet that used potato protein concentrate as the major protein source. The determined trypsin inhibitor activity increased with the increasing content of non-toasted soya bean: 1.90, 6.21, 8.46 and 3.72 mg/g for the three soya bean diets (0, 100 and 200 g of non-toasted soya bean/kg) and the potato protein diet respectively.

2. Although increasing amounts of the non-toasted full-fat soya bean increased the feed intakes of the birds, there was a marked reduction in protein digestibility, weight gain and feed conversion efficiency.

3. There was a linear increase in sub-clinical NE lesions in the duodenum, jejunum, mid small intestine and ileum with increasing non-toasted soya bean. Caecal *Clostridium perfringens* counts increased with the increasing dietary content of non-toasted soya bean. Serum α-toxin antibodies were higher in the birds fed the 200 g non-toasted soya bean/kg diet compared with the other diets.

4. The results demonstrated that variation in the amount of non-toasted dietary soya bean not only affects growth performance of broilers but also affects the incidence of sub-clinical necrotic enteritis in the flock. Ensuring the lowest possible trypsin-inhibitor activity in soya bean samples is a valuable tool to improve the health and welfare of birds and in reducing the financial losses from this disease.

INTRODUCTION

Subclinical necrotic enteritis (NE) is an economically important bacterial disease in modern broiler flocks (Kaldhusdal, 2000). The condition is not usually detected owing to the absence of clear clinical signs and persitsunnoticed and untreated. However, the disease may be suspected when there are poor growth (Lovland and Kaldhusdal, 2001) and wet litter conditions (Williams, 2005). Sub-clinical NE also increases the risk of contamination of poultry products for human consumption (Craven *et al.,* 2001). The financial cost of the disease to the world's poultry industry has been estimated as US$2.6 billion per year (van der Sluis, 2000). The causative organism, *C. perfringens*, is ubiquitous in the environment and the intestines of most healthy animals and humans (Wages and Opengart, 2003). *C. perfringens* is also a commensal bacterium of chicken intestines (Ewing and Cole, 1994) and there are further predisposing factors that alter the intestinal balance to favour its proliferation and toxin production. Intestinal *C.perfringens* and alpha-toxin levels (Hofshagen and Stenwig, 1992; Si *et al*., 2007) are increased in the disease, so causing major pathological changes in the small intestine (Gholamiandehkordi *et al*., 2007) and liver (Lovland and Kaldhusdal, 2001).

NE has been reproduced experimentally by oral or intra-duodenal administration of birds with pathogenic strains of *C. perfringens* isolated from clinically diseased birds (Gholamiandehkordi *et al*., 2007; Pedersen *et al*., 2008). The majority of these experiments have involved regular administration of the pathogen into a small number of birds kept in cages (Drew *et al*., 2004; Gholamiandehkordi *et al*., 2007). These conditions are different from practical broiler production methods. However, Lovland *et al.* (2003) found that sub-clinical NE would develop spontaneously (without dosing the birds with pathogenic *C. perfringens*) if appropriate predisposing factors (un-medicated diets, putative predisposing feeding regimens, housing birds on litter) were provided. This method of reproducing sub-

clinical NE provides the possibility of studying the effects of production variables under conditions that are directly related to commercial production methods.

Apajalathi *et al*. (2001) identified the diet as the strongest determinant of the caecal bacterial community, so diet composition may influence the susceptibility of broilers to sub-clinical NE. There is evidence that different dietary protein sources affect the proliferation of *C. perfringens* within the caecum (Drew *et al*., 2004) and in the ileum (Wilkie *et al*., 2005) when birds are orally dosed with these bacteria. The effect of different protein concentrates on the incidence of spontaneously-occurring sub-clinical NE was studied in an earlier experiment and there was a higher incidence of NE in birds fed potato protein-based diets compared with soya bean-based diets (Palliyeguru *et al*., 2010). High trypsin inhibitor activity (TIA) in this diet was identified as a probable causative factor.

Soya bean meal is the most common protein concentrate used in proprietary poultry feeds. Soya bean must be heat-treated to improve protein digestibility due to the presence of a heat-labile trypsin inhibitor. Heat denatures the trypsin inhibitor, but the process has a variable efficiency that depends on the initial concentration of TIA, the moisture content of the seeds, the temperature and time of heating (Liener, 1994). Precise control of heating conditions is needed to prevent under or over-heating of the soya bean (Liener and Kakade, 1980). TIA has been identified as the best *in vitro* predictor of the nutritional value of processed full-fat soya bean for chickens (Perilla *et al*., 1997).

A survey of commercially-available soya bean meals found there to be varying levels of TIA (0.4-6.8 mg/g) (Probert, 2004) even though there is a European Union import threshold of TIA for soya bean meal of 4 mg/g. Therefore, the quality of soya bean meal is a factor that could affect the protein digestibility of the overall diet. Perilla *et al*. (1997) showed that a TIA of 2.8 mg/kg of diet was associated with poor growth performance in broilers. Hill *et al*. (1957) demonstrated that the lower growth performance of broilers fed an unheated soya

bean (100 g/kg of diet) could be eliminated by the addition of dietary antibiotics. This indicates that small changes in TIA may affect the gut microflora of broiler chickens.

The objective of this experiment was to compare the effect of three different levels of TIA (achieved by varying the amount of unheated full fat soya bean in replacement for heat-treated full fat soya bean of the same original source) in nutritionally-complete diets, on the incidence of spontaneously-occurring, sub-clinical NE. A fourth dietary treatment compared the effect of a diet that used potato protein concentrate as the major protein source. All 4 diets had nutrient compositions typical of those fed to commercial broilers.

MATERIALS AND METHODS

Dietary treatments and experimental design

Four nutritionally complete (Aviagen, 2007) maize-based, broiler-grower diets were formulated. All 4 diets were formulated to have similar calculated contents of metabolisable energy (13.1 MJ/kg), crude protein (210 g/kg), lysine (13 g/kg), methionine and cysteine (10 g/kg) and threonine (10 g/kg) (Table 1). Celite (20 g/kg) was included in each diet as an indigestible marker for determining nutrient digestibility. Three increasing inclusion rates (0, 100 or 200 g/kg) of unheated full-fat soya bean (with high TIA) were included in replacement for a toasted full fat soya bean (from the same batch and with the same proximate composition). A nutritionally-complete diet that included potato protein concentrate was also formulated (Table 1). No antibiotic growth promoters or anti-coccidial drugs were used in the diets. The formulated diets were mixed and steam-pelleted (3 mm diameter pellets). Chemical analysis of representative samples of the prepared diets indicated that there were only relatively small differences in dry matter, ash, protein and amino acid contents.

Table 1 near here

The dietary treatments were fed to 6 replicate pens of male broilers. Twenty four pens were used within the environmentally controlled house. The dietary treatments were randomly allocated to pens within positional blocks.

Broiler chicken management and feeding

The experiment was conducted under the guidance of the Research Ethics Committee of Harper Adams University College. A total of 1900 1-d-old male Ross 308 broilers was reared as a single flock in a solid-floored pen in an environmentally-controlled house. The birds were fed a proprietary, nutritionally-complete, broiler-starter diet that contained no antibiotic or anticoccidial drugs for the first 16 d and provided with adequate feeders and drinkers for the age and the number of birds.

At 16 d of age, the birds were weighed and 74 were randomly allocated to each of 24 pens within an environmentally-controlled house, so that 1776 birds were used in the experimental design (24 pens x 74 birds). Each of the 24 pens had a solid concrete floor with an area of 1.5 m x 3 m covered with a bedding of 4 parts new wood shavings to one part reused litter material. The reused litter was from a previous flock reared on the same poultry unit. This flock had no clinical NE but some sub-clinical NE and sub-clinical coccidiosis would have been expected.

For the following 16 d, the birds were fed the experimental diets. *Ad libitum* feed and water were provided during the experimental period. Feed was provided in hanging tube feeders and the levels were managed to minimise wastage. House temperatures and humidity were controlled to provide optimum growing conditions for the age of bird using the current (online) Management Guide for Ross 308 broilers (Aviagen, 2009). Light was provided for 23 h/d. Over the experimental period, the growth and feed intakes of the birds were recorded. In addition, the birds in each pen were inspected daily and mortalities recorded.

Data collection

Lesion scoring

Randomly-selected birds were sampled from each of the 24 pens on d 5 and at the end of the feeding period (d 12, 13, 14 and 15). On d 5, 4 birds from each pen were killed by cervical dislocation and 5 ml of blood collected from the jugular vein and transferred into glass tubes, and ileal contents were collected and pooled for each pen. At 12, 13, 14 and 15 d, 8 birds from each replicate pen (two birds per day) were randomly selected, killed by cervical dislocation and 5 ml of blood collected from the jugular vein and transferred into glass tubes. The intestinal tract and liver were removed and the ileal contents collected from individual birds. Each liver was inspected for the lesions of hepatitis or cholangiohepatitis which are consistent with the pathological changes of NE (Lovland and Kaldhusdal, 1999; Sasaki *et al*., 2000). Four 8-cm intestinal sections were taken; the first of these sections was the duodenal loop, the second was proximal jejunum beginning immediately after the distal end of the duodenal loop, the third was either side of Meckel's diverticulum (mid small intestine) and the fourth terminated at the ileo-caecal junction (distal ileum). All 4 sections were immediately incised, washed in normal saline and the mucosal surfaces were inspected and any necrotic lesions recorded. A scoring system originally established by Truscott and Al-Sheikhly (1977), but modified by Gholamiandehkordi *et al*. (2007), was further slightly modified to score intestinal lesions. The scoring system was: score 0: no lesions, score 1: focal necroses (1-10 mm diameter), score 2: necrotic patches (1-2 cm diameter), score 3: coalesced necroses and score 4: pseudo-membrane covering the whole epithelial surface. The mucosal surfaces of each of the 4 gut wall samples were scored for necrotic lesions.

Enumeration of Clostridium perfringens and coccidial oocysts

The duodenal sections were used for the quantification of *C. perfringens* and coccidial oocysts. Each duodenal sample was washed 4 times in sterile Phosphate Buffered Saline (PBS) at pH 7.2, weighed, added to the same weight of dilution buffer (Bio-X Diagnostics, Jemelle, Belgium), stomachised in a sterile stomacher bag and the resulting liquid frozen at -20°C. Duodenal extracts were subsequently thawed at room temperature and vortexed for the quantification of coccidial oocysts. The extracts were diluted (x 6) with saturated NaCl, placed into both sides of a McMaster counting chamber and allowed to settle for 5 min. The number of coccidial oocysts (all species found in the sample) was counted at 10-times magnification.

C. perfringens in the caecal contents and in the duodenal mucosal sample were quantified with a double antibody sandwich ELISA (McCourt *et al.,* 2005), using the BIO K 086 – *Closridium perfringens* antigen detection kit (Bio-X Diagnostics, Jemelle, Belgium). Caecal contents were collected in a sterile container and diluted (x2) with dilution buffer (Bio-X Diagnostics, Jemelle, Belgium), vortexed and frozen at -20°C before analysis. Both duodenal extracts and diluted caecal contents were thawed and vortexed before being allowed to settle for 10 min for the enumeration of *C. perfringens*. Standardisation of the ELISA was done as described previously (Palliyeguru *et al*., 2010).

Quantification of serum α-toxin antibodies

The blood collected from each bird was allowed to clot for 8-10 h at room temperature. The serum was separated from the coagulum and stored at -20^{o}C. Twelve serum samples from each pen were compared for antibody levels developed against the alpha toxin of *C. perfringens* using an indirect ELISA according to the method described by Heier *et al.* (2001) and Lovland *et al.* (2003) with slight modifications as described below. Each well of the Nunc-immunoplates (F96 Maxisorp, 735-0083, Thermo Fisher Scientific, Rochester, NY)

was coated with antigen, phospholipase C type XIV (Sigma-Aldrich P 4039, St. Louis, MO) 10 µg/ml in sodium carbonate and bicarbonate coating buffer (pH 9.6) by incubating the 100 µl of antigen in each well overnight at 4°C. The plates were washed three times with PBS at pH 7.2 with 0.05% Tween 20 (PBST). A volume of 150 µl of 1% bovine serum albumin in PBST was added into each well as a blocking buffer and incubated for 2 h. The plates were washed again three times with PBST.

Serum samples were diluted 1:250 in PBST and 50 ml of each sample was added into duplicate wells. After leaving the plates at 4°C overnight, the contents were washed with PBST. Rabbit anti-chicken immunoglobulin IgY (IgG) whole molecule conjugated with alkaline phosphatase (Sigma-Aldrich A9171, St. Louis, MO) was diluted (10^{-4}) in blocking buffer and 50 µl of diluted anti-chicken antibodies were added to each well and the plates incubated at 37°C. After 2 h, the plates were washed with PBST and tapped on absorbent paper to remove all droplets.

The plates were incubated with 150 µl of para-nitrophenyl phosphate (Sigma-Aldrich P 7998, St. Louis, MO) for 1 h at 37°C, and the reaction stopped with 50 µl of 2M NaOH. The optical density (OD) was determined at 405 nm using a microplate reader (Bench Mark 170-6850, Bio-Rad, Hercules, CA). Resulting OD values were pooled for each pen. The indirect ELISA was not standardised using chicken α-toxin antibodies, so the pooled OD values were compared instead of the antibody titres.

Feed and digesta analyses

The experimental feed samples were oven dried to a constant weight at 105°C to determine the dry matter content. Nitrogen (N) was determined with an automatic analyser (LECO FP-528 N; LECO Corp. St Joseph, MI) by AOAC 968.06 (Dumas method) using EDTA as the standard (AOAC International, 2000). TIA was determined by measuring the inhibition of mg

of bovine trypsin per 1 g of sample using the method described by Smith *et al*. (1980). Dietary amino acid samples were determined by ion exchange chromatography (Biochrom 20 analyser, Amersham Pharmacia Biotech, Pittsburgh, PA) with post-column ninhydrin reaction as previously described (Palliyeguru *et al*., 2010).

Ash and acid insoluble ash contents in feed and digesta samples were measured using a modified method described by Atkinson *et al*. (1984). Approximately 5 g of diet (dry) or 1.0 g of digesta (dry) was placed into a previously-weighed crucible and ashed at 610°C for 13 h. The contents of the cooled crucibles were weighed and washed into a glass tube with 50 ml of 4M HCl. The tubes were boiled at 110°C for 30 min. The cooled contents were filtered and washed with de-ionised water through an ash-less filter paper and the filter paper was again ashed in the same crucible at 610°C for 13 h. Subsequently the crucibles were cooled in a desiccator and weighed.

Crude protein content in each feed sample was measured in 6 replicate samples and the crude protein content in ileal digesta and the acid insoluble ash (indigestible marker) contents in both feed and ileal digesta were measured in duplicate samples and the protein digestibility coefficient of the lieal digesta calculated.

Statistical analysis

The variables of growth performance, necrotic lesion scores in the intestinal sections, proportion of birds that had liver and gall bladder lesions, *C. perfringens* in the caecal contents and duodenal mucosa, serum antibody levels for alpha toxin and the number of coccidial oocysts on the duodenal mucosa were compared using a randomised block analysis of variance. The 24 pens were used as the experimental units and individual bird or sample data from within a pen were pooled before statistical analysis. The treatment sums of squares of the three soya bean diets were partitioned using a polynomial contrast with the determined

TIA in dietary treatments as the explanatory variate using *Genstat 10* (Lawes Agricultural Trust, 2007)

RESULTS

There were no major differences in the determined nutrient compositions of the three soya bean diets (Table 1). The determined TIA of the non-toasted full fat soya bean was 27.62 mg/g. The TIA of the treatment diets increased with increasing inclusion rate of non-toasted soya bean (0, 100 and 200 g/kg). Determined TIA with the three increasing non-toasted soya bean diets and the potato protein diet were 1.90, 6.21, 8.46 and 3.72 mg/g respectively. Although increasing the content of non-toasted full-fat soya bean significantly increased feed intake, there was a marked reduction (P <0.001) in protein digestibility, weight gain and feed conversion efficiency. Mortality was not significantly affected (Table 2).

Tables 2 & 3 near here

There was a linear increase (P <0.001) of sub-clinical NE lesions in the duodenum, jejunum, mid small intestine and ileum with increasing dietary non-toasted soya bean (Table 3). There was a significant increase in the number of liver lesions in the birds fed all other dietary treatments compared with the toasted soya bean diet. Caecal *C. perfringens* counts increased with the increasing dietary non-toasted soya bean. Serum α-toxin antibodies were higher (P <0.001), on d 5 in the birds fed the 200 g/kg non-toasted soya bean diet compared with the other two soya bean diets, although there were no significant treatment differences at the end of the feeding period. Dietary treatment had no significant effect on the number of *C. perfringens* colonised on the duodenal mucosa. Coccidial oocyst counts decreased (P <0.01) with increasing dietary non-toasted soya bean (Table 3).

There was a strong negative correlation (P <0.001) between the necrotic lesion scores of the intestine and the growth and protein digestibility coefficient of the broilers.

DISCUSSION

Although the toasting may have had other effects on the chemical composition of the full fat soya bean, the high levels of TIA were probably the major cause of the reduced protein digestibility in the non-toasted soya bean diets; Perilla *et al*. (1997) concluded that TIA gave the best prediction of the feeding value of soya beans. The growth performance responses of the broilers to the dietary treatments in the present experiment were expected, but the size of the reduction in growth was relatively large in comparison with the results of Perilla *et al*. (1997), who observed a 6.5% increase in feed intake and a 4.9% decrease in growth rates for an increase in dietary TIA from 0 to 9.4 mg/g. In the present experiment, there was a 4.3% increase in feed intake but a 22% decrease in growth rate from the low to high TIA diets.

When the non-toasted soya bean was fed to the broilers, the effect of trypsin inhibitors on protein digestibility did not fully explain the reduction in growth performance, as reported by Liener and Kakade (1980). Dietary antibiotics have beneficial effects on the growth performances of birds fed non-toasted soya bean diets (Hill *et al*., 1957), and there is a lack of growth depression in germ-free birds fed dietary trypsin inhibitors (Coates *et al*., 1970; Hewitt and Coates, 1973); all these experiments indicated that microbial activity in the intestine may exacerbate the negative effects on growth performances caused by poor protein digestion. However, in the present experiment, the broilers were reared in conditions that predisposed sub-clinical NE and it is possible that the increased severity of sub-clinical NE in these dietary treatments further contributed to the poor growth rate and feed conversion efficiency.

The results (Table 3) are clear evidence that dietary non-toasted soya bean produced an increased incidence and severity of sub-clinical NE, although there was no significant increase in the *C. perfringens* numbers in the duodenum. Proliferation of this causative organism can be restricted to localised sites in the small intestine, so it is difficult to

consistently demonstrate a difference in the numbers of *C. perfringens* by sampling a representative amount of mucosal surface. Conversely, there was a good correlation between the numbers of caecal *C. perfringens* and the intestinal necrotic lesion scores in the present experiment, as has been reported in other studies (Kaldhusdal *et al*., 1999; Elwinger *et al*., 1992). Although the serum antibody levels for *C. perfringens* alpha toxin were increased following 5 d of feeding 200 g/kg non-toasted soya bean, the birds fed this diet had the lowest antibody levels at the end of the experimental period (Table 3). Malnutrition generally has negative effects on antibody production in farm animals (Lawrence and Fowler, 2002), and, according to Klasing (2007), nutritional deficiencies can seriously reduce the immune responses of chickens. In the present experiment, the birds fed the highest (200 g/kg) rate of non-toasted soya bean had the greatest damage in their intestinal mucosa. This may have caused poorer nutrient absorption and impeded the humoral immune responses. In addition to poor nutrition, damage to the gut-associated lymphoid tissues could have contributed to depressed immune responses. Chickens do not have peripheral lymph nodes similar to those of mammals, therefore gut-associated lymphoid tissues are important for protection against intestinal pathogens (Sklan, 2004). B lymphocytes in the laminapropria initiate the humoral antibody responses to pathogens that enter into the body through the gut wall, so the damage to the intestinal mucosa could have greatly suppressed gut-associated immune mechanisms (Sklan, 2004; van Dijk *et al*., 2002) and contributed to the lower antibody levels in the 200-g/kg non-toasted soya bean treatment. Controversially, increased gut damage could have improved the immune response allowing more systemic access to toxin. In such an instance, it is possible that the antibody response in this treatment group could have increased, come to an earlier peak and then declined between the two sampling days.

The factors in non-toasted soya bean that predispose the proliferation of *C. perfringens* or influence their toxins are not clear. However, the causative agent of NE, *C.*

perfringens, is a commensal bacterium of chicken intestines (Ewing and Cole, 1994). When protein digestibility is low, the ileal contents will have a relatively high content of protein. The growth of *C. perfringens* under *in vitro* conditions needs 11 amino acids (Sebald and Costilow 1975). *C. perfringens* that are producing α-toxin need specific amino acids and peptides for their proliferation (Nakamura *et al*., 1968), so it is possible that the low protein digestibility in the small intestine directly predisposed the more rapid multiplication of *C. perfringens*. There is also a possibility that trypsin inhibitors may stabilise the *C. perfringens* toxins and so increase the severity of their toxigenic effects.

Soya beans contain lectins that are known anti-nutritive factors (Probert, 2004) which have the ability to damage the intestinal epithelial cells (van Dijk *et al*., 2002) and also possibly mediate the adhesion of pathogenic bacteria on to the epithelial cell membrane. Lectin concentration can be 10-20 mg/g of non-toasted soya bean (Clarke and Wiseman, 2000). However, the lectins are heat labile and Armour *et al*. (1998) and Probert (2004) found that lectins in soya bean denature more readily than trypsin inhibitors.

A negative correlation of coccidial oocysts counts in the duodenal mucosa with the severity of lesion scores was found in this experiment and also in an earlier experiment (Palliyeguru *et al*., 2010). This may be due to their exclusion from the proliferation sites when *C. perfringens* invade the damaged intestinal epithelium (Williams *et al*., 2003). It is therefore possible that the coccidial oocysts in the mucosa of the small intestine reduced with an increasing severity of NE, even though the initial coccidial damage in the mucosal epithelium may have predisposed (Al-Sheikhly and Al-Saieg, 1980; Shane *et al*., 1985) and aggravated (Park *et al*., 2008) the *C. perfringens* damage.

The birds fed the potato protein diet had significantly poorer growth performance (weight gain, feed intake and gain:feed) than the birds fed the soya bean diet with the lowest TIA. The non-significant trend of lower protein digestibility of the potato protein diet was

consistent with the determined TIA of this diet, even though it was not as low as the two soya bean diets that contained some non-toasted soya bean and had much higher trypsin inhibitor activities. The relatively low TIA in the potato protein diet was not expected, despite the trypsin inhibitor activities of different samples of potato protein concentrate being known to vary with the variety (Jadhav and Kadam, 1998) and the method of processing (Knorr, 1980, 1982). There were no significant differences in caecal *C. perfringens* counts and serum α-toxin antibody concentrations between the potato protein and the soya bean diet with the lowest TIA. This was consistent with the relatively small difference between these two diets in protein digestibility and TIA. However, the birds fed the potato protein diet had a significantly higher incidence of liver lesions, increased severity of duodenal lesions and a lower feed intake compared with the low trypsin inhibitor soya bean diet. The heat-resistant glycoalkaloids (Jadhav and Kadam, 1998) that are also contained within potato protein (Lokra *et al*., 2008) may have contributed to the severity of the disease in the potato protein fed birds.

The results of this experiment have demonstrated that variation in the amount of non-toasted dietary soya bean not only affects growth performance of broilers but also affects the incidence of sub-clinical NE in the flock. Although the maximum recommended TIA of soya bean is 4 mg/g, there is considerable variation in activity below this level in commercially-available samples (Probert, 2004). Many countries have now adopted a complete ban on the use of antimicrobial growth promoters and so there is potential for an increase in the incidence of sub-clinical NE in their commercial broiler flocks. Ensuring the lowest possible TIA in soya bean samples would appear to be a valuable tool for improving bird health and welfare and reducing the risk of serious financial losses from NE.

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Table 1. *Feed ingredients, calculated and determined chemical compositions of experimental diets containing 0, 100 or 200 g/kg non-toasted soya bean or potato protein*

Ingredients (g/kg)	Experimental diet supplementation			
	Non-toasted soya bean protein (g/kg)			Potato protein
	0	100	200	
Maize	504.7	504.7	504.7	696.0
Rapeseed meal	073.0	073.0	073.0	073.0
Dehulled soya bean meal	142.3	142.3	142.3	-
Full fat soya bean	200.0	100.0	-	-
Ground raw soya bean	-	100.0	200.0	-
Potato protein concentrate	-	-	-	163.1
Soya bean oil	23.25	23.25	23.25	12
Lysine HCl	2.75	2.75	2.75	1
Methionine	2.5	2.5	2.5	1.3
Threonine	2.5	2.5	2.5	-
Tryptophan	0.1	0.1	0.1	-
Limestone	12.6	12.6	12.6	11.4
Di Calcium Phosphate	8.8	8.8	8.8	13.0
Salt	5.0	5.0	5.0	5.0
Choline chloride	-	-	-	1.7
Vitamin and trace element premix[1]	2.5	2.5	2.5	2.5
Celite	20.0	20.0	20.0	20.0
Calculated chemical composition[2]				
Metabolizable energy (MJ/kg)	13.1	13.1	13.1	13.1
Calcium	8.9	8.9	8.9	8.9
Phosphorus	5.7	5.7	5.7	5.7
Sodium	2.1	2.1	2.1	2.1
Determined chemical composition				
Dry matter	928.1	925.2	916.3	927.8
Ash	50.0	49.6	49.2	57.8
Crude protein	226.5	226.6	229.5	216.0
Alanine	9.8	9.9	9.8	10.8
Arginine	12.9	12.7	12.8	10.0
Aspartic acid	19.9	19.7	19.9	20.5
Cystine	3.4	3.4	3.4	3.2
Glutamic acid	35.2	35.3	35.2	27.4
Glycine	8.6	8.7	8.7	9.3
Histidine	5.4	5.4	5.4	4.9
Isoleucine	8.5	8.1	8.5	9.1
Leucine	16.7	16.5	16.7	19.8
Lysine	13.1	13.1	13.1	13.3
Methionine	5.3	5.3	5.4	5.3
Phenylalanine	10.0	9.8	9.8	11.2
Proline	11.9	11.6	11.4	11.9
Serine	10.2	10.4	10.2	10.6
Threonine	10.3	10.5	10.4	10.4
Tyrosine	7.3	7.1	7.3	9.0
Valine	9.7	9.1	9.9	11.4

[1]Vitamin-trace mineral premix for broilers (Target Feeds Ltd., Whitchurch, UK) added per kg: 800 mg Retinol, 150 mg Cholecalciferol, 1.3 g Tocopherol, 150 mg Thiamin, 500 mg

Riboflavin, 150 mg Pyrodoxine, 750 mg Cyanocobalamin, 3 g Nicotinamide, 0.5 g Pantothenic acid, 75 mg Folic acid, 6.25 g Biotin, 12.5 g Choline chloride, 1 g Iron, 50 mg Cobalt, 5 g Manganese, 0.5 g Copper, 4 g Zinc, 50 mg Iodine, 10 mg Selenium, and 25 mg Molybdenum

[2]Chemical composition was calculated with UK derived nutrient composition tables (Premier Nutrition, 2008)

Table 2. *Growth performance[1] and protein digestibility[2] in male broiler chickens fed nutritionally complete diets with different protein concentrates for 16 d (16-32 d of age)*

	Soya bean diets Level of un-heated soya bean (g/kg)			Potato protein diet	SED	Probability of differences	
	0	100	200				
	Trypsin inhibitor activity mg/g (TIA)			(TIA 3.72)		Treatment differences	Linear effect of non-toasted soya bean
	1.90	6.21	8.46				
Weight gain (kg)	1.290	1.179	0.995	1.142	0.0208	< 0.001	< 0.001
Feed intake (kg)	1.887	1.936	1.969	1.764	0.0342	< 0.001	< 0.05
Gain:feed	0.684	0.609	0.506	0.647	0.0115	< 0.001	< 0.001
Mortality %	1.6	1.8	1.6	5.0	1.56	NS	NS
Protein digestibility coefficient	0.653	0.561	0.544	0.614	0.0204	< 0.001	< 0.001

[1]Data are means of 6 pens of 74 broilers per pen.
[2]Data are means of 6 replicate pens of 12 sampled broilers per each pen.
NS=not significant.

Table 3. *Necrotic lesion scores,* Clostridium perfringens *counts cocidial oocyst counts and serum antibody levels for* Clostridium perfringens *alpha-toxin*[1] *in male broiler chickens fed nutritionally complete diets with different protein concentrates for 16 d (16-32 d of age)*

	Soya bean diets Level of un-heated soya bean (g/kg)			Potato protein diet	SED	Probability of differences	
	0	100	200				
	Trypsin inhibitor activity mg/g (TIA)						
	1.90	6.21	8.46	(TIA 3.72)		Treatment differences	Linear effect of non-toasted soya bean
Necrotic lesion scores							
Duodenum	2.38	2.92	3.19	2.63	0.078	< 0.001	< 0.001
Jejunum	2.13	2.44	2.58	2.27	0.085	< 0.001	< 0.001
Mid small intestine	2.02	2.19	2.25	1.97	0.064	< 0.01	< 0.01
Ileum	1.65	1.88	2.02	1.71	0.096	< 0.05	< 0.01
Proportion of birds with liver or gall bladder lesions	0.69	0.85	0.88	0.88	0.062	< 0.05	< 0.01
C.perfringens counts (log_{10}cfu/g)							
Caecum	7.82	7.99	8.32	7.91	0.138	< 0.01	< 0.01
Duodenum	7.53	7.71	7.60	7.62	0.152	NS	NS
Serum α-toxin antibody (OD_{405}) in the feeding period							
Intermediate (d 5) [2, 3]	0.357	0.354	0.509	0.340	0.0370	< 0.01	< 0.001
End (d 12-15)[4]	0.554	0.584	0.509	0.582	0.0691	NS	NS
Coccidial oocyst counts induodenal mucosa (n/g) [2]	4564	3193	2530	3188	456.7	< 0.01	< 0.001

[1]Data are means of 6 replicate pens with 8 broilers sampled from each pen.
[2] Data are means of 6 replicate pens with 4 broilers sampled from each pen.
[3] Birds were sampled on d 5 of the feeding period (20 d of age)
[4] Birds were sampled for 4 consecutive days; from d 12-15 of the feeding period (28-31 d of age).