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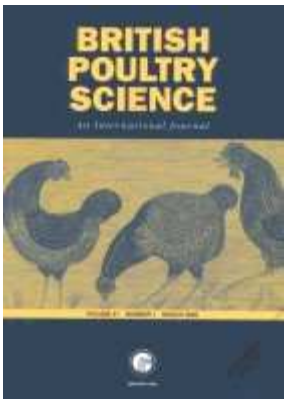

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Phytase effects on the efficiency of utilisation and blood concentrations of phosphorus and calcium in Pekin ducks

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Abstract 1. The objective was to study the effects of a supplementation of a 6-phytase derived from the *Peniophora lycii* gene in the White Pekin duck.

2. In two balance studies, low-phosphorus (P) diets consisting mainly of maize, solvent extracted soybean meal and solvent extracted sunflower meal were supplemented with phytase up to concentrations of 1500 U/kg (Study 1) or 2000 U/kg (Study 2). Each diet (phytase level) was fed to 8-10 individually penned ducks. The intake and excretion of each animal was measured for 5 consecutive d when ducks were in their third week of life. Responses were described by nonlinear regression.

3. Although the basal diets from the two studies were similar in ingredient composition, efficiencies of P utilisation (P accretion/P intake × 100) for the unsupplemented basal diets were 39% in Study 1 and 30% in Study 2. Phytase supplementation significantly improved P utilisation up to levels of about 55% in both studies. A plateau in P utilisation with an increase in phytase supplementation was achieved in Study 2, but not in Study 1. The enzyme was more efficient in Study 2 than in Study 1 at low rates of supplementation. Utilisation of calcium (Ca) was significantly improved by phytase supplementation. Accretions of P and Ca increased at a constant ratio.

4. In a 5-week growth study, diets with an intentionally marginal P level were used. Diets were fed either unsupplemented or supplemented with 1000 or 10000 U/kg of phytase. Eight pens of 10 sex-separated ducks each (4 pens per sex) were allocated to each dietary treatment.

5. Phytase significantly improved the growth of ducks of both sexes between d 1 and 21, but not between d 22 and 35. Feed conversion rate was not affected by treatment. Blood serum phosphate concentrations, but not calcium, were significantly increased by phytase supplementation. Blood concentrations of creatinine, aspartate aminotransferase, and lactate dehydrogenase remained unaffected while alanine aminotransferase was significantly reduced by phytase supplementation.

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6. It was concluded that the efficacy of a microbial phytase varies even under similar experimental conditions. Differences in intrinsic phytase activity of maize-soybean meal-based diets may be responsible for this. The 6-phytase used has the potential to improve the utilisation of plant P in duck feeding. A plateau in response was reached above 1500 U/kg.

INTRODUCTION

It is well known that phosphorus (P), present as phytate in plant feedstuffs, is hardly available to poultry in the absence of the enzyme phytase. Supplementing diets with products containing microbial phytase is a tool to improving the utilisation of phytate P and, combined with a reduced need for P supplementation, reducing the amount of P in excreta.

Duck meat production is continuously increasing on the global and European markets. Average annual growth in duck meat production between 1998 and 2003 was 3% in Europe and 6% worldwide (FAO, 2005). Yet, effective nutrition of ducks must not only meet the animal's requirements for growth and health; with particular relevance in the case of P, avoiding excessive intake and minimising excretion is also relevant for economic and ecological reasons (Rodehutscord, 2005). Consequently, the role of phytase as a feed supplement in ducks has been of increasing interest, especially the use of a 3-phytase derived from the *Aspergillus niger* gene (Farrell *et al.*, 1993; Martin *et al.*, 1998; Orban *et al.*, 1999; Attia, 2003; Wendt *et al.*, 2003). Because 3-phytases and 6-phytases show differences *in vitro* properties, such as optimum pH, temperature stability, resistance to proteolytic enzymes (Simon and Igbasan, 2002), and in animal studies in efficacy to improve P utilisation (Augspurger *et al.*, 2003; Paditz *et al.*, 2004; Payne *et al.*, 2005), each phytase preparation needs to be studied for its particular efficacy before supplementation levels can be recommended for diet formulation. Changes in P accretion due to phytase supplementation, determined at marginal dietary concentrations of P, are commonly regarded as a sensitive measure of phytase efficacy. Also, growth and blood phosphate concentrations are often taken

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into consideration. Our objective was to study the efficacy of a 6-phytase derived from the *Peniophora lycii* gene[1] based on balance, growth, and blood data.

MATERIALS AND METHODS

Two balance studies and one growth study were conducted. They were approved by the animal welfare authorities in accordance with the German Animal Welfare Act.

Balance study 1

A basal diet that was adequate in ME and all nutrients with the exception of P and calcium (Ca) was formulated. This was based on NRC (1994) recommendations and results of recent amino acid requirement studies with Pekin ducks (Timmler and Jeroch, 1999; Bons *et al.*, 2002; Timmler and Rodehutscord, 2003). Ingredients were selected to achieve a combination of low total P concentration with a high proportion of phytate P and low intrinsic phytase activity (Table 1). Limestone was added to give a Ca concentration of about 6.5 g/kg of diet. Analysed concentrations of P and Ca were 4.4 and 6.6 g/kg of diet. Ingredients were mixed in one batch to ensure consistency. This mix was divided into 6 portions. Phytase was then added to achieve the following activities (U/kg diet): 0, 250, 500, 750, 1000, and 1500. Diets were mixed again and pelleted through a 3 mm die without using steam. Intended phytase activities were confirmed by analysis with the exception of the highest supplementary level (Table 2).

Tables 1 and 2 near here

One-d-old male White Pekin ducklings (Duck-Tec Brüterei GmbH, Belzig, Germany) were raised in floor pens on straw bedding with a commercial duck starter feed containing adequate P. After 10 d, 60 of the 130 animals were selected for the experiment based on appearance and body weight (BW). Each bird was individually penned in a balance crate that was (in mm) 370 high, 360 wide, and 550 deep. Trays and crates were specially constructed and closed at the bottom to avoid any loss of excreta. Crates were part of a system in an animal house at the institute. Ten birds were allocated to each treatment diet (phytase

[1] The product used was Ronozyme P 5000 (CT), DSM Nutritional Products, Basel.

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concentration) by BW. Diets were offered for 10 consecutive days with a quantitative collection of excreta during the last 5 d. Excreta were collected from the trays before the morning feeding time and bulk-stored at –18°C for each individual bird. During collection and the 3e d prior to collection, feed allowance was slightly restricted in order to avoid feed refusals. The amount (130 g/d) was chosen on the basis of the previous *ad libitum* intake. The feed was offered in two meals per d (at around 09:00 h and 15:00 h). De-mineralised water was always available from nipple drinkers with attached cups. The diet was, therefore, the only source of P and Ca intake. Body weights were determined at the beginning and end of the excreta collection period prior to the morning feeding time. Data from three ducks (one from the treatment with 250 U/kg and two from the treatment with 500 U/kg) were not considered because of incomplete feed intake (<90 % of feed allowance).

Thawed excreta samples were homogenised. All chemical analyses were run in duplicate. Diets and excreta samples were analysed for dry matter (105°C) and ash content (incineration at 550°C). Diets were also analysed for crude protein content (macro Kjeldahl-N) according to official standards (Naumann and Bassler, 1976). Concentrations of P and Ca were determined from filtered ash solutions after acid hydrolysis with an Inductively Coupled Plasma Spectrometer (ICP-OES, JY 24, Jobin Yvon GmbH, Grassbrunn, Germany) as described by Rodehutscord and Dieckmann (2005). Phytase activity was measured in accordance with the method of Engelen *et al.* (1994), in which one phytase unit (U) is defined as the amount of enzyme which, at 37 °C and pH 5.5, liberates one µmol of inorganic phosphate per minute from 0.0051 mol/l sodium phytate.

Accretions of P and Ca (mg/d) were calculated as the differences between intake (mg/d) and the amount recovered in the excreta (mg/d). Efficiency of utilisation is accretion as a percentage of intake. The content of utilised P (P_u) in the diet is analysed P content (g/kg) multiplied by the efficiency of P utilisation (%) divided by 100. Unexcreted organic matter (UOM) was expressed as a percentage of OM intake.

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Results were subjected to routine ANOVA procedures using the software package SPSS for Windows (version 11.0, SPSS Inc., Chicago, IL, USA). Responses to phytase supplementation were described by the following exponential equation as given by Robbins *et al.* (1979):

$$y = a + b\,(1 - e^{-k\,x}) \qquad \text{[Eq. 1]}$$

where a is estimated response (y-value) at zero phytase supplementation

b is maximum response to supplemented phytase ($a + b$ = upper asymptote)

k is parameter describing the steepness of the curve

y is response criterion (efficiency of P utilisation or P_u concentration in the diet)

x is supplemented phytase (U/kg).

In order to describe the marginal efficacy of phytase, the first derivative of the above equation was used when applied to the P_u concentration in the diet:

$$y' = b\,k\,e^{(-k\,x)} \qquad \text{[Eq. 2]}$$

This derivative describes the additional amount of P that is released by each incremental unit of phytase ($\Delta P_u/\Delta U$) (Paditz *et al.*, 2004).

A linear regression was calculated to describe the relation between Ca accretion (y, mg/d) and P accretion (x, mg/d) using all phytase levels:

$$y = a + m\,x \qquad \text{[Eq. 3]}$$

where a is a constant, and m is the slope of the regression line.

Nonlinear and linear regression analysis was performed with the program GraphPad Prism 4.02 (GraphPad Software Inc., San Diego, CA, USA). As parameters for the goodness of fit, r^2 and $s_{y.x}$ are presented. The $s_{y.x}$ values are the standard deviations of the residuals, which are the distances between the individual points and the calculated line.

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Balance study 2

A basal diet very similar to that of Balance Study 1 was used (Table 1). Phytase was applied at the following concentrations (U/kg): 0, 250, 500, 750, 1000, 1500, and 2000. The highest level was included because no saturation of response was detected in Study 1. Intended differences in the supplementary levels were confirmed by analysis (Table 2). Analysed concentrations of P and Ca in the diets were 4.2 and 6.0 g/kg of diet. Details of diet and pellet preparation were as described for Balance Study 1.

One-d-old male White Pekin ducklings (Seddiner Zucht- und Mastenten GmbH, Westerscheps, Germany) were raised until they were 10 d old as described for Balance Study 1. Birds were then individually penned in the balance crates. With the exception of the unsupplemented basal diet, for which 10 ducks were used, each diet was allocated to 8 ducklings. The daily feed allowance during the collection period and the 3 d preceding was 90 g. All other experimental details, including excreta sampling, sample treatment and chemical and statistical analysis, were as described for Balance Study 1. Two animals were excluded from data evaluation due to incomplete feed intake (<90 % of feed allowance; one from the treatment without phytase supplementation and one belonging to the highest phytase level).

Growth study

The growth study comprised two phases: phase one from hatch to d 21 and phase 2 from d 22 to the end (d 35). Two basal diets were mixed for each phase (Table 1). They were calculated to meet the requirements as specified for the balance studies with the exception of P and Ca. Phosphorus concentrations were calculated to be slightly below the estimated requirement, as suggested by Rodehutscord (2005). The calculated concentrations of ME were 12.2 and 12.5 MJ/kg in phase one and two, respectively. In order to ensure consistency of the mixes, the total amount of feed needed for the respective phase was mixed in one lot and subsequently divided into three portions. Phytase was then supplemented to achieve the following activities

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(U/kg diet): 0, 1000 and 10000. Diets were mixed again and pelleted through a 3 mm die without using steam. Intended phytase activities were confirmed by analysis (Table 2).

One-d-old ducklings (120 of each sex) of a White Pekin duck hybrid (Duck-Tec Brüterei GmbH, Belzig, Germany) were penned in groups of 10, sex-separated, on straw bedding. Eight pens (4 with male and 4 with female ducklings) were randomly allocated to each dietary treatment. Feed was offered *ad libitum* throughout the study. Troughs were manually re-filled on demand from pre-weighed buckets. During the first week, pellets were also offered from a flat plastic tray on the ground. Tap water was available from three drinkers per pen. Drinkers had an attached cup. The room temperature was 32°C from d 1 to 4, and was then successively reduced to 16°C on d 16, and kept constant from then on. Continuous lighting was provided during the first week, and a 20 h light:4 h dark pattern was applied from d 8 onwards.

Ducks were weighed at the onset of the experiment and at weekly intervals thereafter. Feed consumption was simultaneously determined on a per-pen basis. Ducks were continuously monitored for general health status, and no abnormalities were observed during the trial. On d 35, blood samples were taken from two ducks per pen by wing vein puncture. Blood samples were centrifuged at 2000 *g* and 20°C for 10 min. The serum samples were spectrophotometrically analysed[2] for inorganic phosphate (P_i) after conversion to ammonium phosphomolybdate, for Ca after conversion to a Ca-o-cresolphthalein complex, and for creatinine after a Jaffé reaction with alkaline picrate. The enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were determined with commercial test kits. Due to some technical problems, which were probably caused by the high viscosity of the serum samples, measurements could not be made for all birds. The minimum number of replicates per treatment and sex was, however, less than 6 in only three cases. Diets were analysed according to the methods described for Balance Study 1.

[2] Conducted by Labor für klinische Diagnostik GmbH&Co. KG, Bad Kissingen, Germany

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Data were subjected to two-way ANOVA procedures using the software package SPSS for Windows (version 11.0, SPSS Inc., Chicago, IL, USA).

RESULTS

Balance studies

In Balance Study 1, ducks weighed 780 g (SD 30 g) and 1079 g (SD 30 g) at the beginning and end of the collection period, respectively. Ducks in Balance Study 2 had lower BW than those in Balance Study 1 (601 g (SD 30 g) at the beginning and 828 g (SD 31 g) at the end of the collection period). Neither study showed any significant effect of phytase supplementation on growth, which was what we expected considering the short period (5 d) and restricted feed supply.

Tables 3, 4 and 5 near here

In Balance Study 1, the efficiency of P utilisation was 39% when no phytase was added (Table 3). Phytase supplementation significantly reduced the excretion of both P and Ca ($P \leq 0.001$). Accordingly, both accretion and efficiency of utilisation of P and Ca were significantly improved by phytase supplementation ($P \leq 0.001$). Results were similar in Balance Study 2 (Table 4). However, efficiency of P and Ca utilisation on the unsupplemented basal diet was lower than in Study 1. While a plateau in the efficiency of P utilisation was not attained in Study 1, it did become obvious with the higher range of phytase supplementation in Study 2 (Figure 1). The greatest P utilisation found in Study 2 was about 54% (Figure 1, Table 5). A higher plateau in efficiency of P utilisation was indicated by a regression analysis for Study 1 (63%, sum of parameters *a* and *b*), but this estimate is based on an extrapolation of the curve without the necessary data for phytase levels above 1500 U/kg. Accordingly, the SE of estimate for parameter *b* was high in Balance Study 1 (Table 5).

The content of P_u in the diet increased by up to 1 g/kg at a high level of phytase supplementation (Figure 2). Marginal efficacy was greater by a factor of 3 in Study 2 than it was in Study 1 at a low level of supplementation (Figure 3). Differences in marginal efficiency between studies decreased with increasing supplementation.

Figs 1, 2, 3, 4 near here

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There was a linear relationship between accretions of P and Ca, calculated at all phytase levels (Figure 4). The Ca:P ratio in accretion caused by phytase supplementation was 1.63:1 in Study 1 and 1.90:1 in Study 2, as indicated by the slopes of the regression lines.

In relation to organic matter intake, about 73% (Study 1) and 70% (Study 2) of organic matter was not recovered in excreta. This proportion was not significantly affected by phytase supplementation (Tables 3 and 4).

Tables 5, 6 and 7 near here

Growth study

A total of 5 ducks had to be culled in the course of the experiment because of mobility restrictions and standing ability (one male in the treatment without phytase, one male and one female each in the treatments with 1000 and 10000 U/kg).

Initial BW was 58.9 g (SE 1.4 g) and 58.1 g (SE 1.1 g) for male and female ducklings, respectively. Growth rate was high, overall. Males grew significantly more rapidly than females, and final BWs of about 2.6 kg (males) and 2.4 kg (females) were achieved at d 35 (Table 6). Phytase supplementation significantly increased growth rate in phase one but not in phase two. The effect on growth for the total experimental period was significant. In both sexes, the BW at any given time was always greatest in birds that were given the diet with 10000 U of phytase per kg. The feed/BW gain ratio was 1.52 in phase one and 2.27 in phase two, without a significant effect of sex and phytase supplementation.

Phosphate concentration in blood serum, but not Ca concentration, was significantly increased by phytase supplementation (Table 7). The concentration of ALT was significantly reduced by phytase supplementation. Concentrations of creatinine, AST and LDH were not significantly affected by phytase supplementation, nor were they significantly different between males and females.

DISCUSSION

The results of Balance Studies 1 and 2 indicated that the efficiency of P utilisation in the basal diet is a critical factor in phytase efficacy studies. In Balance Study 1, the P utilisation values

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found for all phytase supplemented diets were similar to those measured in Study 2 for the corresponding treatments but the basal level was much higher (39 *vs.* 30%). Rodehutscord and Dieckmann (2005) described a range in P utilisation for plant-based diets of from 28 to 49% in ducks, 40 to 73% in broilers, and 46 to 63% in turkeys. This was based on a review of studies using 3 to 5-week-old birds and diets with low P content, as well as low or undetectable intrinsic phytase activity. The wide ranges in P utilisation are, therefore, difficult to explain. The differences found between the two basal diets in the present studies were remarkable because of the very similar experimental conditions and diets. Dietary ingredients were, however, from different batches. Concentrations of phytate P, which were not analysed, may have been different. A difference existed in intrinsic phytase activity, which was higher in Study 1 than in Study 2, yet close to the detection limit. This difference corresponds to the ranking found in the efficiency of P utilisation for the two basal diets. Phytase was most effective at a low level of supplementation. Therefore, small differences in the intrinsic phytase activity of the ingredients may become relevant for P utilisation. Furthermore, intrinsic phytase activity may be underestimated by the method of Engelen *et al.* (1994), which was applied in the present study and was developed for supplemental microbial phytase. Zimmermann *et al.* (2002) determined intrinsic phytase activity either in direct incubation or after extraction in a buffer with pH 5.5. Extraction caused phytase activities that were only about 0.3 (wheat) and 0.5 (barley, rye) of those measured in direct incubation. We hypothesise that variation in intrinsic phytase activity of maize, although at a low level, contributes to the variation found in P utilisation of maize/soybean meal-based diets. As the present studies indicate a great relevance for efficacy studies with microbial phytases – not only in ducks – this hypothesis should be investigated in future studies.

Intestinal phytase activity was detected in brush border vesicles obtained from duodenal and jejunal mucosa cells of 18-d-old Pekin ducks (Rush *et al.*, 2005). Alkaline phosphatase, which is involved in phytate hydrolysis, was also found in the small intestine

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mucosa of Pekin ducks (King *et al.*, 2000). Whether these intestinal enzyme activities are high enough to be relevant for intestinal phytate hydrolysis is unclear. Along this line, it remains uncertain whether the differences in P utilisation found between Studies 1 and 2 can be explained by differences in intestinal enzyme activities.

Supplements of *Aspergillus niger* 3-phytase were found to positively affect P utilisation in ducks (Farrell *et al.*, 1993; Wendt *et al.*, 2003). The 6-phytase that was used in the present studies improved the efficiency of P utilisation, although there were some differences in efficacy between the two studies. The responses to phytase were nonlinear in the present studies, as they were in the study with 3-phytase by Wendt *et al.* (2003), using a similar experimental approach. Lowest and highest P utilisation values in their study were 28 and 57%, which basically corresponds with results from Studies 1 and 2 of the present paper. As outlined above, the efficiency of P utilisation in the basal diet is critical in the determination of microbial phytase efficacy. Interaction may occur between the efficacy of supplemented phytase and efficiency of P utilisation in the basal diet. In our opinion, this weakens the comparison of efficacy values from different studies and makes it almost impossible to compare phytase efficacy across different papers even when the experimental approaches are similar.

Supplementing with 1000 U/kg provided an additional 0.5 (Study 1) and 0.9 (Study 2) g P_u per kg of diet. Inorganic P sources were found to differ in P utilisation in ducks between 77 and 100 % (Wendt and Rodehutscord, 2004). Equivalence values for phytase can be calculated based on these values and the phytase efficacy determined in the present studies. Results of the two balance studies and serum P_i concentrations measured in the growth study indicate that an increase in P utilisation, although small, can still be achieved at inclusions greater than 1000 U/kg. In studies with turkeys, supplementing the diet with 10000 instead of 1000 U/kg of a so-called consensus 3-phytase still increased the efficiency of P utilisation and

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tibia ash (Esteve-Garcia *et al.*, 2005). Augspurger and Baker (2004) found that chickens still responded in tibia ash to inclusions 10 to 20 times higher than usual.

Phytase supplementation increased the efficiency of Ca utilisation. It is not clear whether this is a direct phytase effect in the sense of a release of Ca from phytate, especially because a high proportion of Ca originated from limestone. Accretion of Ca correlated closely with P accretion (Figure 4). This indicates that Ca excretion was reduced with phytase supplementation simply because of a higher intermediary demand for Ca as a consequence of increased P accretion.

Phytase improved precaecal P digestibility and tibia ash content in some studies, but growth was not always positively effected, depending on the level of P supply and age (Martin *et al.*, 1998; Orban *et al.*, 1999; Attia, 2003). In the present growth study, the P supply in phase one was not high enough to meet the duck's requirement for available P. Consequently phytase supplementation had effects on growth. In phase two, no effect on growth was detected, indicating that P supply was sufficient for growth without phytase. Similarly, Orban *et al.* (1999) found no significant effect on the growth of 3 to 6-week-old ducks when they supplemented a diet containing 4.8 g P/kg with 750 U phytase/kg, but plasma P concentration was almost doubled. While the increase in the serum P_i level indicates that phytate hydrolysis in the present study was further improved when 10000 instead of 1000 U/kg were used, no beneficial effects were measured with regard to growth and feed conversion. Lower basal P levels should be applied if growth effects are to be further studied.

Data on the activity of liver enzymes or creatinine in the blood of Pekin ducks were not found in the literature, but some data from other ducks were reported. Generally, reported blood concentrations for ducks appear difficult to compare because they show a large variation between and within studies, the reasons for which are only partly known. Mule ducks had significantly increased blood plasma concentrations of LDH and ALT after a 12-d force-feeding period (Tardieu *et al.*, 2004) or after an 11-week application of high

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concentrations of fumonisin B_1 (Tran *et al.*, 2005). Laying Tsaiya ducks developed higher blood serum levels of LDH and AST when exposed to dietary supplements of 3-nitro-4-hydroxyphenylarsonic acid (Chen *et al.*, 2000). Olayemi *et al.* (2002) reported that, in the Nigerian duck, plasma concentrations of ALT and AST were lower in younger ducks than in adults. While overall ALT levels were similar in the Pekin ducks from our study and those from the other papers (Tardieu *et al.*, 2004; Tran *et al.*, 2005), the LDH level was much lower. In contrast, activities of ALT and AST in our study were lower and activity of LDH was higher level than the values for adult mule ducks given by Samour (2000). We found no explanation as to why phytase supplementation significantly reduced serum ALT concentrations in our study. This observation corresponds, however, with the finding that relative liver weights were reduced due to phytase supplementation (data not shown). Values for creatinine lie within the range reported by Samour (2000) for adult mule ducks.

Conclusions

Phytase is a promising tool for improving the utilisation of plant P and reducing P excretion in ducks through a reduced use of inorganic P supplements. The efficacy of microbial phytase decreases with an increasing level of supplementation. A plateau in P utilisation is attainable with this phytase between 1500 and 2000 U/kg. Intrinsic phytase activity in maize/soybean meal-based diets influences the efficacy of supplemental microbial phytase.

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Table 1. *Composition of the basal diets (g/kg unless otherwise stated)*

	Balance study		Growth study	
	1	2	Week 1-3	Week 4-5
Ingredients:				
Maize	622.7	656.0	605.5	660.7
Soybean meal, solvent extracted	240.0	200.0	245.0	200.0
Sunflower meal, solvent extracted	100.0	100.0	100.0	100.0
Soybean oil	12.0	25.0	10.0	10.0
Premix[1], P-free	10.0	10.0	10.0	10.0
Limestone ($CaCO_3$)	8.0	4.0	12.5	11.0
Monocalcium phosphate ($Ca(H_2PO_4)_2$)	-	-	10.0	2.0
Salt (NaCl)	3.5	3.5	2.5	3.0
L-lysine·HCl	2.5	-	2.5	1.5
L-threonine	-	-	1.2	-
DL-methionine	1.3	1.5	0.8	1.8
Analysed:				
Total phosphorus	4.4	4.2	7.1	4.9
Phytate P (calculated)	2.8	2.6	2.8	2.4
Calcium	6.6	6.0	10.2	7.8
Crude protein	188	193	192	178
ME (calculated), MJ/kg	12.4	12.5	12.2	12.5

[1]Premix contained (per kg): 233 g Ca, 3.6 g Mg, 80 g Na, 2 g Fe, 10 g Zn, 10 g Mn, 3 g Cu, 50 mg Co, 100 mg I, 50 mg Se, 10 g butylated hydroxytoluene (antioxidant), 172 mg retinol acetate, 5 mg cholecalciferol, 3 g DL-alpha tocopheryl acetate, 200 mg menadione sodium bisulphite, 200 mg thiamine mononitrate, 300 mg riboflavin, 200 mg pyridoxine hydrochloride, 1.5 mg cyanocobalamin, 1000 mg Ca-D-pantothenate, 3 g nicotinic acid, 10 mg D-biotin, 100 mg folic acid, 50 g choline chloride.

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Table 2. *Intended and analysed phytase activity in the pelleted diets*[1] *(U/kg)*

Intended	0	250	500	750	1000	1500	2000	10000
Analysed								
Balance study 1	83	131	416	734	822	1126		
Balance study 2	56	191	437	694	862	1520	1759	
Growth study wk 1-3	95				1040			9600
Growth study wk 4-5	50				910			8970

[1] Values given for the supplemented diets are the analysed total activities minus the intrinsic activity determined in the unsupplemented diet.

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Table 3. *Intake, excretion and efficiency of utilisation of phosphorus and calcium in three-week-old Pekin ducks given diets with different phytase levels in Balance Study 1*

	Phytase supplementation (U/kg)						Pooled	*P*
	0	250	500	750	1,000	1,500	SEM	(ANOVA)
Sample size (n)	*10*	*9*	*8*	*10*	*10*	*10*		
Phosphorus								
Intake (mg/d)	577	577	577	577	577	577	0.0	
Excretion (mg/d)	349	331	312	278	287	257	5.3	≤0.001
Accretion (mg/d)	226	247	264	296	289	320	5.3	≤0.001
Utilisation (%)	39.4	42.8	45.8	51.6	50.2	55.5	0.92	≤0.001
Calcium								
Intake (mg/d)	853	853	853	853	853	853	0.0	
Excretion (mg/d)	512	516	492	424	445	387	9.1	≤0.001
Accretion (mg/d)	340	340	360	426	407	468	9.1	≤0.001
Utilisation (%)	39.9	39.7	42.3	50.1	47.8	54.8	1.07	≤0.001
UOM[1] (%)	73.1	73.6	73.1	73.6	73.3	73.4	0.13	0.788

[1] UOM = unexcreted organic matter in relation to organic matter intake.

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Table 4. *Intake, excretion and efficiency of utilisation of phosphorus and calcium in three-week-old Pekin ducks given diets with different phytase levels in Balance Study 2*

	Phytase supplementation (U/kg)							Pooled	*P*
	0	250	500	750	1000	1500	2000	SEM	(ANOVA)
Sample size (n)	*9*	*8*	*8*	*8*	*8*	*8*	*7*		
Phosphorus									
Intake (mg/d)	374	374	374	374	374	374	374	0.0	
Excretion (mg/d)	263	224	195	190	184	176	171	4.9	≤0.001
Accretion (mg/d)	112	150	179	184	191	199	204	4.9	≤0.001
Utilisation (%)	29.8	40.1	47.9	49.3	50.9	53.1	54.4	1.30	≤0.001
Calcium									
Intake (mg/d)	541	541	541	541	541	541	541	0.0	
Excretion (mg/d)	353	269	208	202	184	169	174	9.5	≤0.001
Accretion (mg/d)	188	272	333	339	357	371	366	9.5	≤0.001
Utilisation (%)	34.7	50.2	61.6	62.6	65.9	68.7	67.8	1.75	≤0.001
UOM[1] (%)	70.5	69.9	70.4	70.4	69.9	70.5	70.0	0.15	0.826

[1] UOM = unexcreted organic matter in relation to organic matter intake.

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Table 5. *Results of parameter estimate (±SE) when a function of the general type $y = a+b\times(1-e^{-kx})$ was fitted to the response data from Balance Studies 1 and 2*

	Estimated parameter				
	a	*b*	*k*	r^2	$s_{y.x}$
P utilisation (%) (Figure 1)					
Study 1	39.17 ±1.29	23.93 ±9.08	0.000746 ±0.000487	0.62	4.4
Study 2	29.82 ±1.67	24.10 ±2.07	0.002375 ±0.000513	0.73	5.2
Dietary content of utilised P (g/kg) (Figure 2)					
Study 1	-0.010 ±0.076	1.320 ±1.030	0.000534 ±0.000622	0.94	0.08
Study 2	-0.001 ±0.035	1.005 ±0.042	0.002363 ±0.000243	0.99	0.04

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Table 6. *Means (standard deviation in italics) for body weight (BW) and BW gain of ducks given different phytase levels in the growth study (n=4 pens of 10 ducks per treatment)*

Phytase (U/kg)	0	1000	10000	0	1000	10000	*P* (ANOVA)		
Sex	Male			Female			Phytase	Sex	Phy×S
Body weight, g/duck									
d 7	226 *5.4*	229 *7.5*	236 *5.3*	215 *4.4*	216 *17.0*	223 *8.9*	0.177	0.004	0.969
d 14	684 *35.7*	690 *27.0*	718 *8.3*	626 *16.7*	671 *17.5*	676 *5.6*	0.003	<0.001	0.203
d 21	1334 *30.1*	1334 *19.0*	1391 *28.2*	1215 *20.5*	1280 *13.4*	1293 *21.0*	<0.001	<0.001	0.028
d 28	1995 *52.8*	1991 *35.3*	2099 *43.4*	1831 *20.8*	1901 *13.0*	1956 *10.3*	<0.001	<0.001	0.102
d 35	2634 *33.0*	2605 *7.0*	2664 *64.4*	2437 *73.5*	2469 *41.7*	2531 *27.1*	0.024	<0.001	0.325
BW gain, g/duck									
d 1-21	1,276 *30.3*	1,274 *18.7*	1,332 *27.0*	1,157 *21.6*	1,222 *14.0*	1,236 *22.0*	<0.001	<0.001	0.031
d 22-35	1,299 *62.0*	1,271 *19.7*	1,273 *38.4*	1,222 *61.9*	1,189 *29.0*	1,237 *10.6*	0.331	0.001	0.490
d 1-35	2,575 *32.9*	2,546 *6.4*	2,605 *63.4*	2,379 *74.0*	2,411 *42.1*	2,473 *28.3*	0.024	<0.001	0.330
Feed/BW gain, g/g									
d 1-21	1.52 *0.032*	1.52 *0.008*	1.51 *0.009*	1.53 *0.026*	1.53 *0.024*	1.53 *0.036*	0.818	0.139	0.944
d 22-35	2.21 *0.155*	2.25 *0.052*	2.28 *0.048*	2.27 *0.082*	2.37 *0.074*	2.21 *0.202*	0.476	0.472	0.289
d 1-35	1.87 *0.080*	1.88 *0.023*	1.89 *0.026*	1.91 *0.048*	1.94 *0.032*	1.87 *0.104*	0.524	0.247	0.431

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Table 7. *Means (standard deviation in italics) of blood serum concentrations and enzyme activities determined in 35-d-old Pekin ducks given different phytase levels*

Phytase (U/kg)	0	1000	10000	0	1000	10000	*P* (ANOVA)		
Sex	Male			Female			Phytase	Sex	Phy×S
PO_4^{3-}, mmol/l	1.53 *0.30*	1.85 *0.39*	2.49 *0.21*	2.06 *0.42*	1.94 *0.30*	2.63 *0.31*	<0.001	0.017	0.177
Ca, mmol/l	2.93 *0.14*	2.96 *0.15*	3.04 *0.10*	3.07 *0.08*	3.01 *0.13*	3.03 *0.12*	0.559	0.150	0.353
Creatinine, µmol/l	24.0 *2.47*	26.0 *0.99*	23.3 *2.31*	25.0 *1.12*	24.7 *2.39*	24.4 *2.16*	0.193	0.697	0.260
LDH,[1] U/l	421 *96*	404 *82*	372 *82*	353 *55*	363 *77*	371 *80*	0.883	0.195	0.609
ALT, U/l	19.3 *4.56*	19.3 *4.03*	16.5 *3.25*	23.2 *4.82*	18.0 *2.92*	17.1 *3.83*	0.025	0.399	0.248
AST, U/l	6.6 *1.17*	6.7 *2.99*	6.8 *1.51*	7.4 *0.80*	6.7 *1.76*	6.8 *2.12*	0.948	0.694	0.837

[1] LDH: lactate dehydrogenase, ALT: alanine aminotransferase, AST: aspartate aminotransferase.

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Figure legends

Figure 1. *Effect of phytase supplementation on efficiency of phosphorus utilisation studied in three-week-old Pekin ducks (means and SD). An exponential equation was fitted to the data and the parameter estimates are summarised in Table 5.*

Figure 2. *Increase in the dietary content of utilised phosphorus (P_u) depending on phytase supplementation. Calculations were made with treatment means based on analysed dietary phosphorus content and measured efficiency of phosphorus utilisation as given in Tables 3 and 4. Estimated parameters of the exponential equations are summarised in Table 5.*

Figure 3. *Marginal efficacy of supplemented phytase in diets for Pekin ducks. The lines show the first derivatives of the functions from Figure 2 according to Equation 2. Marginal efficacy is the increment in the content of utilised phosphorus (P_u) achieved with each increment in phytase supplementation.*

Figure 4. *Relationship between phosphorus accretion and calcium accretion in Pekin ducks given diets with variable phytase levels. The equations show the estimated parameters (± SE of estimate) of linear regressions fitted to the data.*

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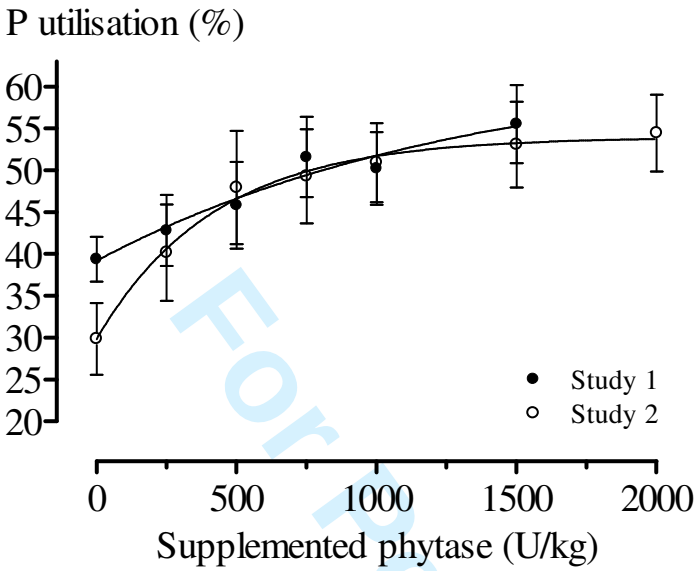

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Fig. 1 of Rodehutscord et al., 2005-023

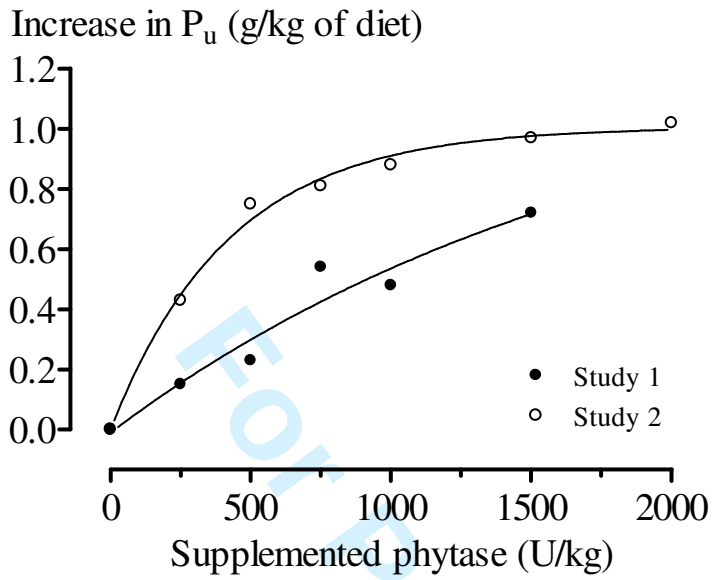

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Fig. 2 of Rodehutscord et al., 2005-023

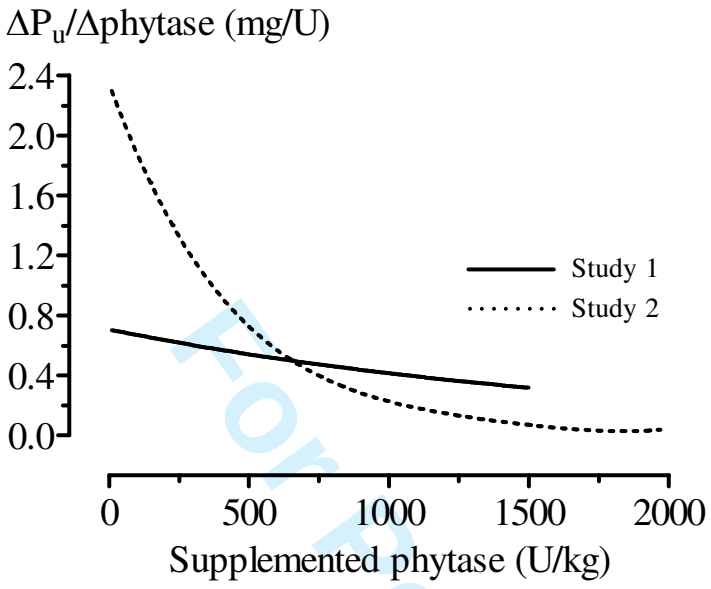

Fig. 3 of Rodehutscord et al., 2005-023

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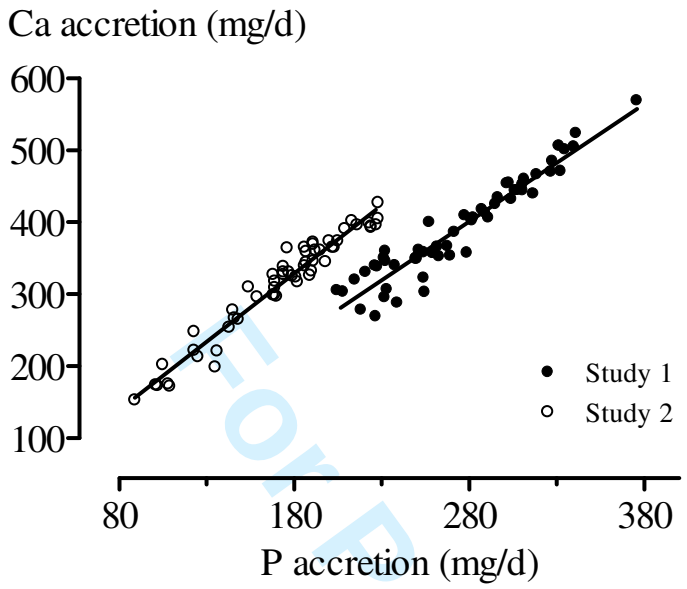

$y_{Study\ 1} = -56(\pm 20) + 1.63(\pm 0.07)\ x$; r^2: 0.91; $s_{y.x}$: 21

$y_{Study\ 2} = -13(\pm 11) + 1.90(\pm 0.06)\ x$; r^2: 0.95; $s_{y.x}$: 16

Fig. 4 of Rodehutscord et al., 2005-023

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