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Effect of phytase dose and reduction in dietary calcium on performance, nutrient digestibility, bone ash and mineralization in broilers fed corn-soybean meal-based diets with reduced nutrient density

Running Header: PHYTASE DOSE AND CALCIUM LEVEL IN BROILERS

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Highlights

- Buttiauxella phytase at 1000 FTU/kg improved BWG, FCR vs. 500 FTU/kg during 0-21d
- Phytase supplementation enhanced ileal and total tract digestibility of P and Ca
- Increase Ca reduction level with phytase increased ileal P and Ca digestibility
- Increase Ca reduction level improved feed efficiency in diets containing phytase
- All phytase treatments reduced feed cost and improved energy efficiency vs PC

ABSTRACT

The effect of reducing dietary Ca level (and Ca:P ratio) in combination with phytase supplementation, on broiler growth performance, nutrient digestibility, bone ash and mineralization, was investigated. A total of 2072 Ross 308 d-old male broilers were allotted

to 7 dietary treatments with 37 birds/pen and 8 pens/treatment in a randomized block design. A positive control corn-soybean meal-based diet was formulated based on breeder's recommendations. Six test diets were evaluated in a 2 x 3 factorial arrangement including two levels of a *Buttiauxella* sp. phytase (500 and 1000 FTU/kg feed) and three Ca reduction levels (equivalent to 1.3, 1.6, 2.3g/kg reduction and 1.6, 1.9 and 2.3g/kg reduction in the 500 and 1000 FTU/kg phytase treatments respectively *vs.* PC). The reductions in available P (AvP) of the test diets were 1.46 and 1.74g/kg in the 500 FTU/kg and 1000 FTU/kg phytase treatments respectively. Test diets were formulated by adding phytase, Ca and P supplements to a basal diet containing a reduction of 68 kcal ME/kg, on average 0.2 g/kg dig AA and 0.3 g/kg Na *vs.* PC. Diets were formulated in four phases. Excreta samples were collected on d 24-26 and 38-40 (from 5 birds transferred to cages on d 21 and 35 respectively) and ileal digesta and tibia samples were collected on d 10, 27 and 41. Compared to PC, feed conversion ratios (FCR) were reduced in growers fed medium or high Ca reduction and high phytase dose diets ($P < 0.05$). All phytase treatments improved energy efficiency (by up to 1.05 MJ/kg BWG) compared with the PC. Factorial analysis revealed a greater BWG and lower FCR with phytase supplemented at 1000 FTU/kg *vs.* 500 FTU/kg during starter and grower phases ($P < 0.05$). The high Ca reduction level (2.3 g/kg reduction *vs.* PC) reduced FCR in the finisher phase, and reduced overall (42 d) calorie consumption and feed cost per kilogram weight gain, compared with the low Ca reduction level ($P < 0.05$). Compared to the PC, phytase supplementation generally enhanced (by 10 to 30%) ileal, and total tract digestibility of P and, to a lesser extent Ca. Ileal digestibility of P at d 10 and d41 and Ca at d 41 were higher in the high- vs. the low-phytase groups. The high Ca reduction groups resulted in higher ileal P digestibility at d 10 and d41, ileal Ca digestibility at d27 and d41 *vs.* low Ca reduction groups ($P < 0.05$). Tibia ash was unaffected by dietary treatments, but bone Zn was increased at d 10 in all but one treatment *vs.* PC ($P < 0.001$) and was higher in the high- vs. the low-phytase groups ($P < 0.01$).

Collectively, these findings indicate that a moderate (1.6 g/kg) to high (2.3 g/kg) reduction in dietary Ca in diets supplemented with 500 to 1000 FTU/kg of *Buttiauxella* phytase improved P and Ca digestibility, energy efficiency and productive performance in broilers fed corn-soybean meal-based diets.

Abbreviations

AIA, acid insoluble ash; AvP, available P; Ca, calcium; FCR, feed conversion ratio; GE, gross energy; ME, metabolizable energy; P, phosphorus; Zn, zinc, Mg, manganese

Keywords: broilers, *Buttiauxella* phytase, calcium, digestible P, production performance

1. Introduction

Phosphorus (P) is essential for optimal growth and bone development in broilers. However, in typical corn-soybean meal based commercial diets up to 80% of P is locked up in the form of phytic acid or its salt: phytate (Taylor and Coleman, 1979; Selle and Ravindran, 2007). Phytic acid and phytate are poorly digested by monogastric animals, meaning that phytate has limited phosphorus availability to poultry (Sebastian *et al.*, 1996). Furthermore, phytate readily binds to minerals such as Ca, Zn, Fe and Mg in the gastrointestinal tract (Selle *et al.*, 2000), forming insoluble complexes which are not readily broken down in the broiler small intestine where the pH is typically 5.5 to 6.6 (Shafey *et al*., 1991). Calcium is the most abundant mineral in poultry diets and is typically present at levels far in excess of those of phytate; a typical commercial broiler starter-diet contains 9 to 10 g/kg Ca, whilst phytate phosphorus levels in corn-soybean meal-based diets are typically 2.4 to 2.6 g/kg. Therefore, in the small intestine, the majority of the phytate will be bound to Ca (Farkvam *et al.*, 1989; Selle

et al., 2009). This substantially reduces the availability of both phytate phosphorus and Ca to the bird. On this basis, the levels of minerals in the diet, including those of Ca, are a key factor in the ability of the bird to utilize phosphorus in the form of phytate (Sandberg *et al.*, 1993).

Microbial phytase is commonly used in poultry diets to breakdown phytate, releasing digestible phosphorus and also increasing the availability of Ca. However, the efficacy of different phytases has been somewhat inconsistent between studies (Selle and Ravindran, 2007). This may be due to a number of factors, including phytase biochemistry and its microbial source, feed composition, and substrate availability, as well as gut physiology (e.g. pH). One key aspect of this is understanding the influence of both the absolute levels and the ratio of Ca to P in the diet on digestibility and performance, and how phytase supplementation impacts on these factors. The relative contributions of P and Ca (as well as of other nutrients such as digestible amino acids) in the formulation of feed with added phytase, is referred to by nutritionists as the 'phytase matrix'. Most feed nutritionists formulate diets with added microbial phytase in order to partially or totally replace the need to add inorganic P, from sources such as monocalcium phosphate (MCP) and dicalcium phosphate (DCP). When applying a Ca matrix that is lower than the dig P matrix, or when the matrix is not in proportion to the requirement of digestible P and Ca, in order to maintain the required Ca level in the diets more limestone is often added. Limestone has an extraordinarily high acid binding capacity and will tend to increase pH along the digestive tract, this may reduce the solubility of phytate and reduce its accessibility by exogenous phytase (Selle *et al*., 2000). Studies suggested that increased dietary Ca content can negatively affect the efficacy of added phytase (Tamim *et al*., 2004; Lei *et al*., 1994), but effects are likely to differ between phytases of different microbial source, pH optima and be related to different dietary compositions. Commercially phytase activity is standardized at pH 5.5, however, the activity in the gizzard and proventriculus varies among different commercial phytases (Menezes-Blackburn *et al*., 2015). A new generation

Buttiauxella sp. phytase is highly active at pH 3.0 (Menezes-Blackburn *et al*., 2015), therefore it is expected that such a phytase will breakdown phytate quickly in the gizzard and proventriculus and will efficiently reduce the formation of Ca and phytate complexes. So its beneficial effect on improving P availability is likely to be mediated through increased hydrolysis of phytate before it enters the small intestine (where it can form insoluble complexes with Ca), rather than by hydrolyzing phytate in the small intestine. However, this will also lead to increased availability of Ca, which may have an impact on Ca and P balance.

It is important to maintain an optimal balance between Ca and P in the diet in order to ensure that the availability of Ca and P to the bird is adequate for optimal growth and bone mineralization, without there being a deficiency or excess of these minerals, both of which can have a negative impact on P and Ca utilization (Qian *et al*., 1996, 1997). Increasing the content of Ca relative to phosphorus in broiler diets can significantly reduce P digestibility (Plumstead *et al*., 2008) and impair performance (Qian *et al*., 1997; Paiva *et al*., 2014), but few previous studies have sought to determine an optimal dietary Ca:P ratio in the context of phytase supplementation at different dose levels. Understanding the effects of individual phytases on Ca and P utlilization will provide valuable information which will help to better maintain Ca to P balance in the diets supplemented with phytase and may lead to improved performance.

The objective of the present study was to determine the effect of reducing dietary Ca content (relative to phosphorus) in the presence of phytase at two doses, on growth performance, nutrient digestibility, tibia ash and mineral content, in broilers. The hypothesis was that a reduced level of dietary calcium (relative to phosphorus) in the presence of phytase supplementation would maintain better Ca:P balance, increase P digestibility and growth performance of broilers, without negatively affecting bone ash or mineral content.

2. Materials and methods

2.1 Experimental and control diets

The experimental design was a 2 x 3 factorial arrangement to evaluate the effects of three dietary Ca reduction levels and two levels of phytase (500 and 1000 FTU/kg feed), against a positive control diet, giving a total of seven dietary treatments (Table 1). One FTU is defined as the quantity of enzyme that releases 1 μmol of inorganic P per minute from 5.0 mM sodium phytate at pH 5.5 at 37°C (AOAC, 2000). All diets were corn and soybean-meal based and were formulated in each of four phases (starter: d 0 to 10, grower: d 11 to 21, finisher 1: d 22 to 35, finisher 2: d 36 to 42). The positive control (PC) diets were formulated to meet the recommended requirement for nutrients of the birds set by the breeder (Table 2). For the remaining 6 dietary treatments, phased negative control basal diets were formulated with a reduction of 0.28 MJ ME/kg, and an average reduction of 0.2 g/kg digestible amino acids and 0.3 g/kg Na *versus* PC, based on the contribution of the phytase at 500 FTU/kg (Table 2). The basal diets were then supplemented with a *Buttiauxella* sp. phytase (Axtra® PHY, Danisco Animal Nutrition, Marlborough, UK) at 500 FTU/kg or 1000 FTU/kg, and with different levels of DCP and limestone (as shown in Table 2) in order to achieve three levels of dietary calcium reduction *vs* PC (low (L), medium (M), and high (H)) (equivalent to 1.3, 1.6, 2.3g/kg reduction and 1.6, 1.9 and 2.3g/kg reduction in the 500 and 1000 FTU/kg phytase treatments respectively *vs.* PC). The high Ca reduction level of 2.3 g/kg was based on an estimated Ca to P release ratio in the broiler intestine which was derived from the Ca binding capacity of phytate and its esters (Selle, 2009), thus it was the same for both 500 and 1000 FTU/kg treatments. An appropriate Ca to available P (AvP) ratio in the diets containing phytase was achieved via a consequent reduction in available P (*vs.* PC) of 1.46 g/kg in the 500 FTU/kg phytase treatments, and of 1.74 g/kg in the 1000 FTU/kg phytase treatments. The levels of phytase inclusion and reduction in dietary Ca and available P (*vs.* PC) among the different treatments are given in

Table 1. A filler material (sand) was used in order to maintain the same overall ingredient composition across all experimental diets (Treatments 2 to 7) except for DCP and limestone (Table 2). Celite, a source of acid-insoluble ash (AIA), was added at 2% to all experimental diets as an indigestible marker. Experimental diets were pelleted at ~ 65°C.

2.2 Birds and housing

The experimental protocols were approved by the Animal Experiments Committee of The University of Queensland, Australia.

Ross 308, one day old male broilers were obtained from a commercial hatchery where they had been vaccinated against infectious bronchitis and assigned to floor-pens based on BW, so that pens contained birds with approximately equal average bird weight. A total of 2072 birds were assigned to 56 pens with 37 birds per pen and 8 pens per treatment, in a completely randomized block design. Pens were located in an environmentally controlled broiler house where temperature was maintained at 34°C for the first 7 days and then gradually reduced to 27°C by d 21, under a light-dark cycle of 23:1 h. Birds were given free access to water and to diets which were provided in a crumbled form for starter phase and pelleted form for other phases.

2.3 Sampling and measurements

Body weight and feed intake (FI) were recorded on d 1, 10, 21 and 42 and used to calculate BWG and mortality corrected FCR. Mortality was recorded daily.

On each of d 21 and d 35, 5 birds per floor-pen were transferred to cages, where temperature and light cycling conditions were identical to those of the floor pens, for the purposes of total tract and ileal nutrient (Ca and P) digestibility measurements. After a three-day acclimation period, feed intake and total excreta output were measured quantitatively per

cage on three consecutive days (d 24 to d 26 and d 38 to d 40, respectively). Daily excreta collections were pooled within a cage, dried in an oven at 80°C for 48 h and then ground to pass through a 0.5 mm sieve prior to analysis and stored at -20°C. On d 27 and d 41 respectively, all caged birds were euthanized by cervical dislocation and the contents of the lower half of the ileum extracted by gentle flushing with distilled water. Ileal digesta samples were also collected on d 10 from 7 birds per replicate floor-pen, using the same methods. Ileal digesta samples were pooled per replicate, freeze-dried and ground to pass through a 0.5 mm sieve prior to nutrient analysis. Ileal digestibility of nutrients was calculated using acid insoluble ash as an indigestible marker.

Left tibia bones were collected from the 7 birds per floor-pen that had been euthanized on d 10 as well as from the 5 birds per replicate cage that had been euthanized on d 27 and 41, and the pooled samples per replicate were used for the determination of tibia ash and mineral content.

Representative sub-samples of all dietary treatments (500 g feed) were taken and analyzed for DM, CP, GE, P, Ca, phytase and phytate content.

2.3 Chemical analysis

Calcium and total phosphorus in feed, ileal digesta samples and tibia ash were determined by ICP-OES analysis (ICPOES method 2011.14) (AOAC, 1990) following microwave assisted acid digestion. The acid insoluble ash content of feed and ileal digesta samples was determined by AOAC method 975.12 (AOAC, 1990). For the quantification of de-fatted tibia ash, the tibia bones were soaked in methanol for 24 h followed by diethyl ether for 48 h in pyrex tubes (Thomas Scientific, US). The de-fatted bones were then left to dry overnight in a fume hood before weighing in beakers and dried in an oven at 100°C for 8 to 12 h. The defatted, dry bones were then weighed and ashed in the same beakers in a muffle furnace

at 600°C for 48 h. (AOAC, 2000). Zinc and Mg in the tibia ash were determined by Inductively Coupled Plasma (ICP) Spectroscopy (AOAC 1985, Official Method 985.01). The nitrogen content of feed samples was determined by the combustion method (AOAC International, 1990; method 990.03) and crude protein was calculated. Gross energy (GE) was determined by Oxygen bomb calorimetry and oxygen bomb combustion methods (Parr Manual 120. 1948). The phytase activity and phytic acid content of feed samples were analyzed by Danisco Innovation Laboratories (Brabrand, Denmark), using the methods described by Yu *et al.* (2012).

2.5 Calculations

Body weight gain (BWG) and feed conversion ratio (FCR) were calculated for starter (0 to 10 d), grower (11 to 21 d), starter + grower (0 to 21 d), finisher (22 to 42 d) and for the overall period of 0 to 42 d.

Average feed intake (FI) per bird per day (corrected for mortality) was calculated according to the following formula:

FI = total feed consumed per pen (g) / (number of birds in that pen x number of days of consumption)

Feed conversion ratio (FCR) was calculated according to the following formula:

FCR = total feed consumed per pen (g) / body mass (including dead birds) in that pen (g)

Apparent ileal digestibility of nutrients was calculated according to the following formula, based on the determined concentration of acid insoluble ash (AIA) in the diet and in the digesta, in accordance with Ravindran *et al.*, 2006:

Apparent ileal or total tract digestibility of nutrients = $[(\text{nutrient/AIA})_{\text{diet}} - (\text{nutrient/AIA})_{\text{ileal digesta or total tract excreta}})/ (\text{nutrient/AIA})_{\text{diet}}]$,

where 'nutrient' refers to either Ca or P, '$(nutrient/AIA)_{diet}$ = ratio of nutrient and AIA in the experimental diet, and '$(nutrient/AIA)_{ileal\ digesta\ or\ total\ tract\ excreta}$ = ratio of nutrient and AIA in ileal digesta or total tract excreta, as appropriate.

2.6 Statistical analysis

Data were based on pen as the experimental unit. Data were analyzed by ANOVA using the Fit Model platform of JMP 11.0 (SAS Institute Inc., Cary, NC, 1989-2013) to investigate the effects of treatments, with dietary treatment as a fixed effect and block as a random effect. Means separation was achieved using Tukey's Honest Significant Difference test. Data from treatments 2 to 7 were additionally analyzed as a 2 x 3 factorial arrangement, to test the main effects of phytase, Ca reduction level and their interaction. Differences were considered significant at $P < 0.05$ and $P < 0.1$ was considered a tendency.

3. Results

3.1 Diet analysis

Analyzed concentrations of P and Ca in the diets were close to formulated values, except in the grower phase where Ca content was both more variable and higher than calculated (Table 3). This may have been due to sampling and analytical errors. However, in all phases, the analyzed Ca:P ratio confirmed that Ca was reduced as expected in low, medium and high Ca reduction diets versus PC. Across dietary phases of starter, grower, finisher 1 and 2, the analyzed phytase was slightly higher than targeted dose at 1000 FTU/kg (Table 3). However, considering the variation caused by feed mixing and the acceptable analytical errors, these analyzed activities are in line with the targeted phytase dose.

3.2 Growth performance

Treatment means for BWG, FI, FCR and mortality during starter, grower and finisher phases are presented in Table 4. Data for the combined starter-grower period (d 0 to 21) and the overall period (d 0 to 42) are presented in Table 5. During the starter phase (d 0 to 10), performance was unaffected by dietary treatments. In growers (d 11 to 21), BWG and FI were unaffected by dietary treatment, FCR was reduced in both medium and high Ca reduction diet groups at the higher phytase dose, compared with the PC ($P < 0.05$). There was no mortality during the starter and grower phases across treatments. Overall, the 0 to 21 day data showed lower FCR in the medium calcium reduction and high phytase dose compared with PC ($P < 0.05$). No significant differences were found in finisher (d 22 to 42) phases for FCR. Overall (d 0 to 42), the high Ca reduction level resulted in reduced FI with phytase at 500 FTU/kg vs low Ca reduction with phytase at 1000 FTU/kg, but BWG and FCR were unaffected. There was some mortality among birds in the finisher phases but no significant difference was found between phytase treatments and PC. Overall, energy conversion expressed as megajoules (MJ) needed per kg BWG was decreased by up to 5% (1.05 MJ/kg BWG) in the Ca-reduced phytase-supplemented diets compared with the PC ($P < 0.05$) (Table 5).

Factorial analysis of the main effects of phytase dose and dietary Ca reduction level on growth performance (excluding PC) showed no interaction between phytase dose and dietary Ca reduction level in effects on growth performance parameters (Table 4, 5). Across Ca reduction levels, the high dose phytase (1000 FTU/kg) reduced FCR (d 0 to 10 and d 11 to 21), increased BWG (d 0 to 10 and 11 to 21), and increased FI (d 22 to 42 and overall) *vs*. the low phytase dose (500 FTU/kg) ($P < 0.05$). No main effects of Ca reduction level on measured growth performance parameters were evident during starter and grower phases. However, in the finisher phase, the high Ca reduction level reduced FCR compared to the low Ca reduction level ($P < 0.05$). Overall, the 0 to 42 day data showed that the high Ca-reduction improved energy efficiency (MJ/kg BWG, indicating less energy is used to produce one kg BWG ($P <$

0.05, Table 5)). Dose response analysis revealed a linear decrease in feed intake during d 11 to 21 (Table 4), FCR during d 22 to 42 (Table 4), and in overall energy consumption (MJ) per kg BWG, with increasing level of Ca reduction (Table 5).

3.4 P and Ca digestibility

Treatment means for ileal P and Ca digestibility are presented in Table 6. At d 10, ileal digestibility of P was increased among birds fed the medium and high dietary Ca reduction diets, at both phytase doses *vs.* PC ($P < 0.001$). By d 27, increases in ileal and total tract P digestibility as percentage of intake were evident in all phytase treatments regardless of Ca reduction levels ($P < 0.001$) when compared with PC. Ileal digestibility of Ca was also higher in the medium Ca reduction group supplemented with the low dose of phytase than PC at this time-point ($P < 0.001$). Factorial analysis of the effects of phytase dose and dietary Ca reduction level on digestibility of P and Ca are also shown in Table 6. An interaction between phytase dose and Ca reduction level was found at d 41, for ileal digestibility of P and Ca ($P < 0.01$), such that the observed beneficial effects of increasing phytase dose on digestibility were magnified by increasing dietary Ca reduction. The same interaction was observed at d 27, for ileal digestibility of Ca, where the high phytase dose reduced digestibility and increasing the Ca reduction level improved digestibility. In addition, both phytase dose and dietary Ca reduction level exerted independent effects on ileal digestibility of P and Ca at one or more time-points (Table 6). Phytase dosed at 1000 FTU/kg increased ileal P digestibility at d 10 and d 41, and increased ileal Ca digestibility at d 41, compared with phytase dosed at 500 FTU/kg. The high Ca reduction level increased ileal P digestibility at d 10 and 41 and increased ileal Ca digestibility at d 27 and 41, compared with the low Ca reduction level. A linear increase in ileal P and Ca digestibility was observed at d 10 (P), 27 (Ca) and 41 (P and Ca) with increasing Ca reduction levels.

Treatment means, factorial analysis for main effect and interaction for total tract P and Ca digestibility and their retention are presented in Table 7. Total tract Ca digestibility as percentage of intake was greatest in the high Ca reduction-low phytase dose (500 FTU/kg) group, which was significantly greater than PC ($P < 0.05$). At d 41, the greatest ileal digestibility of P and Ca was seen in the high phytase dose-high dietary Ca reduction group, which was significantly greater than PC and other test groups ($P < 0.001$). Total tract digestibility of Ca was significantly improved with high Ca reduction at both phytase doses *vs.* the PC ($P < 0.01$). Total tract digestibility of P was numerically greater in all treatment groups versus PC, but statistically only showed a tendency to be increased in the high Ca reduction-low phytase group ($P = 0.07$). Total tract retention of P and Ca as g/kg feed were unaffected by treatment, except at d 41 where retention of Ca was increased in the low phytase-high dietary Ca reduction group ($P < 0.01$).

There was no significant main effect of phytase dose on total tract digestibility of P or Ca as g/kg feed at any time-points. However, dietary Ca reduction level positively affected total tract digestibility of Ca at both d 27 and d 41 ($P < 0.05$). A significant effect of dietary Ca reduction level on retention of Ca was found at d 41 ($P \leq 0.05$), where a lower Ca retention (as g/kg feed) was also observed with the higher phytase dose (Table 7). A linear increase in total tract digestibility of Ca with increasing Ca reduction level was observed at d 27 and 41, and in P and Ca retention at d 41 ($P < 0.05$).

3.5 Tibia ash and mineral content

Treatment means for tibia ash and mineral content at d 10, 27 and 41, are presented in Table 8. There were no differences in tibia ash quantities among dietary treatments at any time-point; even in birds given a diet highly reduced in Ca (in combination with phytase supplementation) tibia ash quantities were similar to those of control diet birds. Differences in

tibia mineral content varied across time points and among minerals; by d 10, concentrations of P in the tibia of birds fed the high phytase dose and high Ca reduction diet were higher than those of birds fed the low phytase dose and low Ca reduction diet, but did not differ from control fed birds ($P < 0.01$). Levels of Zn in the tibia were higher in all phytase treatment groups compared with control-fed birds, except those fed the low phytase dose and medium Ca reduction diet ($P < 0.001$). No differences in bone mineral content were seen at d 27. At d 41, Mg levels were increased in birds given the high phytase dose, medium and high dietary Ca reduction diets, compared with control birds ($P < 0.01$).

Factorial analysis of the effects of phytase dose and dietary Ca reduction level on bone mineralization are presented in Table 8. No interactions between phytase dose and dietary Ca reduction level on bone mineralization were evident. At d 10, there was a significant effect of phytase dose on bone mineral content for P, Mg and Zn levels of these minerals were higher in birds fed the greater dose than the lower dose of phytase ($P < 0.05$). At d 27 and d 41, this effect was seen only for Mg ($P = 0.06$ and $P < 0.01$, respectively). At d 41, a linear increase in bone Mg was found with increasing Ca reduction levels ($P < 0.05$).

4. Discussion

4.1 Performance

Whilst Ca is an essential nutrient, up to a third of dietary Ca may be bound to phytate in the digesta (Selle *et al*., 2009), thereby limiting the availability of both phytate-P and Ca to the animals. Phytate that is bound to Ca in insoluble form is less readily hydrolyzed by exogenous phytase. Therefore, dietary levels of Ca and Ca:P ratios are considered to be critical to the efficacy of exogenous phytase in poultry nutrition (Angel *et al*., 2002). Previous studies that have investigated the effects of dietary Ca level and/or dietary Ca:P ratios in broilers fed

corn-soybean meal-based diets have demonstrated that relative increases in dietary Ca or Ca:P ratio can adversely affect performance and digestibility of both Ca and P: Qian *et al.* (1997) found that increasing dietary Ca (from 5.6 to 10.2 g/kg) and Ca:P ratios (from 1.1 to 2.0:1) suppressed broiler weight gain at 21d; Paiva *et al*. (2013) showed that broilers fed a high dietary Ca level (9 g/kg vs 6 g/kg) exhibited increased mortality associated with necrotic enteritis and reduced weight gain; Plumstead *et al*. (2008) found that increasing dietary Ca from 4.7 to 11.6 g/kg decreased ileal phytate-P digestibility by 71% in broilers. However, information regarding the effects of reducing dietary Ca levels and/or Ca:P ratios in phytase supplemented diets is limited. There is a need to characterize the precise effects of these factors – and how they are influenced by phytase supplementation - on growth performance, digestibility and other relevant production measures, in order to inform and optimize recommended calcium contribution ('matrix') values assigned to the use of exogenous phytases in broilers.

In the current study, phytase supplementation at either 500 or 1000 FTU/kg feed appeared to compensate for nutrient reduction in the test diets on growth performance. In fact, in the presence of phytase supplementation, birds fed diets reduced in Ca (from 9.7 g/kg (PC) to 7.2 g/kg (starter phase-high Ca reduction diet) and from 6.5 g/kg (PC) to 4.4 g/kg (finisher 2 phase-high Ca reduction diet (analyzed values)) and in AvP, AA, energy and Na, performed equally to or numerically better than birds in the PC group whose diets were not deficient in any nutrients and did not contain phytase. This suggests that in the presence of phytase (at 500 or 1000 FTU/kg) the birds were able to utilize the feed more efficiently, which was supported by observations of increased feed-derived energy efficiency of the phytase supplemented diets compared with the control diet.

Qian *et al*. (1997) found that the graded addition of phytase between 0 and 900 FTU/kg to corn-soybean meal-based broiler diets containing four different ratios of Ca:tP, linearly increased BWG and FI during starter and grower phases, and concluded that Ca:tP ratios

between 1.1:1 and 1.4:1 were critical to phytase efficacy. The analyzed Ca:tP ratios in the starter-grower diets of the present study fell broadly within that range (1.20 to 1.37 for the dietary treatments with medium and high Ca reduction, Table 3). Our findings are consistent with those of Qian *et al.* (1997) in that a dose-response effect of phytase on measures of performance (BWG and FCR) during these early growth phases was evident from the factorial analysis (in which the PC was excluded), with the higher dose of phytase (1000 FTU/kg), resulting in increased BWG and reduced FCR than the lower dose (500 FTU/kg). However, Qian *et al.* also observed an independent, dose-response type effect of Ca:tP ratio on performance, whereby effects on performance were negatively influenced by widening the dietary Ca:tP ratio (equivalent to increasing dietary Ca level), independent of phytase addition. A similar effect was also noted in a study by Lei *et al.* (1994) in swine. Both of these authors suggested that higher Ca levels and wider Ca:P ratios depressed phytase efficacy due to Ca progressively precipitating phytate in insoluble complexes in the intestine. Schoner *et al.* (1993) reported that in the presence of phytase supplementation, a Ca-reduced diet (6 g/kg Ca) produced higher broiler body weights than diets containing 9 g/kg Ca. Such dose-response type effects of dietary Ca reduction in the presence of phytase supplementation were not evident in the present study in relation to growth performance measures in starter phase. However, at a high phytase dose of 1000 FTU/kg, medium and high Ca reduction reduced FCR *vs.* PC during the grower phase (d 11 to 21). Based on the factorial analysis, during starter-grower phases, there was a tendency towards a linear decrease ($P = 0.058$) in FCR with increasing Ca reduction levels during the grower phase. The absence of a stronger dose-response effect of Ca reduction on starter-grower performance as reported by Lei *et al.* (1994) and Qian *et al.* (1997) may be due to differences in the phytase used and the breadth of dietary Ca reduction levels employed (and therefore of Ca:P ratios); these were narrower in the present study. The choice of dietary Ca reduction levels and Ca:P ratios was purposeful, in order to test whether the dietary

incorporation of Ca in the presence of phytase supplementation could be reduced to below current 'matrix' formulation recommendations, without incurring adverse effects on growth performance. But it may have impaired the ability to detect a stronger dose-response type effect of reducing dietary Ca level on growth performance in the starter phase. In a further study design involving two levels of phytase (0 and 1000 FTU/kg feed), and four dietary Ca:AvP ratios (in which dietary Ca levels were set at 4, 6, 8 and 10 g/kg), Amerah *et al.* (2014) similarly reported an interaction between phytase addition and Ca:AvP ratio in effects on starter-grower performance; increasing dietary Ca:AvP ratio decreased BWG and FI, increased FCR, and these effects were minimized in birds fed the phytase-supplemented diets. Again, the dietary Ca levels in that study were considerably wider than those in the present study (which ranged from a minimum of 6.7 g/kg to a maximum of 8.9 g/kg among the test diets (treatment 2-7) offered to starter-grower birds (analyzed values)). In addition, the results may also indicate that the *Buttiauxella* phytase is less impacted by high dietary Ca, due to its high active at pH 3.0 (Menezes-Blackburn et al., 2015), which lead to more efficient breakdown of phytate in the gizzard and proventriculus and reduced the formation of Ca and phytate complexes (Mejldal, personal communications).

There is an absence of published studies on effects of dietary Ca reduction or Ca:P ratio in phytase supplemented diets on broiler growth stages beyond the starter-grower period. Information on effects in the finisher period is also needed in order to be able to optimize dietary nutrient formulation recommendations across all phases. In the present study, in finisher phases (d 22 to 42), a high level of Ca reduction significantly reduced FCR compared to low Ca reduction. Furthermore, linear decreases in FCR during the finisher phase as well as in energy cost and feed cost per kg BWG (data not shown) during the overall period (d 1 to 42) were observed. These findings suggest there are both performance and production benefits of increasing the Ca reduction level in the presence of phytase. Reducing the Ca level did not have

any impact on mortality during the starter and grower phases, however some mortality was evident in the finisher stages. Whilst this was not significantly above that of the PC group at the $P < 0.05$ level, the analyzed Ca level in the high Ca reduction treatments of 4.4 g/kg could have been low enough to have increased the risk of Ca deficiency in the diets, which may have influenced mortality. Tibia ash was not affected and no leg weakness was observed. Nevertheless, the tendency ($P = 0.091$) towards higher mortality in the high Ca reduction diet groups compared with the medium and low Ca reduction groups may suggest that dietary analyzable Ca levels in the finisher phases should be maintained above a level of 5 g/kg incorporation (Angel, personal communication).

4.2 Phosphorus and calcium digestibility and retention

The effects of the dietary treatments on ileal and total tract digestibility of P and Ca were more pronounced than, but broadly supportive of, effects on growth performance. There was a general trend of phytase supplementation producing enhanced P and Ca digestibility (as a percentage of intake) in the Ca reduced diets compared with the PC diets. The factorial analysis revealed an apparent effect of phytase dose on ileal and total tract digestibility of P and Ca, particularly at d 10 and 41. The lack of a significant response on digestible P at d 27 may be because the analyzed P levels in the phytase treatments were higher than targeted levels. When Ca retention was calculated as g/kg feed, it was observed that the higher phytase dose reduced Ca retention versus the lower dose, which could be related to the lower Ca content in the high phytase dose diets. In addition, there was evidence that, by d 27 and d 41, greater reductions in dietary Ca (and thereby in Ca:digP ratio) led to increased ileal and total tract digestibility of P (increased by up to 30%) and, to a lesser extent, Ca (increased by up to 21%). At these time-points, there was also an interaction between effects of Ca reduction and phytase dose on ileal digestibility of Ca and/or P, such that beneficial effects of increasing phytase dose were enhanced by increasing the reduction in dietary Ca. The enhancing effect of

supplementary phytase in broiler diets on ileal digestibility of Ca and P is well established (Ravindran *et al.* 2006; Ravindran *et al*., 2008). A positive effect of decreasing dietary Ca levels on ileal P digestibility – or rather a negative effect of increasing dietary Ca levels - has also been described previously; Plumstead *et al.* (2008) showed that increasing dietary Ca in soybean meal based diets, from 4.7 to 11.6 g/kg feed - a wider range than that employed in the present study - linearly decreased phytate-P digestibility coefficient by 71%. Tamim *et al.* (2004) reported a 63% decrease in phytate P (PP) disappearance from the ileum when dietary Ca increased from 1.8 to 6.5 g/kg, and Amerah *et al.* (2014) similarly reported a reduction in ileal phytate-P disappearance in broilers fed diets containing 6.8 g/kg Ca *vs.* 5.1 g/kg Ca during starter-grower phases. Some of these studies have also investigated the effect of phytase supplementation in Ca reduced diets on nutrient digestibility, although few have considered a possible dose effect of phytase. Tamim *et al.* (2004) showed that addition of 5 g/kg Ca from limestone to maize-soybean broiler diets reduced the ileal disappearance of phytate-P (from 69.2 to 25.4%) and reduced Ca and P absorption, whilst inclusion of 500 FTU/kg phytase mitigated these effects but still showed lower phytate P digestibility at high Ca level. The aforementioned study by Amerah *et al.* (2014) observed increases in phytate degradation and P digestibility (and digestibility of energy and amino acids) in response to 1000 FTU/kg of *Buttiauxella* phytase addition at all tested levels of Ca:AvP. A further recent study, by Li *et al.* (2016), evaluated the effects of two Ca levels and 3 phytase levels (0, 500 and 1000 FTU/kg) and reported decreased ileal IP6 disappearance at a higher Ca level (10 g/kg) *vs.* a lower Ca level (7 g/kg), that was improved by addition of phytase. No interactions between phytase dose and dietary Ca level in effects on ileal digestibility were evident in those studies, but that may be because they focused on the starter-grower phases only (0 to 21 d); such interactions were only evident in the present study at d 27 and/or d 41. In the present study, increasing Ca reduction also linearly increased P and Ca retention at d 41. Overall, the results of the existing

literature and the present findings would appear to underline the efficacy of phytase in supporting favorable P and Ca digestibility and retention in moderate to highly Ca-reduced corn-soybean meal broiler diets, at a phytase level of 500 to 1000 FTU/kg.

There are a number of potential mechanisms by which dietary Ca may impede the activity of exogenous phytase in the broiler gut. Any combination of these might explain the observed effects of increased growth performance and/or nutrient digestibility when phytase was administered in increasingly Ca-reduced diets. Clearly, a lower concentration of Ca in the diet, and therefore in the digesta, may reduce the likelihood of formation of insoluble Ca-phytate complexes (Selle *et al*., 2009). However, these predominantly form in the pH of the small intestine. *Buttiauxella* phytase is highly active in the proximal gut regions of poultry (Menezes-Blackburn *et al*., 2015), where an acidic pH tends to increase phytate solubility and susceptibility to hydrolysis by phytase (Campbell and Bedford, 1992). So it is more likely that the action of the phytase used in the present study was to increase phytate hydrolysis in the proximal gut regions, releasing P for absorption in the small intestine and reducing the formation of insoluble Ca-phytate complexes. It has been observed that 1000 FTU/kg *Buttiauxella* phytase was able to break down up to 90% of phytate in the proventriculus + gizzard in broilers fed corn and soybean meal based diets (Bello *et al.*, 2016; Li *et al.*, 2016). *In vitro* testing has also shown that pre-hydrolyzing phytate by incubation with *Buttiauxella* phytase for 60 min. at pH 2.5 (to mimic the pH in the proventriculus and gizzard) reduced Ca-phytate complex formation by 75% at pH 6.5 (to mimic the pH in the small intestine) (Mejldal, personal communications). It might therefore have been expected that increasing dietary Ca level would have a minimal effect on the efficacy of a phytase that is highly active in the proventriculus and gizzard, such as *Buttiauxella* phytase, as demonstrated by Amerah *et al*. (2014). However, there are different views regarding the pH ranges (and therefore gut regions) in which Ca-phytate binding occurs (Selle *et al*., 2009). Therefore, it is not necessarily the case

that Ca-phytate complexes are limited to the small intestine. Since limestone binds acid in the proximal gut (Lawlor *et al*., 2005), an increased presence of Ca in the diet may increase pH in these regions. This will have a negative impact on protein digestion and may increase the likelihood of binding of minerals, such as Ca, to phytate (Maenz *et al*., 1998). In addition, it has also been suggested that Ca may directly inhibit phytase activity via competitive inhibition at active sites (Qian *et al.*, 1996). Excessive Ca in the small intestine may also precipitate phosphates and reduce the availability of P for absorption (Selle *et al*., 2009). Furthermore, there are other factors associated with dietary Ca, independent from its level of incorporation in the broiler diet, that may impact on the efficacy of phytase. These need to be further evaluated. For example, there is *in vitro* and *in vivo* evidence to suggest that particle size is important; smaller, more soluble, Ca particles have been shown to be more likely to form Ca-phytate complexes and to inhibiting the hydrolyzing actions of phytase on phytate (Manangi and Coon, 2007). In the current study, a fine limestone was used (50-80%: 20-75 microns; 80-90%: 75-150 microns; 90-100%: 150-300 microns), it may be expected that such fine limestone is more soluble and may have a bigger impact on P digestibility than a coarse limestone.

On comparison of apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) on d 27 and 41, it seems the ATTD P is lower than AID P, this indicates that the dietary P may exceed the requirement and some absorbed P could be excreted in the urine. This might also be due to the imbalance between Ca and P. Increasing Ca reduction level linearly improved P and Ca retention (expressed as g/kg feed) at day 41, which may imply an improved Ca and P balance. This study suggested that lower dietary Ca level may maintain a better Ca:P balance and lead to improved P digestibility, in the presence of phytase.

4.3 Tibia ash and minerals

No effects of dietary Ca reduction in combination with phytase supplementation on tibia bone ash were seen in the present study. Even birds whose diets were relatively highly deficient in Ca (2.3 g/kg lower compared with breeder recommendations) did not exhibit reductions in bone density when supplemented with 500 FTU/kg or 1000 FTU/kg phytase, suggesting that the balance of Ca:P in these treatments was sufficient despite the reduced levels of Ca in the diet. In fact, phytase supplementation appeared to improve bone mineralization in starter birds whose diets were reduced in Ca, compared with the PC, as evidenced by an increase in Zn deposition. Conversely, Yan *et al.* (2006) reported increasing dietary Ca (from 5 to 9 g/kg) to be a limiting factor in maximizing bone density in broiler chicks at a low level of non-phytate P (below 2.5 g/kg of the diet) in the absence of phytase, suggesting that the higher Ca levels created an imbalance between Ca and P when P was deficient in the diet. The same authors observed that this effect was ameliorated by 1000 FTU/kg phytase supplementation, which they speculated may have increased P availability for incorporation into bone by improving the balance between Ca and P. Our findings would seem to support this in that the reduced Ca diets containing phytase did not adversely affect tibia ash quantities compared with the control diet that met breeder recommendations. A recent study by Kim *et al*. (2017), involving similar dietary Ca reduction levels to those used in the present study, has also reported no effect of reducing dietary Ca concentrations (from 10 to 6 g/kg of the diet) on bone density or breaking strength when diets were supplemented with 1000 FTU/kg phytase. The present study has supported and extended these findings by showing that bone density can be maintained right through to the finisher stages of growth (d 41) in highly Ca reduced diets when supplemented with phytase.

In practice, when applying phytase matrix values, some feed producers are currently matching Ca to digestible or available P reduction ratio around 1:1. The findings from this study suggest that in diets that are formulated based on broiler breeder's recommendation and

supplemented with 500 to 1000 FTU/kg *Buttiauxella* phytase, applying a total Ca to available P downspec ratio of higher than 1:1 may improve Ca:P balance leading to improved performance, nutrient digestibility, bone mineralization and production benefit.

In conclusion, supplementation of corn-soybean meal based broiler diets with a *Buttiauxella* phytase at 500 and 1000 FTU/kg compensated for a reduction of nutrients (available P, AA, ME, Na and Ca) and maintained growth performance and bone ash over a 42 d period compared with diets based on breeder recommendations. Furthermore, the phytase treatments increased conversion of feed-derived energy into BWG compared with the control. Increasing phytase dose from 500 to 1000 FTU/kg increased BWG and reduced FCR during starter and grower phases, increased ileal P and Ca digestibility and bone mineral content. Across phytase doses, reducing Ca levels (or increasing Ca matrix values) in the diets linearly reduced FCR during the finisher phase, increased P and Ca digestibility and retention, and improved overall energy efficiency.

Conflict of Interest

None.

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References

Amerah, A.M., Plumstead, P.W., Barnard, L.P., Kumar, A. 2014. Effect of calcium level and phytase addition on ileal phytate degradation and amino acid digestibility of broilers fed corn-based diets. Poult. Sci. 93, 906-915. doi: 10.3382/ps.2013-03465

Angel, R., Tamim, N. M., Applegate, T.J., Dhandu, A.S., Ellestad, L.E., 2002. Phytic acid chemistry: influence on phytin –phosphorus availability and phytase efficacy. J. Appl. Poult. Res. 11, 471-480. doi: 10.1093/japr/11.4.471

AOAC 1985. Official Method 985.01. Metals and Other Elements in Plants and Pet Foods

AOAC International. 1990. Official Methods of Analysis of AOAC International 15th ed. AOAC Int., Washington, DC, USA.

AOAC International. 2000. Official Methods of Analysis of AOAC International 16th ed. AOAC Int., Washington, DC, USA

AOAC 2000. Method 2000.12: Phytase activity in feed: Colorimetric enzymatic method. In Official Methods of Analysis of AOAC International. 17th edition. Association of Official Analytical Chemists, Arlington, VA

Bello, A., Dersjant-Li, Y., Korver, D. R., 2016. The efficacy of two phytases on inositol phosphate degradation in different segments of the gastrointestinal tract and bone quality of broilers. Poult. Sci. 95 (Suppl. 1), 30. (Abstr.).

Campbell, G. L., Bedford, M. R., 1992. Enzyme applications for monogastric feeds: a review. Can. J. Anim. Sci. 72, 449-466. doi: 4141/cjas92-058

Farkvam, D. O., Nelson, T. S., Kirby, L. K. 1989. Calcium and phytic acid in chick nutrition. Nutrition Reports International. 40(1), 33 – 42.

1. Kim, J. H., Han, G. P., Shin, J. E., Kil, D. Y. 2017. Effect of dietary calcium concentrations in phytase-containing diets on growth performance, bone mineralization, litter quality, and

footpad dermatitis score in broiler chickens. Animal Feed Science and Technology 229, 13-18. doi: 10.1016/j.anifeedsci.2017.04.008

Lawlor, P. G., Lynch, P. B., Caffrey, P. J., O'Reilly, J. J., O'Connell, M. K., 2005. Measurements of the acid-binding capacity of ingredients used in pig diets. Irish Vet. J. 58, 447-452. doi: 10.1186/2046-0481-58-8-447

Lei, X.G., Ku, P.K., Miller, E.R., Yokoyama, M.T., Ulrey, D.E., 1994. Calcium level affects the efficacy of supplemental microbial phytase in corn-soybean meal diets of weanlings pigs. J. Anim. Sci. 72, 139-143.

Li, W., Angel, R., Kim, S. W., Brady, K., Yu, S., Plumstead, P. W., 2016. Impacts of dietary calcium, phytate, and nonphytate phosphorus concentrations in the presence or absence of phytase on inositol hexakisphosphate (IP6) degredation in different segments of broilers digestive tract. Poult. Sci, 95, 581-589. doi: 10.3382/ps/pev354

Maenz, D. D., Classen, H. L., 1998. Phytase activity in the small intestinal brush border membrane of the chicken. Poult. Sci. 77, 557-563.

Manangi, M. K., Coon, C. N. 2007. The effect of calcium carbonate particle size and solubility on the utilization of phosphorus from phytase for broilers. International Journal of Poultry Science. 6(2), 85-90.

Menezes-Blackburn, D., Gabler, S., Greiner, R., 2015. Performance of seven commercial phytases in an in vitro simulation of poultry digestive tract. J. Agr. Food Chem. 63, 6142-6149. doi: 10.1021/acs.jafc-5b01996

NRC, 1994. Nutrient Requirements of Poultry. 9th rev. ed. National Academy of Science, Washington, DC.

Paiva, D. M., Walk, C. L., McElroy, A. P. 2013. Influence of dietary calcium level, calcium source, and phytase on bird performance and mineral digestibility during a natural necrotic enteritis episode. Poultry Science. 92(12): 3125-33. doi: 10.3382/ps.2013-03298.

Parr Manual 120. 1948. Oxygen bomb calorimetry and oxygen bomb combustion methods. Parr Instrument Company, Moline, IL, USA.

Plumstead, P.W., Leytem, A.B., Maguire, R.O., Spears, J.W., Kwanyuen, P., Brake, J., 2008. Interaction of calcium and phytate in broiler diets. 1. Effects on apparent prececal digestibility and retention of phosphorus. Poult. Sci. 87, 449–458. doi: 10.3382/ps.2007-00231

Qian, H., Gregory, E. M., Kornegay, E. T., 1996. Characterization of Aspergillus niger phytase and investigation of the inhibitory effect of cations on the phytase activity. J. Anim. Sci. 74 (Suppl. 1), 8 (Abstr.).

Qian, H., Kornegay, E.T., Denbow, D.M., 1997. Utilization of phytate phosphorus and calcium as influenced by microbial phytase, cholecalciferol, and the calcium:total phosphorus ratio in broiler diets. Poult. Sci. 76, 37–46. doi: 10.1093/ps/76.1.37

Ravindran, V., Morel, P.C.H., Partridge, G.G., Hruby, M., Sands, J.S., 2006. Influence of an E. coli-derived phytase on nutrient utilization in broilers fed starter diets containing varying concentrations of phytic acid. Poult. Sci. 85, 82-89.

Ravindran, V., Cowieson, A. J., Sell, P. H. 2008. Influence of dietary electrolyte balance and microbial phytase on growth performance, nutrient utilization and excreta quality of broiler chickens. Poult. Sci. 87, 677-688. doi: 10.3382/ps.2007-00247

Sandberg, A.S., Larsen, T., Sandstrom, B., 1993. High dietary calcium level decreases colonic phytate degradation in pigs fed a rapeseed diet. J. Nutr. 123, 559-566.

Schoner, F.J., Hoppe, P. P., Schwarz, G. and Wiesche, H., 1993. Effects of microbial phytase and inorganic phosphate in broiler chickens: performance and mineral retention at various calcium levels. J. Anim. Physiol. Anim. Nutr. 69:235-244.

Sebastian, S., Touhburn, S. P., Chavez, E. R., Lague, P. C. 1996. The effects of supplemental microbial phytase on the performance and utilization of dietary calcium, phosphorus, copper, and zinc in broiler chickens fed corn-soybean diets. Poult. Sc. 74:729-736.

Selle, P.H., Ravindran, V., Caldwell, R.A., Bryden, W.L.., 2000. Phytate and phytase: consequences for protein utilisation. Nutr. Res. Rev. 13, 255–278. doi: 10.1079/095442200108729098

Selle, P.H., Ravindran, B., 2007. Microbial phytase in poultry nutrition. Anim. Feed Sci. Technol. 135, 1-41. doi: 10.1016/j.anifeedsci.2006.06.010

Selle, P.H., Cowieson, A.J., Ravindran, V., 2009. Consequences of calcium interactions with phytate and phytase for poultry and pigs. Livest. Sci. 124, 126–141. doi: 10.1016/j.livsci.2009.01.006

Shafey, T. M., and McDonal, M. W. 1991. The effects of dietary calcium, phosphorus and protein on the performance and nutrient utilization of broiler chickens. Poult. Sci. 70:548-533.

Taylor, T.G., Coleman, J.W., 1979. A comparative study of the absorption of calcium and the availability of phytate-phosphorus in the golden hamster (Mesocricetus auratus) and the laboratory rat. Br. J. Nutr. 42, 113-119.

Tamim, N.M., Angel, R., Christman, M., 2004. Influence of dietary calcium and phytase on phytate phosphorus hydrolysis in broiler chickens. Poult. Sci. 83, 1358–1367.

Yan, F., Kersey, J. H., Fritts, C. A., Waldroup, P. W., 2006. Effect of phytase supplementation on the calcium requirement of broiler chicks. International Journal of Poultry Science 5(2): 112-120. doi: 10.3923/ijps.2006.112.120

Yu, S., Cowieson, A., Gilbert, C., Plumstead, P., Dalsgaard, S., 2012. Interactions of phytate and myo-inositol phosphate esters (IP1-5) including IP5 isomers with dietary protein and iron and inhibition of pepsin. J. Anim. Sci. 90, 1824-1832. doi: 10.2527/jas.2011-3866

Table 1

Dietary treatments.

Treatment number	Phytase* FTU/kg	Ca reduction level	Reduction in AvP (g/kg)**	Reduction in Ca (g/kg)	Ratio of reduction in Ca:AvP
1	0	None	0	0	0
2	500	Low (L)	1.46	1.34	0.92
3	500	Medium (M)	1.46	1.64	1.12
4	500	High (H)	1.46	2.34	1.60
5	1000	Low (L)	1.74	1.59	0.91
6	1000	Medium (M)	1.74	1.89	1.09
7	1000	High (H)	1.74	2.34	1.34

* *Buttiauxella* sp. phytase (Axtra® PHY, Danisco Animal Nutrition, Marlborough, UK) at 500 FTU/kg or 1000 FTU/kg.

**AvP = available P, calculated based on a reduction in digestible P of 1.34 and 1.59 g/kg at 500 and 1000 FTU/kg phytase supplementation, respectively.

Table 2. Ingredient and nutrient composition (g/kg, as fed basis) of the control and experimental diets in the starter, grower, finisher 1 and finisher 2 phases

	T1	T 2	T 3	T 4	T 5	T 6	T7
Starter, d 1 to 10							
Ingredient (g/kg)							
Maize	512	556	556	556	556	556	556
Soybean meal	339	320	320	320	320	320	320
Canola meal	40	40	40	40	40	40	40
Wheat bran	20	20	20	20	20	20	20
Soy oil	27.7	9.0	9.0	9.0	9.0	9.0	9.0
Celite	20	20	20	20	20	20	20
Dicalcium phosphate	18.9	10.7	10.7	10.7	9.2	9.2	9.2
Limestone	10.2	11.7	10.9	9.1	12.0	11.2	10.0
NaCl	3.8	3.0	3.0	2.9	2.9	2.9	2.9
Filler[1]	-	-	0.8	2.7	1.3	2.1	3.3
Lys-HCl	2.3	2.6	2.6	2.6	2.6	2.6	2.6
DL-Met	2.8	2.7	2.7	2.7	2.7	2.7	2.7
L-Thr	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Arginine	0.08	0.034	0.034	0.034	0.034	0.034	0.034
Isoleucine	0.20	0.26	0.26	0.26	0.26	0.26	0.26
Coccidiostat	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin-mineral[2]	2	2	2	2	2	2	2
Calculated nutrients (g/kg)							
Dry matter	892	888	888	888	888	888	888
Crude protein	229	225	225	225	225	225	225
Crude fibre	34	35	35	35	35	35	35
Fat	54	36	36	36	36	36	36
Linoleic acid	26	17	17	17	17	17	17
ME (MJ/kg)	12.6	12.3	12.3	12.3	12.3	12.3	12.3
dig. Lys	12.8	12.6	12.6	12.6	12.6	12.6	12.6
dig. Met	6.1	6.0	6.0	6.0	6.0	6.0	6.0
dig. M + C	9.5	9.3	9.3	9.3	9.3	9.3	9.3
dig. Ile	8.6	8.4	8.4	8.4	8.4	8.4	8.4
dig. Thr	8.6	8.4	8.4	8.4	8.4	8.4	8.4

dig. Trp	2.5	2.4	2.4	2.4	2.4	2.4	2.4
dig. Val	9.6	9.4	9.4	9.4	9.4	9.4	9.4
dig. Arg	13.7	13.5	13.5	13.5	13.5	13.5	13.5
Ca	9.6	8.3	8.0	7.3	8.0	7.7	7.3
P (available)	4.8	3.3	3.3	3.3	3.1	3.1	3.1
P (total)	7.6	6.1	6.1	6.1	5.8	5.8	5.8
Cl	3.0	2.5	2.5	2.5	2.5	2.5	2.5
K	9.9	9.6	9.6	9.6	9.6	9.6	9.6
Na	1.8	1.5	1.5	1.5	1.5	1.5	1.5
Phytate P	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Grower, d 11 to 21							
Ingredient (g/kg)							
Maize	559	600	600	600	600	600	600
Soybean meal	286	270	270	270	270	270	270
Canola meal	40	40	40	40	40	40	40
Wheat bran	20	20	20	20	20	20	20
Soy oil	36.6	18.2	18.2	18.2	18.2	18.2	18.2
Celite	20	20	20	20	20	20	20
Dicalcium phosphate	16.8	8.6	8.6	8.6	7.1	7.1	7.1
Limestone	9.5	11.0	10.2	8.4	11.3	10.5	9.3
NaCl	3.7	2.9	2.9	2.9	2.9	2.9	2.9
Filler[1]	-	-	0.8	2.7	1.3	2.2	3.3
Lys-HCl	2.3	2.5	2.5	2.5	2.5	2.5	2.5
DL-Met	2.5	2.4	2.4	2.4	2.4	2.4	2.4
L-Thr	1.0	0.9	0.9	0.9	0.9	0.9	0.9
Arginine	0.16	0.39	0.39	0.39	0.39	0.39	0.39
Isoleucine	0.28	0.33	0.33	0.33	0.33	0.33	0.33
Coccidiostat	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin-mineral[2]	2	2	2	2	2	2	2
Calculated nutrients (g/kg)							
Dry matter	891	887	887	887	887	887	887
Crude protein	209	205	205	205	205	205	205
Crude fibre	34	34	34	34	34	34	34
Fat	63	46	46	46	46	46	46
Linoleic acid	31	23	23	23	23	23	23
ME (MJ/kg)	13.0	12.7	12.7	12.7	12.7	12.7	12.7
dig. Lys	11.5	11.3	11.3	11.3	11.3	11.3	11.3
dig. Met	5.6	5.5	5.5	5.5	5.5	5.5	5.5
dig. M + C	8.7	8.6	8.6	8.6	8.6	8.6	8.6

dig. Ile	7.8	7.7	7.7	7.7	7.7	7.7	7.7
dig. Thr	7.7	7.6	7.6	7.6	7.6	7.6	7.6
dig. Trp	2.2	2.2	2.2	2.2	2.2	2.2	2.2
dig. Val	8.7	8.5	8.5	8.5	8.5	8.5	8.5
dig. Arg	12.3	12.2	12.2	12.2	12.2	12.2	12.2
Ca	8.7	7.4	7.1	6.4	7.1	6.8	6.4
P (available)	4.4	2.9	2.9	2.9	2.6	2.6	2.6
P (total)	7	5.4	5.4	5.4	5.2	5.2	5.2
Cl	3.0	2.5	2.5	2.5	2.5	2.5	2.5
K	8.9	8.7	8.7	8.7	8.7	8.7	8.7
Na	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Phytate P	2.4	2.4	2.4	2.4	2.4	2.4	2.4

Finisher 1, d 22 to 35

Ingredient (g/kg)

Maize	606	647	647	647	647	647	647
Soybean meal	233	217	217	217	217	217	217
Canola meal	40	40	40	40	40	40	40
Wheat bran	20	20	20	20	20	20	20
Soy oil	36.6	18.2	18.2	18.2	18.2	18.2	18.2
Celite	20	20	20	20	20	20	20
Dicalcium phosphate	14.8	6.5	6.5	6.5	5.0	5.0	5.0
Limestone	8.7	10.3	9.5	7.7	10.6	9.8	8.6
NaCl	3.7	2.9	2.9	2.8	2.9	2.8	2.8
Filler[1]	-	-	0.8	2.7	1.3	2.1	3.3
Lys-HCl	2.3	2.5	2.5	2.5	2.5	2.5	2.5
DL-Met	2.5	2.4	2.4	2.4	2.4	2.4	2.4
L-Thr	1.0	0.9	0.9	0.9	0.9	0.9	0.9
Arginine	0.25	0.48	0.48	0.48	0.48	0.48	0.48
Isoleucine	0.28	0.33	0.33	0.33	0.33	0.33	0.33
Coccidiostat	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin-mineral[2]	2	2	2	2	2	2	2

Calculated nutrients (g/kg)

Dry matter	890	886	886	886	886	886	886
Crude protein	188	185	185	185	185	185	185
Crude fibre	33	33	33	33	33	33	33
Fat	73	56	56	56	56	56	56
Linoleic acid	37	28	28	28	28	28	28
ME (MJ/kg)	13.4	13.1	13.1	13.1	13.1	13.1	13.1

dig. Lys	10.2	10.0	10.0	10.0	10.0	10.0	10.0
dig. Met	5.2	5.0	5.0	5.0	5.0	5.0	5.0
dig. M + C	8.0	7.9	7.9	7.9	7.9	7.9	7.9
dig. Ile	7.0	6.9	6.9	6.9	6.9	6.9	6.9
dig. Thr	6.8	6.7	6.7	6.7	6.7	6.7	6.7
dig. Trp	2.0	1.9	1.9	1.9	1.9	1.9	1.9
dig. Val	7.8	7.6	7.6	7.6	7.6	7.6	7.6
dig. Arg	10.9	10.8	10.8	10.8	10.8	10.8	10.8
Ca	7.8	6.5	6.2	5.5	6.2	5.9	5.5
P (available)	3.9	2.4	2.4	2.4	2.2	2.2	2.2
P (total)	6.3	4.8	4.8	4.8	4.5	4.5	4.5
Cl	2.9	2.5	2.5	2.5	2.5	2.5	2.5
K	7.9	7.7	7.7	7.7	7.7	7.7	7.7
Na	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Phytate P	2.2	2.2	2.2	2.2	2.2	2.2	2.2

Finisher 2, d 36 to 42

Ingredient (g/kg)

Maize	649	691	691	691	691	691	691
Soybean meal	201	185	185	185	185	185	185
Canola meal	40	40	40	40	40	40	40
Wheat bran	20	20	20	20	20	20	20
Soy oil	39.9	20.5	20.5	20.5	20.5	20.5	20.5
Celite	20	20	20	20	20	20	20
Dicalcium phosphate	11.7	3.5	3.5	3.5	1.9	1.9	1.9
Limestone	7.8	9.3	8.5	6.7	9.6	8.8	7.6
NaCl	3.7	2.8	2.8	2.8	2.8	2.8	2.8
Filler[1]	-	-	0.8	2.7	1.3	2.1	3.3
Lys-HCl	2.01	2.23	2.23	2.23	2.23	2.23	2.23
DL-Met	1.88	1.8	1.8	1.8	1.8	1.8	1.8
L-Thr	0.47	0.47	0.47	0.47	0.47	0.47	0.47
Arginine	0.52	0.75	0.75	0.75	0.75	0.75	0.75
Isoleucine	0.45	0.52	0.52	0.52	0.52	0.52	0.52
Coccidiostat	0.50	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin-mineral[2]	2	2	2	2	2	2	2

Calculated nutrients (g/kg)

Dry matter	888	884	884	884	884	884	884
Crude protein	176	176	176	176	176	176	176
Crude fibre	32	33	33	33	33	33	33

Fat	68	51	51	51	51	51	51
Linoleic acid	34	25	25	25	25	25	25
ME (MJ/kg)	13.4	13.1	13.1	13.1	13.1	13.1	13.1
dig. Lys	9.2	9.0	9.0	9.0	9.0	9.0	9.0
dig. Met	4.6	4.5	4.5	4.5	4.5	4.5	4.5
dig. M + C	7.3	7.1	7.1	7.1	7.1	7.1	7.1
dig. Ile	6.6	6.5	6.5	6.5	6.5	6.5	6.5
dig. Thr	6.2	6.0	6.0	6.0	6.0	6.0	6.0
dig. Trp	1.8	1.7	1.7	1.7	1.7	1.7	1.7
dig. Val	7.3	7.1	7.1	7.1	7.1	7.1	7.1
dig. Arg	10.3	10.2	10.2	10.2	10.2	10.2	10.2
Ca	6.6	5.3	5	4.3	5.1	4.8	4.3
P (available)	3.3	1.9	1.9	1.9	1.6	1.6	1.6
P (total)	5.7	4.1	4.1	4.1	3.8	3.8	3.8
Cl	2.9	2.4	2.4	2.4	2.4	2.4	2.4
K	7.3	7.1	7.1	7.1	7.1	7.1	7.1
Na	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Phytate P	2.2	2.2	2.2	2.2	2.2	2.2	2.2

[1] Sand, used to maintain the overall ingredient composition among treatments.

[2] Supplied per kilogram of diet: Retinyl Acetate 10000 IU, Cholecalciferol 4000IU, Alfa tocopheryl 40 mg, Menadione 3mg, Thiamine 1.5 mg, Riboflavin 5 mg, Pyridoxin 3 mg, Cyanocobalnine 0.02 mg, Calcium Pentothenate 12 mg, folic acid 1 mg, Niacin 50mg, Biotine 0.20mg, Copper (from copper sulphate) 20 mg, Iron (from Ferrous sulphate) 20 mg, Iodine 1mg, Manganese 120 mg, Selenium 0.1 mg, Zinc 100 mg, Ethoxyquin 50 mg.

Table 3. Analyzed nutritional values (g/kg) of the dietary treatments for each of the four dietary phases.

	T 1	T 2	T 3	T 4	T 5	T 6	T 7
Starter, d 0 to 10							
Ca	9.7	8.9	8.2	7.4	7.9	7.5	7.2
P	7.3	5.8	6	5.8	5.5	5.5	5.4
Ca: P ratio	1.33	1.53	1.37	1.28	1.44	1.36	1.33
DM	893	901	897	903	901	906	898
CP	231	226	224	226	228	223	224
GE (MJ/kg)	17.2	16.9	16.9	16.9	17.0	17.0	16.9
Grower, d 11 to 21							
Ca	9.3	8.6	7.9	7.7	7.7	7.4	6.7
P	7.1	6	5.8	5.8	5.4	5.6	5.6
Ca: P ratio	1.31	1.43	1.36	1.33	1.43	1.32	1.20
DM	906	892	901	906	905	898	899
CP	207	204	205	203	206	203	204
GE (MJ/kg)	17.9	17.4	17.5	17.5	17.5	17.4	17.4
Finisher 1, d 22 to 35							
Ca	7.9	6.5	6.3	5.6	6.4	5.8	5.4
P	6.3	5	5	5	4.6	4.6	5.1
Ca: P ratio	1.25	1.30	1.26	1.12	1.39	1.26	1.06
DM	910	901	897	897	915	896	903
CP	191	185	184	186	185	186	187
GE (MJ/kg)	18.4	18.0	18.1	18.0	18.2	18.2	18.1
Finisher 2, d 36 to 42							

Ca	6.5	5.4	5.2	4.4	5.2	4.8	4.4
P	5.7	4.2	4.2	4.2	3.9	3.9	3.9
Ca: P ratio	1.14	1.29	1.24	1.05	1.33	1.23	1.13
DM	901	903	906	899	897	901	903
CP	178	176	177	179	175	178	176
GE (MJ/kg)	18.4	18.0	18.1	18.1	18.0	18.0	18.0
Analyzed phytase (FTU/kg) (mean of 4 phases)[1]	96	559	592	664	1207	1216	1217

[1] The average values from 4 phases

Table 4. Effect of dietary Ca and phytase level on growth performance of broilers, by growth phase: comparison of treatment means and factorial analysis[1] of main effects and interaction.

	Phytase (FTU/kg)	Ca reduction level	BWG (g/bird)	FI (g/bird)	FCR (g/g)	Mortality (%)
Starter (d 0 to 10)						
Comparison of treatment means:						
T1	0	None	290	349	1.206	0.0
T2	500	L	291	354	1.217	0.0
T3	500	M	292	356	1.220	0.0
T4	500	H	289	350	1.211	0.0
T5	1000	L	294	348	1.184	0.0
T6	1000	M	297	352	1.185	0.0
T7	1000	H	293	354	1.207	0.0
SEM			2.45	4.00	0.01	
P-value			0.325	0.687	0.313	
Factorial analysis:						
Phytase dose (FTU/kg)	500		290.5^{b}	353.2	1.216^{a}	0.0
	1000		294.8^{a}	351.4	1.192^{b}	0.0
Ca reduction level		L	293	351	1.200	0.0
		M	294	354	1.203	0.0
		H	291	352	1.209	0.0
P – value, phytase dose			0.037	0.574	0.045	
P – value, Ca reduction level			0.422	0.798	0.820	
P – value, phytase x Ca interaction			0.839	0.380	0.485	
P – value, linear Ca			0.420	0.960	0.540	
Grower (d 11 to 21)						
Comparison of treatment means:						
T1	0	None	783	1036	1.323^{a}	0.0
T2	500	L	778	1025	1.317^{ab}	0.0
T3	500	M	774	1020	1.318^{ab}	0.0
T4	500	H	774	1012	1.308^{ab}	0.0
T5	1000	L	789	1035	1.313^{ab}	0.0
T6	1000	M	799	1033	1.293^{b}	0.0
T7	1000	H	781	1009	1.292^{b}	0.0
SEM			7.86	8.90	0.01	
P-value			0.112	0.103	0.004	
Factorial analysis:						
Phytase dose (FTU/kg)	500		776^{b}	1019	1.314^{a}	0.0
	1000		790^{a}	1026	1.300^{b}	0.0

Ca reduction level		L	783	1030	1.315	0.0
		M	787	1027	1.305	0.0
		H	777	1010	1.300	0.0
P – value, phytase dose			0.037	0.362	0.020	
P – value, Ca reduction level			0.502	0.081	0.120	
P – value, phytase x Ca interaction			0.470	0.623	0.390	
P – value, linear Ca			0.410	0.027	0.058	

Finisher (d 22 to 42)|

Comparison of treatment means:

T1	0	None	2272	3923[ab]	1.728	3.0
T2	500	L	2232	3846[ab]	1.724	3.5
T3	500	M	2280	3875[ab]	1.700	1.5
T4	500	H	2292	3809[cb]	1.664	6.0
T5	1000	L	2277	3973[a]	1.748	1.0
T6	1000	M	2259	3902[ab]	1.729	2.0
T7	1000	H	2309	3916[ab]	1.698	4.0
SEM			29.36	29.39	0.02	1.21
P-value			0.493	0.009	0.090	0.091

Factorial analysis:

Phytase dose (FTU/kg)	500		2268	3843[b]	1.696	3.67
	1000		2282	3930[a]	1.725	2.33
Ca reduction level		L	2255	3909	1.736[a]	2.3
		M	2269	3888	1.715[ab]	1.8
		H	2300	3862	1.681[b]	5.0
P - phytase dose			0.584	0.001	0.116	0.0259
P – value, Ca reduction level			0.316	0.309	0.050	0.1947
P – value, phytase x Ca interaction			0.548	0.232	0.973	0.4384
P – value, linear Ca			0.120	0.170	0.010	0.0224

[a,b,c] Means in the same column with no common superscripts are significantly different ($P < 0.05$)

[1]The factorial analysis was a 2 x 3 factorial analysis that excluded Treatment 1 (PC).

Table 5. Effect of dietary Ca and phytase level on growth performance of broilers over 0-21 and 0-42 days; comparison of treatment means and factorial analysis[1] of main effects and interaction.

	Phytase (FTU/kg)	Ca reduction level	BWG (g/bird)	FI (g/bird)	FCR (g/g)	FCRc (g/g)	Energy conversion, MJ/kg BWG[2]
d 0 to 21							
Comparison of treatment means:							
T1	0	None	1073	1386	1.292[a]		
T2	500	L	1070	1379	1.290[a]		
T3	500	M	1066	1375	1.291[a]		
T4	500	H	1063	1362	1.281[ab]		
T5	1000	L	1083	1384	1.278[ab]		
T6	1000	M	1096	1385	1.264[b]		
T7	1000	H	1074	1363	1.269[ab]		
SEM			8.73	9.26	0.01		
P-value			0.072	0.283	0.003		
Factorial analysis:							
Phytase dose (FTU/kg)	500		1066[b]	1372	1.287[a]		
	1000		1084[a]	1377	1.270[b]		
Ca reduction level		L	1076	1381	1.284		
		M	1081	1380	1.277		
		H	1068	1362	1.275		
P – value, phytase dose			0.015	0.516	0.002		
P – value, Ca reduction level			0.372	0.085	0.363		
P – value, phytase x Ca interaction			0.489	0.894	0.415		
P – value, linear Ca			0.350	0.030	0.230		

d 0 to 42

Comparison of treatment means:

T1	0	None	3344	5309[ab]	1.595	1.595	21.06[a]
T2	500	L	3301	5225[ab]	1.611	1.624	20.54[ab]
T3	500	M	3346	5250[ab]	1.584	1.583	20.36[ab]
T4	500	H	3355	5170[b]	1.597	1.594	20.01[b]
T5	1000	L	3360	5356[a]	1.623	1.619	20.70[ab]
T6	1000	M	3355	5287[ab]	1.611	1.608	20.46[ab]
T7	1000	H	3383	5279[ab]	1.610	1.598	20.26[b]
SEM			27.14	32.38	0.01	0.01	0.178
P-value			0.470	0.010	0.463	0.647	0.003

Factorial analysis:

Phytase dose (FTU/kg)	500		3334	5215[b]	1.597	1.600	20.30
	1000		3366	5307[a]	1.615	1.608	20.47
Ca reduction level		L	3331	5291	1.617	1.621	20.62[a]
		M	3350	5269	1.597	1.596	20.41[ab]
		H	3369	5225	1.603	1.596	20.13[b]
P – value, phytase dose			0.168	0.002	0.139	0.615	0.255
P – value, Ca reduction level			0.398	0.141	0.369	0.329	0.031
P – value, phytase x Ca interaction			0.669	0.349	0.833	0.735	0.911
P – value, linear Ca			0.170	0.070	0.400	0.220	0.007

[a,b,c] Means in the same column with no common superscripts are significantly different ($P < 0.05$)

[1]The factorial analysis was a 2 x 3 factorial analysis that excluded Treatment 1 (PC).

[2] Energy conversion = energy consumption (MJ) per kg BWG.

Table 6. Effects of dietary Ca and phytase level on apparent ileal digestibility (AID, %) of P and Ca in broilers; comparison of treatment means and factorial analysis[1] of main effects and interaction.

			d 10		**d 27**		**D41**	
	Phytase dose (FTU/kg)	Ca reduction level	AID P (%)	AID Ca (%)	AID P (%)	AID Ca (%)	AID P (%)	AID Ca (%)
Comparison of treatment means:								
T1	0	None	35.1c	28.6	50.5^{b}	41.2^{bc}	47.0^{c}	30.9^{bc}
T2	500	L	45.9bc	37.8	64.3^{a}	42.3^{bc}	59.3^{b}	34.0^{b}
T3	500	M	51.3^{ab}	38.3	61.1^{a}	50.5^{a}	61.5^{b}	35.0^{b}
T4	500	H	51.3^{ab}	41.6	67.0^{a}	48.7^{ab}	63.7^{b}	34.4^{b}
T5	1000	L	48.1^{bc}	27.7	63.8^{a}	38.0^{c}	60.0^{b}	24.1^{c}
T6	1000	M	57.9^{ab}	34.6	66.0^{a}	37.8^{c}	64.5^{b}	40.6^{b}
T7	1000	H	64.1^{a}	38.7	67.1^{a}	46.6^{ab}	77.7^{a}	51.8^{a}
SEM			3.17	3.64	1.68	1.91	1.72	2.37
P - value			<.0001	0.079	<.0001	<.0001	<.0001	<.0001
Factorial analysis:								
Phytase dose (FTU/kg)	500		49.5b	39.2	64.1	47.2a	61.5b	34.5b
	1000		56.7a	33.6	65.7	40.8b	67.4a	38.8a
Ca reduction level		L	47.0b	32.7	64	40.2b	59.6b	29.1b
		M	54.6ab	36.5	63.6	44.1ab	63.0b	37.8a
		H	57.7a	40.1	67.1	47.7a	70.7a	43.1a
P – value, phytase dose			0.011	0.074	0.281	< .001	< .001	0.033
P – value, Ca reduction level			0.007	0.151	0.103	0.002	<.001	<.001
P – phytase x Ca interaction			0.282	0.575	0.244	0.029	0.001	<.001
P – value, linear Ca			0.006	0.056	0.07	0.003	<.001	< .001

[a,b,c] Means in the same column with no common superscripts are significantly different ($P < 0.05$).

[1]The factorial analysis was a 2 x 3 factorial analysis that excluded Treatment 1 (PC).

Table 7. Effects of dietary Ca and phytase level on apparent total tract digestibility (ATTD, %) of P and Ca, and their retention (g/kg feed), in broilers; comparison of treatment means and factorial analysis[1] of main effects and interaction.

			d 27				D41			
	Phytase dose (FTU/kg)	Ca reduction level	ATTD P (%)	ATTD Ca (%)	P retention (g/kg)	Ca retention (g/kg)	ATTD P (%)	ATTD Ca (%)	P retention (g/kg)	Ca retention (g/kg)
Comparison of treatment means:										
T1	0	None	39.2^{b}	36.2^{b}	2.78	3.48	27.7^{b}	20.1^{c}	1.75	1.60^{ab}
T2	500	L	50.7^{a}	40.1^{ab}	3.06	3.57	36.6^{ab}	28.1^{abc}	1.84	2.16^{ab}
T3	500	M	48.0^{a}	40.6^{ab}	2.79	3.45	34.8^{ab}	27.6^{abc}	1.76	2.13^{ab}
T4	500	H	51.3^{a}	44.4^{a}	2.96	3.39	40.4^{a}	35.0^{ab}	2.02	2.44^{a}
T5	1000	L	50.7^{a}	38.4^{ab}	2.74	3.07	33.6^{ab}	22.9^{bc}	1.53	1.45^{b}
T6	1000	M	51.7^{a}	40.9^{ab}	2.88	3.23	35.1^{ab}	27.9^{abc}	1.62	1.69^{ab}
T7	1000	H	51.0^{a}	43.7^{ab}	2.86	3.43	38.3^{ab}	36.4^{a}	1.94	2.34^{ab}
SEM			1.57	1.81	0.09	0.15	2.65	2.76	0.13	0.20
P - value			<.0001	0.034	0.229	0.268	0.071	0.003	0.191	0.008
Factorial analysis:										
Phytase dose (FTU/kg)	500		50	41.7	2.9	3.5	37.2	34.5	1.9	2.25^{a}
	1000		51.1	41.0	2.8	3.2	35.7	38.8	1.7	1.82^{b}
Ca reduction level		L	50.7	39.2^{b}	2.9	3.32	35.1	29.1^{b}	1.69	1.80^{b}
		M	49.9	40.8^{ab}	2.84	3.34	34.9	37.8^{b}	1.69	1.91^{ab}
		H	51.1	44.0^{a}	2.91	3.41	39.4	43.1^{a}	1.98	2.39^{a}
P – value, phytase dose			0.401	0.634	0.13	0.065	0.482	0.638	0.112	0.019
P – value, Ca reduction level			0.719	0.035	0.69	0.815	0.198	0.003	0.052	0.019

P – phytase x Ca interaction	0.392	0.868	0.086	0.207	0.825	0.493	0.684	0.363
P – value, linear Ca	0.708	0.008	0.798	0.545	0.095	0.001	0.027	0.009

[a,b,c] Means in the same column with no common superscripts are significantly different ($P < 0.05$).

[1]The factorial analysis was a 2 x 3 factorial analysis that excluded Treatment 1 (PC).

Table 8. Effects of dietary Ca and phytase level on tibia ash and bone mineral content of broilers; comparison of treatment means and factorial analysis[1] of main and interactive effects.

	Phytase dose (FTU/kg)	Ca reduction level	Tibia ash (g/kg)	Bone minerals (g/kg) P	Ca	Zn	Mg
d 10							
Comparison of treatment means:							
T1	0	None	479	182.1^{ab}	340.2	0.47^{b}	9.24
T2	500	L	485	180.2^{b}	339.5	0.50^{a}	8.93
T3	500	M	483	180.5^{ab}	339.6	0.49^{ab}	9.15
T4	500	H	472	180.5^{ab}	339	0.50^{a}	9.23
T5	1000	L	487	182.9^{ab}	340.7	0.51^{a}	9.39
T6	1000	M	479	182.5^{ab}	338.9	0.51^{a}	9.36
T7	1000	H	496	183.3^{a}	342.7	0.51^{a}	9.36
SEM			9.6	0.64	1.3	0.01	0.16
P - value			0.649	0.004	0.414	<0.001	0.422
Factorial analysis:							
Phytase dose (FTU/kg)	500		480	180.4^{b}	339.3	0.49^{b}	9.10^{b}
	1000		488	182.9^{a}	340.8	0.51^{a}	9.37^{a}
Ca reduction level		L	486	181.5	340.1	0.50	9.25
		M	481	181.5	339.2	0.5	9.16
		H	484	181.9	340.8	0.5	9.3
P – value, phytase dose			0.314	<.0001	0.185	0.007	0.024

P – value, Ca reduction level			0.874	0.752	0.462	0.918	0.625
P – value, phytase x Ca interaction			0.270	0.802	0.237	0.381	0.483
P – value, linear Ca			0.888	0.595	0.496	0.864	0.430

d 27

Comparison of treatment means:

T1	0	None	472	179.3	324.3	0.39	8.13
T2	500	L	486	178.0	345.3	0.40	7.94
T3	500	M	469	179.4	346.0	0.40	7.97
T4	500	H	473	176.9	341.8	0.39	7.91
T5	1000	L	474	178.9	345.5	0.39	8.12
T6	1000	M	469	177.3	340.2	0.40	7.93
T7	1000	H	476	178.9	345	0.39	8.16
SEM			4.56	1.06	2.19	0.01	0.09
P - value			0.198	0.103	0.071	0.457	0.172

***Factorial analysis*:**

Phytase dose (FTU/kg)	500		476	178.1	344.4	0.39	7.94[b]
	1000		473	178.4	343.4	0.39	8.07[a]
Ca reduction level		L	480	178.5	345.4	0.39	8.03
		M	469	178.4	342.9	0.4	7.95
		H	475	177.9	343.4	0.39	8.03
P – value, phytase dose			0.433	0.765	0.622	0.895	0.061
P – value, Ca reduction level			0.081	0.874	0.512	0.119	0.528
P – value, phytase x Ca interaction			0.267	0.159	0.13	0.551	0.199
P – value, linear Ca			0.378	0.613	0.410	0.649	0.953

d 41

Comparison of treatment means:

T1	0	None	444	185.2	347.8	0.34	7.90[c]
T2	500	L	430	187.3	346.5	0.35	7.96[bc]
T3	500	M	436	185.4	345.3	0.34	7.93[bc]
T4	500	H	432	186.2	343.4	0.36	8.20[abc]
T5	1000	L	432	185.9	347.5	0.35	7.98[bc]
T6	1000	M	434	185.7	345.7	0.35	8.35[ab]
T7	1000	H	429	186.4	345.1	0.37	8.46[a]
SEM			6.31	0.77	1.45	0.01	0.10
P - value			0.721	0.499	0.441	0.185	0.001

Factorial analysis:

Phytase dose (FTU/kg)	500		433	186.3	345.1	0.35	8.0[b]
	1000		432	186	346.1	0.36	8.3[a]
Ca reduction level		L	431	186.6	347	0.35	8.0[b]
		M	435	185.6	345.5	0.35	8.1[ab]
		H	430	186.3	344.2	0.36	8.3[a]
P – value, phytase dose			0.883	0.685	0.418	0.305	0.006
P – value, Ca reduction level			0.747	0.454	0.207	0.088	0.003
P – value, phytase x Ca interaction			0.894	0.486	0.912	0.373	0.147
P – value, linear Ca			0.823	0.838	0.083	0.060	0.002

a,b,c means in the same row with no common superscripts are significantly different ($P < 0.05$).

[1]The factorial analysis was a 2 x 3 factorial analysis that excluded Treatment 1 (PC).