# The interplay of dietary nutrient level and varying calcium to phosphorus ratios on efficacy of bacterial phytase: 2. Ileal and total tract nutrient utilization

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# The interplay of dietary nutrient level and varying Ca to phosphorus ratios on efficacy of a bacterial phytase: 2. Ileal and total tract nutrient utilization

**ABSTRACT** A 14-d broiler experiment was conducted to assess the effects of two dietary variables on efficacy of a bacterial 6-phytase expressed in *Aspergillus oryzae* on nutrient and phytate phosphorus (PP) utilization. Diets were formulated with or without nutrient matrix values (matrix) for phytase as negative control (NC) or positive control (PC), respectively and with two Ca:tP levels (2:1 or 2.5:1). The diets were supplemented with 0, 1,000 or 2,000 FYT/kg phytase thus producing a 2×2×3 factorial arrangement. Excreta were collected on d 19 to 21 and ileal digesta on d 21. There was no three-way interaction on digestibility of any nutrient. There was matrix × phytase ($P < 0.01$) interaction for Ca and DM digestibility and Ca:tP × phytase interaction ($P < 0.05$) for acid hydrolyzed fat, Ca and P digestibility. Pre-cecal flow of Mn, Zn and Na was greater ($P < 0.05$) in NC diets whereas phytase increased ($P < 0.05$) pre-cecal flow of Mg, Fe, Mn, and Zn but decreased ($P < 0.05$) pre-cecal Na flow. Total tract PP disappearance and total tract Ca retention increased ($P < 0.05$) with phytase supplementation in diets with 2:1 Ca:tP whereas there was no effect of phytase supplementation on PP disappearance or Ca retention in diets with 2.5:1 Ca:tP. Total P and Ca retention were reduced ($P < 0.05$) in PC and NC diets when Ca:tP increased to 2.5:1 but the depression was more pronounced in the NC diet. In addition, PP disappearance decreased ($P < 0.05$) with increasing Ca:tP in the PC diets but there was no effect of widening Ca:tP on PP disappearance in NC diets. It was concluded from the current study that the effect of phytase supplementation on P utilization is reduced when diets contain adequate P as exemplified in the PC diets and that the negative impact of wide Ca:tP is more pronounced in diets with phytase matrix allowance as exemplified in the NC diets.

**Key words:** broilers, calcium:phosphorus, nutrient utilization, phytase matrix

## INTRODUCTION

The use of phytase in non-ruminant diets and the effects of different nutritional variables on the efficacy of phytase have been well studied in recent years (Adeola and Cowieson, 2011). However, there is continued interest in understanding the various factors that mitigate the efficacy of phytase or that may improve its effect in poultry diets because of the sheer amount of phytate present in typical poultry diets. A typical corn-soybean meal diet for poultry formulated using conventional (i.e. non low-phytate varieties) may contain up to 4 g/kg phytate-P (Selle and Ravindran, 2007) which are largely unavailable to birds without the action of phytase (endogenous and exogenous). Liberation of 60% of the P tied up in phytate will be a considerable saving in terms of reducing both the cost for inorganic P supplementation and environmental impact of P excretion. Therefore it is imperative to understand factors that may hinder or enhance the efficacy of phytase.

The negative effect of wide Ca:P on phytase efficacy is well known (Tamim et al., 2004; Adeola et al., 2006) and this is related to the formation of recalcitrant calcium-phytate (Taylor, 1965, Nelson and Kirby, 1987) or Ca-phosphate complexes (Long et al., 1984). In addition it is common practice to reduce dietary levels of inorganic P, Ca, Na, energy and some digestible amino acids (phytase matrix) in phytase-supplemented diets (Shelton et al., 2004). It would seem that supplementation of phytase to diets that already meet birds' requirements for these minerals can be both wasteful and counterproductive. However, supplementation of phytase at high levels (Shirley and Edwards, 2003; Cowieson et al., 2006) or to diets that already meet nutrient requirements of broilers has produced improvement in animal performance presumably via mitigation of anti-nutritive effects of phytate rather than supply of limiting nutrients (Walk et al., 2013).

The current study examines the interplay of the variation in Ca:tP (tp, **total P**) and dietary nutrient levels on efficacy of phytase added at low and high doses. There have been considerable amount of investigations on the former and much less on the latter. Therefore, the objective of the current experiment was to investigate how the use of a nutrient replacement values for phytase (phytase matrix) affects phytase efficacy on nutrient utilization (with particular focus on utilization of Ca, tP and phytate P), and especially within the context of variable dietary Ca:tP. The companion article considers how these dietary factors influence growth performance and bone mineralization in broilers.

## MATERIALS AND METHODS

All the animal experimentation procedures used in the current study were approved by the Scotland's Rural College's Animal Experimentation Committee.

### *Diets and experimental design*

A total of 576 birds were used for the 14-d experiment to study the influence of nutrient specification and Ca:tP on efficacy of phytase on nutrients and minerals utilization in broilers. The birds were brooded together in a floor pen for the first 7 days of age during which they received a standard diet that meets NRC (1994) nutrient requirement for broilers. On day 7, the birds were weighed and allocated to 12 dietary treatments in a randomized complete block design and a 2×2×3 factorial arrangement of treatments. Each treatment had 8 replicate cages and 6 birds per replicate cage. The factors were two levels of nutrient specifications (explained below), two levels of Ca:tP (2:1 and 2.5:1) and three levels of phytase supplementation (0, 1,000 and 2,000 FYT/kg). Excreta were collected on d 19 to 21 of the birds' age and ileal digesta were collected on d 21 after euthanasia of the birds.

The composition of the experimental diets is presented in Table 1. Nutrient specification was used to define the diets that were formulated to meet all the nutrient requirements for broilers (full nutrient specification without phytase matrix or positive control, **PC**) and another

set of diets with reduced nutrient specification formulated to be deficient in P, Ca, crude protein (**CP**), amino acids, and energy (down specification or negative control, **NC**). The nutrients and energy levels in the NC diets were reduced relative to the PC diets on the basis of the amount of nutrients and energy that the phytase was expected to release (nutrient matrix values for phytase). The matrix values used per kg feed for 1,000 FYT were approximately, 75 kcal ME, 1.5 g for available P, 1.8 g for Ca, 0.26 g for CP, 0.11, 0.07, 0.04, and 0.07 g for digestible lysine, total sulphur amino acids, methionine, and threonine, respectively. One phytase (FYT) unit is defined as the activity that releases 1 μmol inorganic phosphate from 5.0 mM phytate per minute at pH 5.5 and 37°C.

***Chemical analysis***

Diets, ileal digesta and excreta were analyzed for dry matter, N, gross energy, Ti, and minerals. Dry matter was determined by drying the samples in a drying oven (Uniterm, Russel-Lindsey Engineering Ltd., Birmingham, England, UK) at 105°C for 24 hours (AOAC Method 934.01; AOAC, 2006). Total N content was determined by the combustion method (Method 968.06; AOAC, 2006). Gross energy was determined in an adiabatic bomb calorimeter (Model 6200, Parr Instruments, Moline, IL) using benzoic acid as an internal standard. Titanium concentration in the samples was determined using the method of Short *et al*. (1996). Minerals content was determined using Inductively Coupled Plasma – Optical Emission Spectroscopy (AOAC Method 990.08; AOAC, 2006) following digestion, in turn, in concentrated $HNO_3$ and HCl. Free fat was determined using extraction by petroleum ether in a Soxhlet apparatus for six hours whereas acid hydrolyzed fat (**AHF**) was determined by acid hydrolysis using 30% HCl followed by ether extraction.

***Statistical analysis***

The data were analyzed by the MIXED procedure of SAS as appropriate for a randomized complete block design and a factorial treatment arrangement. For ease of reference, the two types of control diets (NC and PC) were coded as matrix (i.e. nutrient matrix for phytase) in the factorial arrangement with PC (as diets without phytase matrix) and NC (as diets with phytase matrix). The three-way interactions were investigated first in the analysis. Where the 3-way interactions were not significant they were dropped from the model and the data re-analyzed. Non-significant interactions were dropped for more thorough investigation of the main effects means. Because the two-way interactions were significant for most of the responses even though the three-way interactions were not, the simple effects means are presented in the tables. Because of the hierarchical arrangement of main effects and interactions, only the interactions are discussed for responses in which all the two-way interactions are significant, whereas main effects means are also discussed in cases where one or more of the two-way interactions are not significant.

## RESULTS

The analyzed nutrients compositions of the experimental diets are shown in Table 2 and show that expected nutrient compositions were met despite some slightly higher recoveries of the phytase.

The data on ileal nutrient digestibility response to the dietary treatments are presented in Table 3. There were no three-way interaction effects on digestibility of any of the nutrients. There were matrix × Ca:tP ($P < 0.05$) interaction for DM and AHF digestibility explained by lower ($P < 0.01$) DM and AHF digestibility in the NC diets with narrow Ca:tP whereas there was no effect of Ca:tP on DM and AHF digestibility in the PC diets. There was also matrix × phytase ($P < 0.01$) interaction for Ca and DM digestibility with lower ($P < 0.01$) DM and Ca

digestibility (drastic reduction observed for Ca digestibility) in phytase-supplemented NC diets whereas such effect was not observed in the PC diets. Ca:tP × phytase interaction was observed ($P < 0.05$) for AHF, Ca and P digestibility with phytase at 2,000 FYT/kg increasing ($P < 0.05$) AHF digestibility in the diets with narrow Ca:tP whereas phytase supplementation had no effect on AHF digestibility in diets with wide Ca:tP. The Ca:tP × phytase interaction for Ca digestibility was characterized by drastic and stepwise reduction ($P < 0.05$) in Ca digestibility with increasing phytase supplemental level in the diets with wide Ca:tP but a less drastic reduction in Ca digestibility at 1,000 FYT/kg in diets with narrow Ca:tP. For P digestibility, the Ca:tP × phytase interaction was manifested in reduced ($P < 0.05$) P digestibility at 1,000 FYT/kg and an increase ($P < 0.05$) at 2,000 FYT/kg in diets with narrow Ca:tP but a reduction ($P < 0.05$) in P digestibility at both 1,000 and 2,000 FYT/kg in diets with wide Ca:tP.

The data on pre-cecal flow of micro-minerals in response to the dietary treatments are presented in Table 4. Pre-cecal flow of Na, Mn and Zn was greater ($P < 0.05$) in NC diets; in addition pre-cecal flow of K and M n was greater ($P < 0.05$) in diets with wide Ca:tP. On the other hand, phytase supplementation increased ($P < 0.05$) pre-cecal flow of Mg, Fe, Mn, and Zn, decreased ($P < 0.01$) flow of Na and had no effect on K flow. There were significant Ca:tP × phytase interactions ($P < 0.05$) for pre-cecal flow of Mg and Mn with a decrease in the pre-cecal flow of the minerals with phytase supplementation in diets with narrow Ca:tP. On the other hand there was an increase in pre-cecal flow of the minerals with phytase supplementation in diets with wide Ca:tP. Ca:tP × matrix interaction was significant ($P < 0.01$) for pre-cecal flow of Na and Mn. Generally pre-cecal flow of Na and Mn was greater ($P < 0.01$) in PC diets with wide Ca:tP whereas Ca:tP had no effect on flow of the minerals in NC diets. Matrix × phytase interaction was observed ($P<0.01$) for pre-cecal Na and K flow. Phytase supplementation

decreased ($P < 0.05$) pre-cecal Na flow but increased ($P < 0.05$) K flow in NC diets however phytase supplementation had no effect on pre-cecal Na flow but decreased ($P < 0.05$) pre-cecal K flow in PC diets.

The effects of the treatments on total tract nutrient retention are shown in Table 5. There were Ca:tP × phytase interaction ($P < 0.05$) for total tract retention of DM, fat, AHF, and N, as well as AME. Dry matter and N retention as well as AME increased ($P < 0.05$) with phytase supplementation in diets with narrow Ca:tP but DM retention decreased ($P < 0.05$) whereas AME, N and fat retention were unaffected by phytase supplementation in diets with wide Ca:tP. The interaction of Ca:tP × matrix was significant ($P < 0.05$) for retention of DM, AHF, N, and AME. In PC diets, retention of DM, AHF and N decreased ($P < 0.05$) whereas AME increased ($P < 0.05$) with widening of Ca:tP. In NC diets, AHF and N retention increased ($P < 0.05$) whereas there was no change in DM retention and AME with widening of Ca:tP.

The effect of the dietary treatments on total tract retention of Ca, P and PP are shown in Table 6. Widening Ca:tP to 2.5:1 decreased ($P < 0.05$) total tract retention of Ca and tP as well as PP disappearance. Ca:tP × phytase interaction was significant ($P < 0.05$) for total tract retention of Ca, tP and PP. In the diets with narrow Ca:tP, only 2,000 FYT phytase increased ($P < 0.05$) tP retention. In the diets with wide Ca:tP, phytase supplementation at 1,000 FYT/kg improved tP retention. Total tract PP disappearance and Ca retention increased ($P < 0.05$) with phytase supplementation in diets with narrow Ca:tP but no effect was observed in diets with wide Ca:tP. There was significant Ca:tP × matrix ($P < 0.05$) on total tract retention of tP and Ca as well as PP disappearance. Total P and Ca retention were reduced ($P<0.05$) in both NC and PC with widening of Ca:tP to 2.5:1 but the depression in P and Ca retention due to widening of Ca:tP was more pronounced in the NC diets. In addition, PP disappearance decreased ($P<0.05$) with

widening Ca:tP in the PC diets but there was no effect of widening Ca:tP on PP disappearance in the NC diets. Matrix × phytase was significant ($P < 0.05$) only for total tract P retention with phytase supplementation increasing P retention only at 2,000 FYT/kg in PC diets and only at 1,000 FYT/kg in NC diets.

## DISCUSSION

There is a preponderance of information on the effects of phytase on nutrient utilization (Selle and Ravindran, 2007; Adeola and Cowieson, 2011) as well as the effect of Ca:P on phytase efficacy (Qian et al, 1997; Selle et al., 2009). In addition, it is a usual practice to reduce nutrient specification in phytase-supplemented diets or provide a nutrient matrix values for phytase (Shelton et al., 2004; Silversides and Hruby, 2009). Therefore the objective of the current experiment was to study the interactivity of varying Ca:tP in PC and NC diets on the efficacy of phytase at low and high doses in promoting nutrient utilization in broilers. Although the effects of the treatments in the current experiment were observed on energy and a large number of nutrients, the main responses that will subsequently be focused on are phytate and total P as well as Ca in view of their association with phytic acid.

### *Effects of use of nutrient matrix for phytase on phytase efficacy*

The use of a phytase matrix in phytase-supplemented feed enables a reduction in nutrient specification, reduces nutrient excretion and increases the chance of being able to observe phytase effects. Two lines of evidence are presented here to show that the use of a phytase matrix differently affects phytase effects on PP and tP.

At the ileal level, the efficacy of phytase in promoting PP disappearance was the same in both PC and NC diets but at the total tract level, phytase supplementation of PC diet marginally

reduced PP disappearance whereas phytase supplementation increased PP disappearance in the NC diets. The PP level was virtually the same across all diets (average of 0.22%) and hence the lower PP disappearance in PC diet without phytase suggests that the higher dietary non-phytate P (**nPP**) level (providing greater quantities of readily available P) in the diet may provide a feedback mechanism inhibiting the degradation of phytic acid. It is not clear if such a mechanism exists, however others have similarly observed reduced PP disappearance in diets with high levels of nPP (Ballam et al. 1982; Olukosi et al., 2013).

Ravindran et al. (2000) observed that hydrolysis of PP increases with an increase in dietary PP level. This is intuitive, up to a point, as higher PP provides more substrate for phytase. But it is also of interest to consider how PP hydrolysis is affected by nPP levels in diets with the same content of PP. In the current study, ileal PP disappearance was similar in both PC and NC diets supplemented with 2,000 FYT/kg even though ileal PP disappearance in the diets without phytase was five percentage units greater in the NC diet. This shows that the effect of phytase on PP disappearance, relative to the control, was greater in the PC diet. The observation that PP disappearance was the same in the diets supplemented with 2,000 FYT/kg in both PC and NC diets indicates that effect of phytase supplementation on PP disappearance did not depend on dietary level of nPP as also observed by Plumstead et al. (2008).

Phytate P disappearance at the total tract level in response to phytase supplementation was greater in both PC and NC diets compared with the disappearance at the ileal level. However the difference in PP disappearance at both levels in diets supplemented with 2,000 FYT/kg was greater for NC diet (11 %) compared with PC diet (5%). Phytase did not improve total tract PP disappearance in the PC diet but improved PP disappearance in the NC diet. Increased PP disappearance in the excreta compared with the ileal level is an indication of either that the

phytase continued to be effective post-ileal or of the possible effects of microorganisms on phytic acid hydrolysis. Overall the observation on PP disappearance and P utilization indicate that reducing the level of nPP is beneficial in promoting greater PP and total P utilization.

Phytase supplementation did not increase ileal P digestibility in both the NC and PC diets but increased total tract P retention by 11% in the NC and had no effect in the PC diets. The numerical increase of 4.3 percentage units for total tract P retention in phytase-supplemented PC diet decreased retained P by 190 mg/kg. On the other hand, phytase supplementation of NC diet increased P retention by 11 percentage units and increased retained P by 830 mg/kg. In spite of the greater retained P in phytase-supplemented NC compared with PC diet, the total retained P at 2,000 FYT/kg was 3.74 and 3.62 g/kg for PC and NC diets, respectively. The hydrolyzed PP at the total tract level for PC and NC diets supplemented with 2,000 FYT/kg were 1.66 and 1.84 g/kg, respectively. Taken together therefore, the data imply that the high nPP content of the PC diets "hinders" phytase from exerting its full effect on phytate. Although the total retained P in PC diet supplemented with 2,000 FYT/kg phytase was greater than retained P in comparable NC diet, this extra retained P could have resulted from the higher dietary P in the PC diet because the amount of PP hydrolyzed was actually lower in the phytase-supplemented PC diet.

***The interplay of Ca:tP and dietary nutrient levels (phytase matrix)***

The use of a phytase matrix in diet formulation enables a reduction in nutrient content of phytase-supplemented diets. This may be a necessary dietary intervention in phytase-supplemented diets in order to optimize phytase effect and maximize reduction in nutrient excretion (Shelton et al., 2004; Silversides et al., 2009). In the current study, at both the ileal and total tract levels, widening Ca:tP decreased P and Ca digestibility in both PC and NC diets but

240 the decrease produced by the wider Ca:tP was more pronounced in NC diets and the depression
241 in Ca utilization due to wide Ca:tP was greater at the total tract level. The decreased digestibility
242 values were also reflected in decreased digestible and retained Ca and P in the diets in response
243 to widening the Ca:tP. Two scenarios emerging from these observations are: 1) a decrease in Ca
244 and P utilization with a widening of Ca:tP irrespective of whether it was in PC or NC diet and, 2)
245 a more pronounced negative effect of widening Ca:tP in NC diets.

246 In the first scenario, the decreased Ca utilization with increased Ca:tP can be associated
247 with increased relative dietary concentration of Ca in the diets with wide Ca:tP because the
248 analyzed Ca in these diets was 27% higher than in diets with narrow Ca:tP. This greater dietary
249 Ca content produced correspondingly higher Ca intake and hence reduced Ca retained as a
250 percentage of intake. Similar observations have been reported in rats (Hoek et al., 1988), pigs
251 (Qian et al., 1996) and chickens (Qian et al., 1997). Thus it seems that the decreased Ca
252 utilization in diets with wide Ca:tP can be largely explained by the presence of an abundance of
253 Ca in the intestine, than can be utilized by the birds, leading to excessive Ca excretion or reduced
254 efficiency of Ca absorption.

255 The decrease in P utilization in the diets with wide Ca:tP ratio is also primarily driven by
256 dietary Ca content because tP content was similar in diets with wide and narrow Ca:tP. Hoek et
257 al. (1988) similarly observed high P excretion in rats receiving diets with high Ca level. This
258 reduced P utilization in diet with wide Ca:tP can be explained by the fact that high concentration
259 of Ca relative to P increases the possibility for negative interaction of Ca and P, leading to
260 greater chances for formation of calcium phosphate (Hurwitz and Bar, 1971). Al-Masri (1995)
261 observed that P digestibility, absorption and endogenous excretion in chickens decreased with
262 increasing Ca:P ratio. Similar effect has been reported by Edwards and Veltmann (1983) and

Qian et al. (1997). Clearly, an increase in Ca:tP increases the concentration of Ca relative to P and hence increases the chances of more Ca being chemically bound and becoming indigestible.

It has been suggested that another way by which high Ca:P reduces P and Ca utilization is by the formation of recalcitrant Ca-phytate complex (Wise, 1983; Maenz et al., 1999). The effect of Ca:tP on PP disappearance was not consistently observed in the current study. It was only at the total tract level that high Ca:tP decreased PP disappearance in the PC diet. The analyzed PP was the same in all diets in the current experiment and the only differences among diets were Ca and P levels. In addition, the depressed PP disappearance observed in the current study was not dependent on Ca:tP per se but rather on dietary concentration of both Ca and P, i.e. the diet with high contents of both Ca and P had depressed PP disappearance.

For the second scenario, it is possible that the reason for the decrease in Ca and P utilization in NC diets with wide Ca:tP relative to similar diets in PC diets was due to the lower Ca and P contents of the NC diets compared with PC diets. Phytate P made up a greater proportion of total P in NC compared with the PC (additional P in the PC diet was supplied by dicalcium phosphate) and hence the P will be less digestible in NC than the more readily digestible P in the inorganic P sources used in the PC diet. Consequently the current data show that the dietary content of Ca and P, not just the ratio, need to be considered in interpretation of the effect of Ca:tP.

In light of the observations in the current experiment, it can be concluded that the effects of wide Ca:tP are more likely to be severe in diets in which nutrient matrix for phytase is used (as exemplified by the NC diets in this experiment) especially as it relates to Ca utilization; and

that the negative effect of high Ca:tP on P and Ca utilization could be mediated via mechanisms independent of phytic acid degradation.

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**Table 1.** Ingredient composition (g/kg) of the experimental basal diets

| Basal diet | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Ca:tP[1] | 2:1 | | 2.5:1 | |
| Control (phytase matrix) | Positive | Negative | Positive | Negative |
| Corn | 482.6 | 477.4 | 466.6 | 499.4 |
| Wheat | - | 50.0 | - | - |
| Soybean meal | 397.5 | 382.5 | 400.5 | 394.5 |
| Soybean oil | 58.0 | 40.0 | 60.0 | 45.0 |
| Corn Starch | 15.0 | 15.0 | 15.0 | 15.0 |
| Dicalcium phosphate | 17.5 | 9.0 | 17.5 | 10.0 |
| Limestone | 17.0 | 15.5 | 28.0 | 24.0 |
| Titanium dioxide | 0.5 | 0.5 | 0.5 | 0.5 |
| L-Lysine·HCl | 1.0 | 0.4 | 1.0 | 0.7 |
| DL-Methionine | 2.8 | 1.9 | 2.8 | 2.8 |
| Threonine | 0.6 | 0.3 | 0.6 | 0.6 |
| Vitamin-mineral premix[2] | 2.5 | 2.5 | 2.5 | 2.5 |
| Salt | 5.0 | 5.0 | 5.0 | 5.0 |
| Phytase premix[3] | To 1,000 | To 1,000 | To 1,000 | To 1,000 |
| Total | 1,000 | 1,000 | 1,000 | 1,000 |
| Calculated nutrients and energy, % | | | | |
| Metabolizable energy, kcal/kg | 3,185 | 3,125 | 3,154 | 3,127 |
| Crude protein | 22.9 | 22.8 | 22.9 | 22.9 |
| Total P | 0.71 | 0.56 | 0.70 | 0.57 |
| Non-phytate P | 0.45 | 0.30 | 0.45 | 0.31 |
| Ca | 1.06 | 0.82 | 1.43 | 1.13 |

[1]Ca:tP based on analyzed chemical composition

[2]Supplied the following per kilogram of diet: vitamin A, 5,484 IU; vitamin D3, 2,643 ICU; vitamin E, 11 IU; menadione sodium bisulfite, 4.38 mg;riboflavin, 5.49 mg; d-pantothenic acid, 11 mg; niacin, 44.1 mg; choline chloride, 771 mg; vitamin B12, 13.2 μg; biotin, 55.2 μg; thiamine mononitrate,2.2 mg; folic acid, 990 μg; pyridoxine hydrochloride, 3.3 mg; I, 1.11 mg; Mn, 66.06 mg; Cu, 4.44 mg; Fe, 44.1 mg; Zn, 44.1 mg; Se, 300 μg.

[3]Phytase premix containing 100 phytase units (FYT)/g replaced corn starch to provide 1,000 or 2,000 FYT/kg.

**Table 2.** Analyzed nutrient composition (%, dry matter basis) and phytase activity in the experimental diets

| Diets | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ca:tP | 2:1 | | | | | | 2.5:1 | | | | | |
| Matrix | Positive Control | | | Negative Control | | | Positive Control | | | Negative Control | | |
| GE, kcal/kg | 4,452 | 4,681 | 4,652 | 4,672 | 4,619 | 4,603 | 5,720 | 5,782 | 4,610 | 4,641 | 4,572 | 4,580 |
| Ether extract | 10.2 | 10.3 | 8.80 | 7.11 | 7.83 | 6.84 | 11.4 | 11.3 | 8.94 | 7.67 | 8.26 | 7.95 |
| Acid hydrolysed fat | 11.1 | 9.49 | 9.80 | 8.03 | 7.96 | 8.03 | 12.1 | 12.9 | 9.70 | 8.30 | 9.37 | 8.37 |
| N | 3.99 | 3.86 | 4.13 | 3.84 | 3.90 | 3.80 | 5.11 | 4.70 | 4.15 | 4.04 | 3.73 | 3.88 |
| Ca | 1.37 | 1.63 | 1.40 | 1.31 | 1.18 | 1.17 | 2.23 | 2.24 | 1.87 | 1.06 | 1.47 | 1.38 |
| P | 0.73 | 0.81 | 0.71 | 0.65 | 0.61 | 0.57 | 0.93 | 0.91 | 0.74 | 0.48 | 0.60 | 0.62 |
| Phytate P | 0.26 | 0.24 | 0.23 | 0.25 | 0.23 | 0.24 | 0.28 | 0.28 | 0.29 | 0.24 | 0.23 | 0.29 |
| non-phytate P[1] | 0.47 | 0.57 | 0.48 | 0.40 | 0.38 | 0.33 | 0.65 | 0.63 | 0.45 | 0.24 | 0.37 | 0.33 |
| Na | 0.23 | 0.26 | 0.24 | 0.26 | 0.25 | 0.23 | 0.24 | 0.28 | 0.23 | 0.17 | 0.23 | 0.19 |
| Mg | 0.18 | 0.18 | 0.16 | 0.18 | 0.18 | 0.16 | 0.23 | 0.22 | 0.18 | 0.15 | 0.18 | 0.19 |
| Cu, mg | 11.3 | 15.8 | 14.7 | 12.5 | 10.2 | 10.2 | 28.2 | 18.2 | 12.4 | 10.2 | 13.6 | 15.8 |
| Fe, mg | 82.4 | 87.0 | 79.2 | 90.7 | 78.5 | 71.3 | 111.3 | 106.5 | 83.3 | 56.6 | 81.4 | 79.0 |
| Mn, mg | 81.3 | 94.9 | 87.1 | 100.9 | 84.2 | 83.8 | 107.1 | 105.1 | 83.3 | 61.1 | 83.7 | 86.9 |
| Zn, mg | 79.1 | 96 | 99.5 | 92.9 | 84.2 | 81.5 | 101.4 | 99.5 | 83.3 | 58.9 | 75.7 | 79.0 |
| K | 1.16 | 1.14 | 1.00 | 1.16 | 1.19 | 0.96 | 1.47 | 1.43 | 1.14 | 0.97 | 1.15 | 1.28 |
| Phytase, FTY/kg[2] | BD | 1,121 | 2,977 | BD | 1,406 | 2,400 | BD | 1,717 | 2,537 | BD | 1,011 | 2,640 |

[1]non-phytate phosphorus level was determined by difference (total P – phytate P)

[2]BD – below detection limit

**Table 3.** Simple effects means for ileal nutrient digestibility response to varying levels of phytase supplementation, dietary Ca:total P broiler diets with or without nutrient matrix values for phytase[1]

| Diet | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | | P-values for interactions[2,3] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ca:tP | 2:1 | | | | | | 2.5:1 | | | | | | SEM | | | |
| M[3] | Positive Control | | | Negative Control | | | Positive Control | | | Negative Control | | | | Ca:tP × M | Ca:tP × Ph | M × Ph |
| Ph[3] | 0 | 1000 | 2000 | 0 | 1000 | 2000 | 0 | 1000 | 2000 | 0 | 1000 | 2000 | | | | |
| DM | 69.9 | 67.8 | 68.6 | 66.1 | 62.7 | 68.5 | 66.2 | 65.4 | 65.4 | 68.2 | 66.3 | 63.1 | 0.897 | 0.003 | 0.635 | 0.001 |
| EE[3] | 89.5 | 88.3 | 87.2 | 83.2 | 83.6 | 83.0 | 87.2 | 88.0 | 86.7 | 85.5 | 86.2 | 85.0 | 1.12 | 0.058 | 0.794 | 0.821 |
| AHF[3] | 82.0 | 79.6 | 82.4 | 76.4 | 74.4 | 78.0 | 81.3 | 81.8 | 81.0 | 78.1 | 81.9 | 78.4 | 1.31 | 0.043 | 0.003 | 0.768 |
| N | 76.9 | 73.8 | 77.1 | 73.9 | 69.9 | 75.0 | 73.5 | 71.4 | 74.8 | 73.8 | 70.3 | 70.0 | 1.12 | 0.636 | 0.156 | 0.447 |
| Ca | 42.8 | 32.5 | 35.9 | 61.7 | 45.9 | 44.9 | 34.0 | 31.9 | 25.8 | 53.5 | 34.8 | 25.9 | 2.56 | 0.062 | 0.029 | 0.001 |
| P | 43.8 | 38.7 | 47.2 | 48.5 | 37.9 | 46.9 | 34.0 | 32.3 | 33.0 | 37.6 | 31.4 | 30.2 | 1.97 | 0.358 | 0.005 | 0.071 |
| PP[3] | 48.5 | 42.7 | 58.7 | 57.7 | 43.3 | 65.4 | 36.0 | 44.1 | 58.6 | 39.3 | 51.1 | 51.7 | 6.03 | 0.493 | 0.088 | 0.784 |

[1]Means were obtained from 8 replicate cages of 6 birds per replicate cage

[2]The main effect for Ca:tP was significant ($P < 0.05$) for all nutrients except EE and PP; The main effect for nutrient matrix was significant for all nutrients except P and PP; The main effect for phytase was significant for all nutrients except EE and AHF; The three-way interaction was not significant for any nutrient

[3]M = nutrient matrix for phytase; Ph = phytase; EE = crude fat; AHF =acid hydrolysed fat; PP = phytate phosphorus

**Table 4.** Simple effects means for pre-cecal flow (g/100 g dry matter intake) of micro-minerals in response to varying levels of phytase supplementation, dietary Ca:total P broiler diets with or without nutrient matrix values for phytase[1]

| Diet | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | | P-values for interactions[2, 3] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ca:tP | 2:1 | | | | | | 2.5:1 | | | | | | SEM | | | |
| M[3] | Positive Control | | | Negative Control | | | Positive Control | | | Negative Control | | | | Ca:tP × Ph | Ca:tP × M | M × Ph |
| Ph[3] | 0 | 1000 | 2000 | 0 | 1000 | 2000 | 0 | 1000 | 2000 | 0 | 1000 | 2000 | | | | |
| Na | 0.271 | 0.235 | 0.232 | 0.409 | 0.346 | 0.292 | 0.257 | 0.242 | 0.260 | 0.362 | 0.290 | 0.294 | 0.017 | 0.119 | 0.032 | 0.008 |
| Mg | 0.155 | 0.166 | 0.158 | 0.168 | 0.190 | 0.157 | 0.164 | 0.178 | 0.171 | 0.156 | 0.171 | 0.186 | 0.006 | 0.003 | 0.096 | 0.620 |
| Fe, mg | 65.8 | 73.6 | 74.5 | 64.7 | 70.9 | 86.9 | 73.3 | 75.3 | 85.5 | 71.7 | 78.0 | 86.6 | 7.55 | 0.965 | 0.804 | 0.717 |
| Mn, mg | 88.0 | 92.7 | 84.5 | 94.0 | 99.4 | 89.4 | 92.6 | 96.9 | 94.2 | 91.6 | 91.9 | 101.0 | 2.20 | < 0.001 | 0.028 | 0.309 |
| Zn, mg | 76.7 | 85.2 | 77.1 | 84.2 | 89.0 | 83.1 | 80.6 | 86.6 | 81.9 | 79.8 | 87.7 | 88.7 | 3.45 | 0.448 | 0.418 | 0.676 |
| K | 0.158 | 0.153 | 0.146 | 0.141 | 0.158 | 0.142 | 0.191 | 0.149 | 0.169 | 0.161 | 0.174 | 0.172 | 0.023 | 0.152 | 0.747 | 0.006 |

[1]Means were obtained from 8 replicate cages of 6 birds per replicate cage

[2]Phytase matrix effect only significant ($P < 0.05$) for Na, Mn and Zn; Ca:tP effect only significant for Mn and K; Phytase effect significant ($P < 0.05$) for all except K; The three-way interaction was not significant for any nutrient

[3]M = nutrient matrix for phytase; Ph =phytase

**Table 5.** Simple effects means for total tract nutrient retention response to varying levels of phytase supplementation, dietary Ca:total P broiler diets with or without nutrient matrix values for phytase[1]

| Diet | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | | P-values for interactions[2, 3] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ca:tP | 2:1 | | | | | | 2.5:1 | | | | | | SEM | Ca:tP × Ph | Ca:tP × M | M × Ph |
| M[3] | Positive Control | | | Negative Control | | | Positive Control | | | Negative Control | | | | | | |
| Ph[3] | 0 | 1000 | 2000 | 0 | 1000 | 2000 | 0 | 1000 | 2000 | 0 | 1000 | 2000 | | | | |
| DM | 71.0 | 68.8 | 71.4 | 67.5 | 66.8 | 70.2 | 69.1 | 67.5 | 66.2 | 68.8 | 70.1 | 67.2 | 0.581 | 0.001 | 0.020 | 0.001 |
| AME | 3,321 | 3,557 | 3,535 | 3,516 | 3,469 | 3,458 | 3,515 | 3,540 | 3,519 | 3,514 | 3,453 | 3,455 | 5.81 | 0.001 | 0.001 | 0.001 |
| EE | 91.7 | 90.4 | 90.0 | 86.7 | 86.5 | 86.4 | 90.6 | 88.7 | 89.7 | 89.0 | 90.3 | 88.4 | 0.636 | 0.001 | 0.063 | 0.876 |
| AHF | 89.5 | 84.4 | 87.2 | 83.4 | 81.8 | 83.3 | 86.1 | 85.0 | 84.9 | 85.9 | 87.8 | 83.5 | 0.818 | 0.001 | 0.014 | 0.001 |
| N | 63.7 | 57.7 | 65.2 | 53.0 | 54.8 | 58.3 | 62.8 | 58.2 | 60.0 | 56.8 | 59.0 | 57.3 | 1.07 | 0.001 | 0.001 | 0.001 |

[1]Means were obtained from 8 replicate cages of 6 birds per replicate cage

[2]Matrix (M) effect was significant for all nutrients except DM; Ca:tP effect was significant for all nutrients except AHF and N; Phytase (Ph) effect was significant for all nutrients except DM and AME; P-values for three-way interaction was not significant for any of the nutrients

[3]M = nutrient matrix for phytase; Ph = phytase; EE = crude fat; AHF =acid hydrolysed fat

**Table 6.** Simple effects means for total tract retention response of calcium, total and phytate phosphorus to varying levels of phytase supplementation, dietary Ca:total P broiler diets with or without nutrient matrix values for phytase[1]

| Diet | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | | P-values for interactions[2, 3] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ca:tP | 2:1 | | | | | | 2.5:1 | | | | | | SEM | Ca:tP × Ph | Ca:tP × M | M × Ph |
| M[3] | Positive Control | | | Negative Control | | | Positive Control | | | Negative Control | | | | | | |
| Ph[3] | 0 | 1000 | 2000 | 0 | 1000 | 2000 | 0 | 1000 | 2000 | 0 | 1000 | 2000 | | | | |
| P | 52.1 | 49.6 | 56.8 | 56.1 | 61.1 | 69.1 | 42.4 | 46.5 | 46.5 | 42.5 | 54.6 | 52.5 | 1.32 | < 0.001 | 0.004 | <.0001 |
| PP[3] | 68.4 | 68.6 | 68.6 | 63.6 | 60.7 | 71.8 | 64.7 | 62.7 | 58.5 | 62.9 | 66.8 | 67.2 | 2.41 | 0.018 | 0.019 | 0.096 |
| Ca | 39.9 | 36.5 | 42.1 | 42.3 | 38.3 | 46.4 | 25.2 | 22.1 | 22.0 | 17.8 | 25.2 | 16.1 | 1.59 | < 0.001 | 0.002 | 0.067 |

[1]Means were obtained from 8 replicate cages of 6 birds per replicate cage

[2]Phytase matrix effect only significant for P; Ca:tP effect significant for all the nutrients; Phytase effect only significant for P only; P-values for three-way interaction was not significant for any of the nutrients

[3]M = nutrient matrix for phytase; Ph = phytase; PP = phytate phosphorus