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# The Effect of Dietary Phytase on Broiler Performance and Digestive, Bone, and Blood Biochemistry Characteristics

■Author(s)

Sousa JPL de[I]
Albino LFT[II]
Vaz RGMV[III]
Rodrigues KF[III]
Da Silva GF[III]
Renno LN[II]
Barros VRSM[II]
Kaneko IN[IV]

[I] Instituto Federal de Educação, Ciência e Tecnologia do Tocantins, Dianópolis, Tocantins, Brazil
[II] Universidade Federal de Viçosa, Department of Animal Science, Viçosa, Minas Gerais, Brazil
[III] Universidade Federal do Tocantins, School of Veterinary Medicine and Animal Science, Department of Animal Science, Araguaína, Tocantins, Brazil
[IV] Universidade Estadual de Maringá, Department of Animal Science, Maringá, Paraná, Brazil

Part of the Ph.D. thesis of the first author

■Mail Address

Corresponding author e-mail address
Sousa JPL de
Rua 132A, Qd45, lote 05, Setor Sul, CEP 74093-220, Goiânia, GO
E-mail: jp-zoot24@yahoo.com.br

■Keywords

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## ABSTRACT

The dietary inclusion of phytase increases nutrient and energy bioavailability for broilers. The effect of phytase increases nutrients and energy bioavailability for either the objective of this experiment was to evaluate the influence of nutrient and energy reduction in diets supplemented with phytase on the performance, gastrointestinal pH, organ and bone composition, and blood biochemistry of broilers between eight and 21 days of age. In the study, 1.120 male Cobb 500® broilers, with 161±1g average weight, were used. At eight days of age, birds were distributed according to a completely randomized experimental design with seven treatments in a 3x2+1 factorial arrangement with eight replicates of 20 broiler each. Treatments corresponded to reduction of calcium (Ca) and phosphorus (P), amino acids and energy, or reduction of Ca, P, amino acids and energy; supplementation or not of phytase; and a positive control treatment. Broiler fed the diet with reduced Ca and P levels and phytase supplementation presented the best performance of all groups. The diet with reduced amino acid and energy levels and phytase addition reduced gizzard and proventriculus pH. Dietary Ca and P reduction increased relative liver and heart weights, as well as albumin blood levels. The bones of broilers fed phytase-supplemented diets presented higher ash content.

Key words: poultry production, enzymes, intestinal pH, bone ash, calcium, available phosphorus, extra-phosporic effect

## INTRODUCTION

The increasing broiler production around the world has led to the application of nutritional strategies that improve nutrient utilization. The supplementation of exogenous enzymes to corn- and soybean meal-based broiler diets allows supplying nutrient deficiencies and to reduce endogenous losses, thereby optimizing performance, particularly during early life.

The dietary supplementation of the exogenous phytase reduces nutrient variability of feedstuffs, and counteracts the antinutritional effects of phytate, increasing the accuracy of feed formulation.

Exogenous phytase is included in feed formulations not only to reduce phosphorus supplementation, but also to release minerals, particularly calcium, as well as amino acids and carbohydrates by the hydrolysis of phytate, improving nutrient utilization (Oluyinka *et al.*, 2007; Slominski, 2011). However, nutrient utilization can also be affected by other factors, including dietary calcium, phosphorus, protein, and energy levels, intestinal pH, environmental temperature, etc.

Dietary calcium content may negatively influence phosphorus utilization, particularly phytic phosphorus, due to the formation of insoluble complexes with calcium in the digestive tract, counteracting

the effect of phytase, which is not able to hydrolyze such complexes (Leeson *et al.*, 1996). Nelson *et al.* (1964) reported that dietary calcium levels higher than 0.70% at pH 6.0 promote the reaction of calcium with phytic acid, resulting in calcium phytate, which precipitates and cannot be broken down by phytase, consequently reducing phosphorus bioavailability. High dietary calcium levels change the pH of the upper sections of the digestive tract, inactivating phytase. Phytase activity is optimal at pH between 5.0 and 6.5, and it is reduced when pH is lower or equal to 3.0 (Casey & Walsh, 2004).

Broilers fed diets with low inorganic calcium and phosphorus levels have higher capacity to hydrolyze phytate than those fed high levels of these minerals (Denbow *et al.*, 1995). Therefore, minimal calcium and phosphorus levels need to be maintained in the diet; however, their interaction should not be overlooked, particularly considering phytase activity (Qian *et al.*, 1997).

Protein utilization and amino acid absorption may also be influenced by dietary phytic acid levels (Lehnen *et al.*, 2011; Walk *et al.*, 2012). These authors observed that phytic acid is a potent chelating agent. Its negative charges react with the positive charges of some amino acids (lysine, arginine, histidine), some proteins (including those involved in protein digestion, such as pepsin and trypsin), and carbohydrates ($\alpha$-amylase), forming insoluble complexes, thereby reducing their availability and digestibility (Maenz, 2001) and consequently affecting metabolism and organ biometry.

Therefore, the objective of this study was to evaluate the supply of feeds containing reduced amino acid, energy, calcium, and phosphorus levels and supplemented or not with phytase on the performance, gastrointestinal pH, relative organ weight, bone mineral content, and blood biochemistry of broilers.

## MATERIALS AND METHODS

The experiment was carried out at the Poultry Sector of the Department of Animal Science of the Federal University of Viçosa. In the study, 1,120 male Cobb 500® broilers, with 161±1g average weight and between eight and 21 days of age, were used. A completely randomized experimental design in a 3x2+1 factorial arrangement (Ca and P reduction, energy and amino acid reduction, and Ca, P, energy, and amino acid reduction; supplementation or not with phytase; and a positive control treatment) with eight replicates of 20 broiler each. Until eight days of age, all broilers were reared in a conventional poultry house according to the recommendations of the genetic company's (Manual, 2009). On day 8, broiler were individually weighed and distributed into 56 pens, measuring 1,5 $m^2$ and equipped with a nipple drinker and a tube feeder each.

The increase in nutrient and energy bioavailability obtained with phytase supplementation was considered when formulating the experimental diets (Table 1). The phytase product was derived from *Escherichia coli*, and presented 500 FTU/kg of feed. It was added at a dose of 100g/ton of feed.

**Table 1 –** Nutritional matrix of the bacterial phytase applied*

| Nutrients and energy | 500 FTU/kg feed |
|---|---|
| Crude protein, % | 0.420 |
| Metabolizable energy, kcal/kg | 52.00 |
| Calcium, % | 0.165 |
| Phosphorus, % | 0.150 |
| Lysine, % | 0.017 |
| Methionine, % | 0.004 |
| Threonine, % | 0.030 |
| Arginine, % | 0.030 |

*Values informed by the manufacturer

The experimental diets were based on corn and soybean meal and were formulated to supply the nutritional requirements of male broilers, as recommended by Rostagno *et al.* (2011) for the starter phase.

Birds, feed offer, and feed residue were weighed per pen to calculate weight gain, feed intake, and feed conversion ratio. Mortality was also recorded to correct performance data.

On day 21, two broiler per stall, with body weight close to the average stall weight (±100 g), were selected, identified, and had their blood collected by heart puncture. Blood was placed in test tubes, centrifuged for 5 min at 1,500 rpm, and the serum was then placed in duly identified Eppendorf tubes and immediately frozen until analyses. Serum calcium, phosphorus, albumin, and total protein were analyzed using specific commercial kits.

The selected birds were identified and sacrificed according to the norms of the Committee of Ethics for the Use of Animals of the Department of Animal Science of the Federal University of Viçosa process nº 76/2013. Broilers were stunned, sacrificed by neck dislocation, and their gastrointestinal tract (GIT) with the digesta was aseptically removed. Before removing

**Table 2 –** Composition of the experimental diet fed to broilers between 8 and 21 days of age (% as fed)

| Ingredients | Control | Treatment with reduced levels | | |
|---|---|---|---|---|
| | | Ca and P | AA and ME | Ca, P, AA, and ME |
| Corn | 58.81 | 60.3 | 61.39 | 62.89 |
| Soybean meal | 35.04 | 34.76 | 33.70 | 33.41 |
| Soybean oil | 2.30 | 1.79 | 1.07 | 0.57 |
| Limestone | 1.06 | 1.15 | 1.06 | 1.15 |
| Dicalcium phosphate | 1.47 | 0.66 | 1.48 | 0.66 |
| Salt | 0.48 | 0.48 | 0.48 | 0.48 |
| L-Lysine HCL 79% | 0.16 | 0.17 | 0.18 | 0.19 |
| DL-Methionine 99% | 0.26 | 0.26 | 0.23 | 0.23 |
| L-threonine 98% | 0.03 | 0.03 | 0.01 | 0.02 |
| Mineral supplement[1] | 0.11 | 0.11 | 0.11 | 0.11 |
| Vitamin supplement[2] | 0.11 | 0.11 | 0.11 | 0.11 |
| Choline chloride 60% | 0.10 | 0.10 | 0.10 | 0.10 |
| Salinomycin, 12% | 0.06 | 0.06 | 0.06 | 0.06 |
| Antioxidant | 0.01 | 0.01 | 0.01 | 0.01 |
| Total | 100 | 100 | 100 | **100** |
| Calculated values | | | | |
| Crude protein, % | 20.8 | 20.8 | 20.38 | 20.38 |
| Metabolizable energy, kcal/kg | 3.000 | 3.000 | 2.948 | 2.948 |
| Calcium, % | 0.86 | 0.70 | 0.86 | 0.70 |
| Available phosphorus, % | 0.38 | 0.23 | 0.38 | 0.23 |
| Digestible lysine, % | 1.14 | 1.14 | 1.12 | 1.12 |
| Total lysine (%) | 1.24 | 1.24 | 1.22 | 1.22 |
| Digestible methionine + cystine (%) | 0.82 | 0.82 | 0.78 | 0.78 |
| Total methionine + cysteine, % | 0.90 | 0.90 | 0.86 | 0.86 |
| Digestible threonine (%) | 0.74 | 0.74 | 0.71 | 0.71 |
| Total threonine, % | 0.85 | 0.85 | 0.81 | 0.81 |
| Digestible arginine, % | 1.31 | 1.31 | 1.28 | 1.28 |

Supplement/kg feed: [1]Manganese 77.0 mg; iron - 55.0 mg; zinc – 71.5 mg; copper - 11.0 mg; cobalt- 1.0 mg; iodine- 1.1 mg; selenium- 0.33 mg.[2]Vitamin A - 8250 UI; Vitamin D3 - 2090 UI; Vitamin E - 31 UI; Vitamin B1 - 2.2 mg; Vitamin B6 – 3.08 mg; pantothenic acid - 11.0 mg; biotin- 0.077 mg; Vitamin K3 - 1.65 mg; folic acid - 0.770 mg; nicotinic acid - 33.0 mg; Vitamin B12 – 0.013 mg.

the digesta, the proventriculus, gizzard, duodenum (from the caudal end of the gizzard until the end of the duodenal loop), jejunum (from the end of the duodenum until Meckel's diverticulum), ileum (from Meckel's diverticulum until the ileal-cecal junction), and the ceca were isolated to prevent the digesta from mixing. Then, the pH was measured sing a digital pHmeter gently introducing the sterilized fine tip of the glass electrode in each GIT section. As recommended by Pang & Applegate (2007), pH was measured twice in each section and the average of the two measurements was used for the statistical analysis. Subsequently, the intestine, gizzard, liver, heart, bursa, and spleen were removed. The intestine was measured using a measuring tape.

The left tibia bone was collected, dehydrated in ethanol, and de-fatted in petroleum ether in Soxhlet apparatus for four hours. Bones were then burnt in a muffle at 600ºC to determine ash content, according to Gardiner *et al.* (1961). Ashes were then used to prepare a mineral solution by dissolving the ashes by digestion on a heated plate for four hours at 200ºC. Calcium content in the ashes was determined by atomic absorption spectrophotometry and phosphorus content was determined by colorimetric spectrophotometry, according to the methods described by Detmann.

Data were submitted to Shapiro Wilk's normality test and then to analysis of variance using the General Linear Model (PROC GLM) procedures of SAS statistical package (1998). Means were compared by orthogonal contrasts. Each contrast was independently tested by the F test a 10% probability level.

The following orthogonal contrasts were analyzed: contrast $C_1$, comparing diets with reduced calcium (Ca) and phosphorus (P) levels supplemented or not with phytase; contrast $C_2$, comparing diets with reduced amino acid (AA) and energy (ME) contents supplemented or not with phytase; contrast $C_3$, comparing diets with reduced AA, ME, Ca and P levels supplemented or not with phytase; contrast $C_4$, comparing the positive-control diet and the diet with reduced AA, ME, Ca and P levels and not supplemented with phytase; contrast $C_5$, comparing the positive-control diet and the diet with reduced AA, ME, Ca and P levels and supplemented with phytase, and contrast $C_6$, comparing the diets with reduced nutrient levels supplemented or not with phytase.

## RESULTS

Contrast $C_1$ shows that the performance of broilers fed the diet with reduced Ca and P levels and supplemented with phytase was better ($P<0.01$) than those fed the same diet with no phytase supplementation, with improvements of 4.40, 11.04, and 7.14% in feed intake (FI), weight gain (WG), and feed conversion ratio (FCR), respectively (Table 3).

**Table 3 –** Performance of broilers fed starter diets (8-21 days of age) with reduced nutrient and energy levels and supplemented or not with phytase.

| Parameter | Treatment with reduced levels | | | | | | | (CV[5] (%) | Orthogonal contrasts[6] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CP[1] | Ca and P[2] | | AA and ME[3] | | Ca, P, AA, and ME[4] | | | | | | | | |
| | | W/o phytase | With phytase | W/o phytase | With phytase | W/o phytase | With phytase | | $C_1$ | $C_2$ | $C_3$ | $C_4$ | $C_5$ | $C_6$ |
| FI (g) | 1085 | 1064 | 1113 | 1100 | 1094 | 1064 | 1110 | 3.01 | * | NS | * | NS | NS | NS |
| WG (g) | 782 | 725 | 815 | 765 | 763 | 684 | 741 | 4.18 | * | NS | * | * | NS | * |
| FCR (g/g) | 1.390 | 1.471 | 1.366 | 1.439 | 1.437 | 1.559 | 1.503 | 4.46 | * | NS | *** | * | NS | * |

[1]Positive control = 3,000 kcal/kg ME, 20.8% CP, 0.38% P and 0.86% Ca; [2]Ca and P = 3,000 kcal/kg ME, 20.8% CP, 0.23% P and 0.69% Ca; [3] AA and ME = 2,948 kcal/kg ME, 20.38% CP, 0.38% P and 0.86% Ca; [4]Ca, P, AA and ME = 2,948 kcal/kg ME, 20.38% CP, 0.23% P and 0.69% Ca; [5] coefficient of variation; [6]orthogonal contrasts: $C_1$ = reduced Ca and P vs. reduced Ca and P + phytase; $C_2$ = reduced AA and ME vs. reduced AA and ME + phytase; $C_3$ = reduced Ca, P, AA and ME vs. reduced Ca, P, AA e ME + phytase; $C_4$ = positive control diet vs. treatments with no phytase; $C_5$ = positive control diet vs. treatments with phytase ; $C_6$ = treatment with phytase vs. treatment with no phytase compared by the F test (P<0.05). NS = not significant at 10% probability level; * p<0.01; **p<0.05; ***p<0.10.

The contrast comparing the supplementation or not of phytase of diets with reduced Ca, P, AA, and ME level (Contrast $C_3$) indicated that broilers receiving phytase presented better FI (p<0.01), WG (p<0.01), and FCR (p<0.10), which improved 4.14, 7.69, and 3.59%, respectively. Interestingly, the improvement in performance with phytase supplementation was more evident in broilers fed reduced Ca and P levels (contrasts $C_1$ and $C_3$).

When the positive-control diet was compared with the diets supplemented with phytase (contrast $C_5$), it was observed that the dietary reduction of nutrients and energy did not influence broiler performance (p>0.05). On the other hand, broilers fed the positive-control diet presented better FI and WG (p<0.01) compared with those fed the diet with reduced nutrient and energy levels and no phytase supplementation (contrast $C_4$).

When the diets with reduced nutrient and energy levels and supplemented or not with phytase were compared (contrast $C_6$), phytase supplementation improved WG and FCR (p<0.01).

The addition of phytase to the reduced AA and ME diet (contrast $C_2$) reduced the pH of the proventriculus (p<0.10) and of the gizzard (p<0.01) in 11.41 and 19.54%, respectively (Table 4).

The pH of the proventriculus (contrast $C_4$) and of the gizzard (contrast $C_6$) increased (p<0.10) when phytase was added to the diets.

There was no influence of treatments on gizzard relative weight (Table 5). However, when the phytase supplementation was evaluated in reduced Ca and P diets (contrast $C_1$), liver (p<0.10) and heart (P<0.01) relative weights increased in 9.06 and 13.33% in broilers fed the diet with no phytase addition.

**Table 4 –** Gastrointestinal tract pH of broilers fed starter diets (8-21 days of age) with reduced nutrient and energy levels and supplemented or not with phytase.

| Parameter | Treatment with reduced levels | | | | | | | (CV[5] (%) | Orthogonal contrasts[6] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CP[1] | Ca and P[2] | | AA and ME[3] | | Ca, P, AA, and 3[4] | | | | | | | | |
| | | W/o phytase | With phytase | W/o phytase | With phytase | W/o phytase | With phytase | | $C_1$ | $C_2$ | $C_3$ | $C_4$ | $C_5$ | $C_6$ |
| Proventriculus | 3.79 | 4.13 | 4.23 | 4.47 | 3.96 | 4.21 | 4.09 | 13.96 | NS | *** | NS | ** | NS | NS |
| Gizzard | 3.87 | 3.93 | 3.83 | 4.40 | 3.54 | 4.26 | 4.25 | 16.01 | NS | * | NS | NS | NS | *** |
| Duodenum | 5.68 | 5.69 | 5.47 | 5.78 | 5.66 | 5.36 | 5.45 | 9.10 | NS | NS | NS | NS | NS | NS |
| Jejunum | 5.92 | 6.10 | 5.78 | 6.03 | 6.04 | 6.10 | 5.89 | 7.13 | NS | NS | NS | NS | NS | NS |
| Ileum | 6.09 | 5.95 | 5.74 | 5.84 | 6.01 | 5.90 | 5.73 | 8.18 | NS | NS | NS | NS | NS | NS |
| Cecum | 5.94 | 5.56 | 5.56 | 6.18 | 6.06 | 6.09 | 5.66 | 9.28 | NS | NS | NS | NS | NS | NS |

[1]Positive control = 3,000 kcal/kg ME, 20.8% CP, 0.38% P and 0.86% Ca; 2Ca and P = 3,000 kcal/kg ME, 20.8% CP, 0.23% P and 0.69% Ca; 3 AA and ME = 2,948 kcal/kg ME, 20.38% CP, 0.38% P and 0.86% Ca; 4Ca, P, AA and ME = 2,948 kcal/kg ME, 20.38% CP, 0.23% P and 0.69% Ca; 5 coefficient of variation; [6]orthogonal contrasts: $C_1$ = reduced Ca and P vs. reduced Ca and P + phytase; $C_2$ = reduced AA and ME vs. reduced AA and ME + phytase; $C_3$ = reduced Ca, P, AA and ME vs. reduced Ca, P, AA e ME + phytase; $C_4$ = positive control diet vs. treatments with no phytase; C5 = positive control diet vs. treatments with phytase ; $C_6$ = treatment with phytase vs. treatment with no phytase compared by the F test (p<0.05). NS = not significant at 10% probability level; * p<0.01; **p<0.05; ***p<0.10.

**Table 5 –** Organ biometrics of broilers fed starter diets (8-21 days of age) with reduced nutrient and energy levels and supplemented or not with phytase.

| Parameter | Treatment with reduced levels | | | | | | | (CV[5] (%) | Orthogonal contrasts[6] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CP[1] | Ca and P[2] | | AA and ME[3] | | Ca, P, AA, and ME[4] | | | | | | | | |
| | | W/o phytase | With phytase | W/o phytase | With phytase | W/o phytase | With phytase | | $C_1$ | $C_2$ | $C_3$ | $C_4$ | $C_5$ | $C_6$ |
| Gizzard (%) | 1.44 | 1.52 | 1.49 | 1.47 | 1.43 | 1.51 | 1.47 | 9.80 | NS | NS | NS | NS | NS | NS |
| Liver (%) | 2.78 | 2.98 | 2.71 | 2.68 | 2.67 | 2.83 | 2.76 | 9.87 | ** | NS | NS | NS | NS | NS |
| Heart (%) | 0.62 | 0.75 | 0.65 | 0.66 | 0.61 | 0.67 | 0.63 | 8.78 | * | NS | NS | * | NS | * |
| Length Small intestine (m) | 1.40 | 1.35 | 1.32 | 1.41 | 1.37 | 1.36 | 1.30 | 5.98 | NS | NS | NS | NS | *** | NS |

[1]Positive control = 3,000 kcal/kg ME, 20.8% CP, 0.38% P and 0.86% Ca; 2Ca and P = 3,000 kcal/kg ME, 20.8% CP, 0.23% P and 0.69% Ca; 3 AA and ME = 2,948 kcal/kg ME, 20.38% CP, 0.38% P and 0.86% Ca; 4Ca, P, AA and ME = 2,948 kcal/kg ME, 20.38% CP, 0.23% P and 0.69% Ca; 5 coefficient of variation; [6]orthogonal contrasts: $C_1$ = reduced Ca and P vs. reduced Ca and P + phytase; $C_2$ = reduced AA and ME vs. reduced AA and ME + phytase; $C_3$ = reduced Ca, P, AA and ME vs. reduced Ca, P, AA e ME + phytase; $C_4$ = positive control diet vs. treatments with no phytase; C5 = positive control diet vs. treatments with phytase ; $C_6$ = treatment with phytase vs. treatment with no phytase compared by the F test (p<0.05). NS = not significant at 10% probability level; * p<0.01; **p<0.05; ***p<0.10.

Heart relative weight increased (p<0.01) when broilers were fed the reduced nutrient diets with no phytase additions (contrasts $C_4$ and $C_6$).

Intestinal length was different (p<0.10) in broiler fed diets containing phytase, as shown in contrast $C_5$.

Tibial ash content (p<0.01, % DM) and phosphorus deposition (p<0.10; % DM) increased 9.53 and 13.56%, respectively, when broilers were fed the diet with reduced Ca and P levels supplemented with phytase (contrast $C_1$; Table 6).

Tibial ash content (%DM) was significantly different (p<0.05), as shown by contrast $C_3$. Broilers were fed the reduced nutrient diets containing phytase than those fed the positive-control diet (contrasts $C_4$ and $C_5$) presented higher tibial ash content (% DM). In addition, when the positive-control diet was compared with the reduced Ca and P diet containing phytase, there was higher Ca and P deposition (% DM), with 5.32 and 13.77%, respectively. Contrast $C_5$ did not reveal any differences (p>0.05) in tibial Ca or P content (% DM).

As determined by contrast $C_6$, tibial Ca and P deposition (% DM) and Ca to P ratio were higher (p<0.05) when the diets were supplemented with phytase.

Blood albumin levels (g/dL) were higher (p<0.10) in birds in the treatments with no phytase supplementation, as shown by contrasts $C_1$ and $C_2$ (Table 7).

Blood P levels (mg/dL) were different (p<0.10) as determined by contrasts $C_3$ and $C_4$, as well as Ca/P ratio detected by $C_1$ (p<0.01), $C_3$ (p<0.01) and $C_4$ (p<0.05).

**Table 6 –** Bone mineral content of broilers fed starter diets (8-21 days of age) with reduced nutrient and energy levels and supplemented or not with phytase.

| Parameter | Treatment with reduced levels | | | | | | | (CV[5] (%) | Orthogonal contrasts[6] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CP[1] | Ca and P[2] | | AA and ME[3] | | Ca, P, AA, and ME[4] | | | | | | | | |
| | | W/o phytase | With phytase | W/o phytase | With phytase | W/o phytase | With phytase | | $C_1$ | $C_2$ | $C_3$ | $C_4$ | $C_5$ | $C_6$ |
| Dry matter (%) | 94.33 | 93.54 | 93.97 | 91.98 | 92.60 | 92.36 | 92.18 | 1.56 | ** | NS | NS | * | ** | NS |
| Ashes (% DM) | 51.22 | 46.48 | 50.92 | 44.94 | 46.87 | 46.96 | 49.85 | 4.86 | * | NS | * | * | * | NS |
| P (% DM) | 12.02 | 12.25 | 13.94 | 10.83 | 10.80 | 10.69 | 10.44 | 5.68 | *** | NS | NS | NS | NS | * |
| Ca (% DM) | 26.66 | 27.31 | 28.16 | 26.94 | 26.94 | 25.48 | 26.21 | 4.78 | NS | NS | NS | NS | NS | * |
| Ca/P (DM) | 2.22 | 2.23 | 2.02 | 2.49 | 2.49 | 2.38 | 2.51 | 7.10 | NS | NS | NS | NS | NS | ** |

[1]Positive control = 3,000 kcal/kg ME, 20.8% CP, 0.38% P and 0.86% Ca; 2Ca and P = 3,000 kcal/kg ME, 20.8% CP, 0.23% P and 0.69% Ca; 3 AA and ME = 2,948 kcal/kg ME, 20.38% CP, 0.38% P and 0.86% Ca; 4Ca, P, AA and ME = 2,948 kcal/kg ME, 20.38% CP, 0.23% P and 0.69% Ca; 5 coefficient of variation; [6]orthogonal contrasts: $C_1$ = reduced Ca and P vs. reduced Ca and P + phytase; $C_2$ = reduced AA and ME vs. reduced AA and ME + phytase; $C_3$ = reduced Ca, P, AA and ME vs. reduced Ca, P, AA e ME + phytase; $C_4$ = positive control diet vs. treatments with no phytase; C5 = positive control diet vs. treatments with phytase ; $C_6$ = treatment with phytase vs. treatment with no phytase compared by the F test (p<0.05). NS = not significant at 10% probability level; * p<0.01; **p<0.05; ***p<0.10.

**Table 7 –** Blood biochemistry of broilers fed starter diets (8-21 days of age) with reduced nutrient and energy levels and supplemented or not with phytase.

| Parameter | Treatment with reduced levels | | | | | | | (CV[5] (%) | Orthogonal contrasts[6] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CP[1] | Ca and P[2] | | AA and ME[3] | | Ca, P, AA, and ME[4] | | | | | | | | |
| | | W/o phytase | With phytase | W/o phytase | With phytase | W/o phytase | With phytase | | | | | | | |
| | | | | | | | | | $C_1$ | $C_2$ | $C_3$ | $C_4$ | $C_5$ | $C_6$ |
| Total proteins (g/dL) | 3.07 | 3.08 | 2.93 | 2.94 | 3.10 | 3.11 | 2.88 | 13.45 | NS | NS | NS | NS | NS | NS |
| Albumin (g/DL) | 1.58 | 1.64 | 1.48 | 1.54 | 1.56 | 1.61 | 1.47 | 13.98 | ** | NS | *** | NS | NS | NS |
| Globulins (g/dL) | 1.49 | 1.43 | 1.45 | 1.40 | 1.53 | 1.50 | 1.41 | 17.39 | NS | NS | NS | NS | NS | NS |
| Alb/glob | 1.09 | 1.20 | 1.05 | 1.12 | 1.02 | 1.09 | 1.06 | 16.85 | ** | NS | NS | NS | NS | *** |
| Ca (mg/dL) | 9.23 | 9.50 | 8.60 | 7.41 | 7.71 | 8.68 | 9.01 | 30.93 | NS | NS | NS | NS | NS | NS |
| P (mg/dL) | 7.84 | 6.96 | 7.45 | 7.03 | 7.04 | 6.21 | 7.50 | 27.32 | NS | NS | *** | *** | NS | NS |
| Ca/P ratio (%) | 1.18 | 1.41 | 1.13 | 1.05 | 1.10 | 1.42 | 1.20 | 16.11 | * | NS | * | ** | NS | NS |

[1]Positive control = 3,000 kcal/kg ME, 20.8% CP, 0.38% P and 0.86% Ca; 2Ca and P = 3,000 kcal/kg ME, 20.8% CP, 0.23% P and 0.69% Ca; 3 AA and ME = 2,948 kcal/kg ME, 20.38% CP, 0.38% P and 0.86% Ca; 4Ca, P, AA and ME = 2,948 kcal/kg ME, 20.38% CP, 0.23% P and 0.69% Ca; 5 coefficient of variation;

[6] Orthogonal contrasts: $C_1$ = reduced Ca and P vs. reduced Ca and P + phytase; $C_2$ = reduced AA and ME vs. reduced AA and ME + phytase; $C_3$ = reduced Ca, P, AA and ME vs. reduced Ca, P, AA e ME + phytase; $C_4$ = positive control diet vs. treatments with no phytase; C5 = positive control diet vs. treatments with phytase ; $C_6$ = treatment with phytase vs. treatment with no phytase compared by the F test ($p<0.05$). NS = not significant at 10% probability level; * $p<0.01$; **$p<0.05$; ***$p<0.10$.

## DISCUSSION

Literature studies show differences in the capacity of phytase of releasing P from phytate due to influence of dietary Ca content. The better performance of broilers fed reduced Ca and P levels and phytase obtained in the present study suggest that phytase promoted better Ca and P utilization. This is consistent with the results of Powell *et al.* (2011), who found that it phytase is used more efficiently when dietary Ca levels are reduced.

These results also confirm that the use of enzymes in corn- and soybean meal-based broiler diets promote better performance ($p<0.01$) by breaking down the antinutritional factors present in the cell wall of plants (Graham *et al.*, 2002), and hence, better nutrient absorption and animal performance (Costa Leite *et al.*, 2011; Powell *et al.*, 2011; Walk *et al.*, 2012).

On the other hand, Alvarenga *et al.* (2011) obtained lower broiler weight gain when the diets contained phytase. Also, in his review, Slominsk (2011) found little effect of phytase supplementation on performance and the utilization of energy and amino acid utilization in broilers. These differences may be related to the different source of phytase currently available in the market (Elkhalil *et al.*, 2007). The positive results obtained in the present study may be possibly attributed to a better efficiency phytase derived from *E. coli*.

Another possible cause of the improved responses may have been the lower dietary Ca levels. Inadequate dietary Ca levels may impair Ca, P, Zn, and Mg absorption. However, Ca reduction in phytase-supplemented diets must be proportional do total phosphorus (tP) reduction in order to maintain the required Ca:P ratio. According to Pereira *et al.* (2012), the inclusion of phytase in feed formulation matrices provides economic and environmental benefits in broiler production.

The lower pH values observed in the gizzard ($p<0.01$) and the proventriculus ($p<0.10$) of broilers fed the reduced nutrient and energy level and no phytase can be attributed to the attempt of birds to balance nutrient intake and energy utilization to supply their requirements.

Phytate is hydrolyzed mainly in the upper gastric tract (proventriculus and gizzard), where the pH favors the action of phytase and its substrate is more water soluble (Selle & Ravidran, 2007). According to Penz *et al.* (1993), GIT pH reduction enhances enzyme activity, thereby increasing dietary nutrient absorption and improving animal performance.

The observed increase in liver ($p<0.10$) and heart ($p<0.01$) relative weights in the broilers fed diets with reduced Ca and P levels and no phytase supplementation is probably due to low phosphorus supply. Heart hypertrophy (increase of heart muscle mass beyond the usual limit) may be caused by

hypophosphatemia due to low phosphorus supply (Rosário *et al.*, 2004; Temprano *et al.*, 2004). Hearts with hypertrophy usually present reduced contraction due to reduced myosin ATPase activity and lower cyclic adenosine monophosphate (cAMP), which may result from hypophosphatemia. This may lead to heart failure, reducing the systolic volume and increasing myocardial fiber diameter (Riedesel, 2006).

Low ATP production stimulates the synthesis of liver enzymes, increasing liver volume (Reis *et al.*, 1999). Oliveira *et al.* (2009) observed reduced liver and heart relative weights when broilers were fed diets with 70% reduction in non-phytic phosphorus level.

The increase in tibial ash ($p<0.01$, % DM) and phosphorus deposition ($p<0.10$; % DM) determined in broilers fed the diet with phytase addition reduced Ca and P levels and in ash content ($p<0.05$) in broilers fed the reduced Ca, P, AA, and ME diet with phytase supplementation was expected, because phytase increases the release of phosphorus and other minerals from the feedstuffs. According to Nelson & Walker (1964) and Pereira *et al.* (2012), bone ash content is the most efficient parameter to estimate the amount of phosphorus released by phytase in corn- and soybean meal-based diets.

Geraldo *et al.* (2006) found 1.88% reduction in tibial ash content for each 1% increase in dietary calcium levels. The dietary supplementation of phytase allows reducing dietary available phosphorus in 0.15% and Ca in 0.15% (Gomide *et al.*, 2011). Consistent with the present study, Schoulten *et al.* (2003) observed that reduced P diets supplemented with phytase allowed reducing Ca requirements to lower values than those present in nutritional tables, but recommended that dietary Ca reduction should be proportional to total P reduction, maintaining adequate Ca to P ratio, as also mentioned by Qian *et al.* (1997).

In the present experiment, average total protein levels in the blood ranged between 2.88 (reduced Ca, P, AA, and ME and no phytase) and 3.11mg/dL (reduced Ca, P, AA, and ME and with phytase) are below those considered normal (3.6 mg/dL). According to Viveiros *et al.* (2002), total protein level in the serum may be reduced in case of malnutrition or low intestinal absorption of nutrients, but the treatments applied in the present study did not cause any of these conditions.

Blood Ca level (mg/dL) was not influenced ($p>0.10$) by the applied treatments, possibly because Ca homeostasis in the extracellular fluid is maintained by the joint effects of the parathyroid hormone (PTH), calcitonin, and 1,25 di-hydroxy vitamin D ($1,25(OH)_2D_3$) in the intestines, kidneys, and bone. Those hormones regulate Ca flow between these organs and the extracellular fluid, resulting in less than 5% variation in Ca blood levels under normal circumstances, and only occurs when hormonal mechanisms fail, secondarily to reduced intestinal absorption (Macari *et al.*, 2002). In the present study, mean Ca serum values were lower than those obtained by Vieites *et al.* (2011), of 6.72 and 6.89mg/dL.

The higher albumin values ($p<0.10$) found in broilers fed reduced nutrient and energy levels and no phytase supplementation are considered normal (1.6-2.0 g/dL; Reece & Swenson, 2006).

Albumins are synthesized in the liver and are very abundant in blood plasma, where they play an essential role in the maintenance of the osmotic pressure (Junqueira & Carneiro, 2011). Differences in serum albumin levels, according to Macari *et al.* (2002) are probably due to acid-base imbalance (metabolic acidosis or alkalosis), because of the buffering action of these proteins that capture or donate $H^+$ ions by their carboxylic terminals (COOH or $COO^-$) attempting to reestablish normal blood pH. This was possibly the case in the present study, and this hypothesis is corroborated by the increase in liver relative weight,

Consistent with this result, the Ca/P ratios presented in contrasts C1, C3, and C4 show higher concentration of P relative to Ca, causing osmotic pressure imbalance (Reece & Swenson, 2006). This difference in the Ca/P ratio is mainly due to P variation (mg/dL), which was different in contrast C3 and C4 ($p<0.10$). According to Vargas Jr. *et al.* (2003), the Ca to P ratio must be constant; any imbalance may affect body functions.

The difference in the glob/alb ratios obtained in contrasts C1 and C6 may be explained by the reduction of this ratio in the treatments with phytase, as shown in the contrasts between treatments with or without phytase and Ca and P reduction (contrast C1). This was caused by variations in the albumin fraction, demonstrating the effect of Ca and P reduction on the metabolism of the liver, which is the only site of albumin synthesis.

The diet with reduced amino acid and energy levels and phytase addition reduced gizzard and proventriculus pH. Dietary Ca and P reduction increased relative liver and heart weights, as well as albumin blood levels. The bones of broilers fed phytase-supplemented diets presented higher ash content. The results of the present study suggest that broiler nutritional requirements need to be reevaluated when the diet is supplemented with phytase.

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