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ANIMAL SCIENCE

Phytate-phosphorus and phytase on performance, bone characteristics, tissue and serum mineral concentration on broilers

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Abstract: Male broiler chickens (384), Cobb 500, were housed in metabolic cages to assess the efficacy of phytase in diets with low and high phytate-phosphorus on the performance, bone physical characteristics, tissue and serum mineral deposits. Birds were distributed in four treatments with a 2 x 2 factorial arrangement in a completely randomized block design. Experimental diets based on maize-soybean meal were T1 – diet low phytate-phosphorus; T2 – diet low phytate-phosphorus and phytase (500 FTU/kg); T3 – diet high phytate-phosphorus; T4 – diet high phytate-phosphorus and phytase (500 FTU/kg). Feed intake, body weight, weight gain and feed conversion ratio were assessed. Two left tibias per experimental unit were analyzed for physical characteristics and mineral concentration; a section of skinless breast muscle and blood were collected to measure the concentration of calcium, phosphorus and sodium. Results showed interaction between bone stiffness and serum calcium. The inclusion of phytase in diets with low and high phytate-phosphorus did not alter performance, bone resistance and flexibility, mineral deposits in the tibia and breast muscle, but increased bone stiffness after 22 days of age. It also provided a higher serum calcium rate in broilers fed diets with low phytate-phosphorus up to 32 days of age.

Key words: Enzyme, mineral deposits, Phytate-phosphorus, wheat bran.

INTRODUCTION

Modern poultry industry is characterized by high production indexes and efficient feed conversion due to great progress in the science of nutrition requirements and contents provided to the animals (Classen & Bedford 2001). The authors underscore that improvement in nutrition should target the best utilization of feedstuffs even in low quality or poorly exploited ingredients due to low availability of nutrients or in feedstuffs with anti-nutritional factors.

The enzyme phytase has been largely studied and used in non-ruminant diets for increasing the bioavailability of phytate-phosphorus (PP) and other minerals and nutrients in low quality feed (Amerah et al. 2014, Liu et al. 2014). The degradation of PP in the digestive tract may occur either due to the phytase produced by microorganisms in the intestine or to natural phytase of raw material, even though the activity of these phytases is only slightly significant and less studied in poultry than in ruminants and swine (Angel et al. 2002, Selle et al. 2009a). Therefore, exogenous enzymes are being extensively studied and used in non-ruminant nutrition and industrial production of phytase from microbial or bacterial strains has been widely increased due to biotechnological progress.

The inclusion of phytase in diets coupled to greater phosphorus bioavailability increases bone quality due to its relevance in the production of broilers aiming at fast bone growth and body weight gain. Lesion problems during capture, transport and slaughter of broilers may become significant when poultry miss a good bone structure, leading to high indexes of carcass rejection and economic losses (Maschio & Raszl 2012, Muchon et al. 2019).

Another important factor for the use of phytase is the inclusion of alternative feedstuffs, such as wheat bran, rich in phytate, substrate for the enzyme´s activities (Wu et al. 2009). Although data on the effects of phytase in broilers´ diets with wheat bran are still scarce, many authors have been studying phytase addition in diets with different feedstuffs to non-ruminants, such as rice, millet, sorghum, canola and sunflower meals (Selle et al. 2010, Agudelo Trujillo et al. 2010, Montanhini Neto et al. 2013, Liu et al. 2015)

However, several authors show that the addition of phytase may increase the degradation of PP of wheat bran and thus its use at higher levels, making available higher proportions of phosphorus and other minerals such as calcium, zinc, copper and others, with a decrease in feed costs, excretion and environmental pollution (Pourreza & Classen 2001, Classen & Bedford 2001, Kerr et al. 2010).

Present data with regard to the bioavailability of phosphorus are qualitative and knowledge on the percentage of the mineral retained by the animal is greatly relevant in the efficient formulation of diets with minimum excretion of nutrients (Shastak & Rodehutscord 2013). In this way, the current trial evaluates the efficiency of phytase in several diets (low and high PP) for broilers on performance, bone traits, tissue and serum mineral deposition.

MATERIALS AND METHODS

All procedures in animal experimentation in the current trial were approved by the Committee for Ethics in Animal Experimentation (CEEA) of the Federal University of Pelotas, Pelotas City, Rio Grande do Sul State, Brazil (Protocol n. 6866).

Animals, installations and experimental management

The assay was conducted at the Brazilian Agricultural Research Corporation – Swine and Poultry in a metabolism room for 32 days. A total of 384 one-day-old Cobb 500 male broilers were distributed in metal metabolic cages with gutter feeder and nipple waterers. Birds had ad libitum access to feed and water. Environmental variables, such as temperature and relative air moisture were registered and adjusted daily, according to the recommendations of the genetic company's (Cobb-Vantress 2009).

Experimental design and diets

The broilers were distributed in four treatments in a 2 x 2 factorial arrangement (two levels of PP and with or without phytase) in a completely randomized block design, with eight replications per treatment and 12 broilers per cage (the experimental unit). The birds were individually weighed at the beginning of the experiment and distributed in blocks according to initial weight, ranging between 40.1 and 51.3 g. A total of eight blocks were used, equivalent to replications, and all the treatments were randomly distributed within each block. Diets had two different levels of PP, or rather, one had a low PP rate (corn and soybean meal) and other had a high PP level due to inclusion of wheat bran (Table I). Diets had similar levels of metabolizable energy, crude protein, calcium, available phosphorus, sodium and digestible amino acids, according to Rostagno et al. (2011). Although the inclusion of filler became necessary, diets with high fiber

Table I. Chemical and nutritional composition of experimental diets for broilers.

Ingredients (kg)	1-10d (Pre-Initial)	11-21d (Initial)				22-32d (Growth)			
	Basal diet	Low PP* without phytase	Low PP with phytase	High PP without phytase	High PP with phytase	Low PP without phytase	Low PP with phytase	High PP without phytase	High PP with phytase
Corn (7.5%)	50.69	63.92	63.92	48.69	48.69	72.42	72.42	52.91	52.91
Soybean meal (45%)	42.01	30.21	30.21	25.45	25.45	24.66	24.66	20.32	20.32
Wheat bran (14%)	0.00	0.00	0.00	20.00	20.00	0.00	0.00	20.00	20.00
Soybean oil	3.14	0.00	0.00	2.90	2.90	0.00	0.00	4.41	4.41
Filler	0.00	2.83	2.83	0.06	0.06	0.58	0.58	0.00	0.00
Bicalcium phosphate[1]	1.80	1.00	1.00	0.76	0.76	0.78	0.78	0.54	0.54
Limestone[2]	0.97	0.71	0.71	0.83	0.83	0.62	0.62	0.73	0.73
Iodate salt	0.53	0.44	0.44	0.56	0.56	0.35	0.35	0.34	0.34
DL-methionine	0.32	0.22	0.22	0.23	0.23	0.16	0.16	0.18	0.18
L-lysine	0.24	0.32	0.32	0.38	0.38	0.22	0.22	0.28	0.28
Vitamin mix[3]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Mineral mix[4]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
L-threonine	0.11	0.13	0.13	0.17	0.17	0.05	0.05	0.09	0.09
Phytase[5]	0.00	0.00	0.05	0.00	0.05	0.00	0.05	0.00	0.05
TOTAL	100	100	100	100	100	100	100	100	100
Nutritional composition									
Metabolizable energy (kcal/kg)	2950	2850	2850	2850	2850	3000	3000	3000	3000
Crude protein (%)	22.98	18.59	18.59	18.58	18.58	16.46	16.46	16.37	16.37
Crude fiber (%)	-	2.70	2.70	4.09	4.09	2.55	2.55	3.89	3.89
Ether extract (%)	-	2.84	2.84	5.78	5.78	3.06	3.06	7.34	7.34
Calcium (%)	0.920	0.601	0.601	0.600	0.600	0.500	0.500	0.500	0.500
Sodium (%)	0.220	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150
Lysine dig. (%)	-	1.100	1.100	1.100	1.100	0.890	0.890	0.900	0.900
Methionine dig. (%)	-	0.460	0.460	0.460	0.460	0.370	0.370	0.390	0.390
Available phosphorus (%)	0.470	0.300	0.300	0.300	0.300	0.249	0.249	0.250	0.250
Total phosphorus (%)	0.734	0.542	0.542	0.622	0.622	0.485	0.485	0.559	0.559
Calculated PP (%)[6]	-	0.242	0.242	0.322	0.322	0.236	0.236	0.309	0.309
Analyzed PP (%)[7]	-	0.225	0.225	0.335	0.335	0.250	0.250	0.327	0.327
Added phytase (FTU/kg)	-	0	500	0	500	0	500	0	500
Determined phytase (FTU/kg)[8, 9]	-	0	690	640	1315	135	725	330	1175

[*]PP – phytate-phosphorus; [1]Calcium: minimum 210g/kg, maximum 250g/kg; Phosphorus: 180g/kg; [2]Calcium: minimum 33%; [3]Guaranteed levels per kg of product: Vitamin A: 9000000.000 UI, Vitamin D3: 2500000.00 UI, Vitamin E: 20000.00 UI, Vitamin K3: 2500.00mg, Vitamin B1: 1500.00mg, Vitamin B2: 6000.00mg, Vitamin B6: 3000.00mg, Vitamin B12: 12000.00mcg, Pantothenic acid: 12g, Niacin: 25g, Folic acid: 800.00mg, Biotin: 60.00mg, Selenium: 250.00mg. [4] Guaranteed levels per kg of product: Copper: 20g, Iron: 100g, Manganese: 160g, Cobalt: 2000.00mg, Iodine: 2000.00mg, Zinc: 100g. [5]Natuphos®, 10000 FTU/g; [6]Calculated phytate-phosphorus = total phosphorus – available phosphorus;[7]Levels obtained according to *Enzyme Services and Consultancy* – AB Vista; [8]Phytase level (FTU/kg feed) according to certificate of Assay CBO – Laboratory Analyses; [9]Expected levels do not usually coincide with supplemented levels due to natural phytase in prime matter; it is supposed that it has no activity at gastrointestinal tract level.

and vegetable oil levels proved to be within physiologically acceptable levels and did not affect both animal performance and the assessed responses. The birds were fed diets in the meal form.

All the broilers received the same basal diet from the start up to the 10th day of age, according to Rodehutscord (2013). Then, the 11-day-old broilers received the experimental diets: T1 – diet with low rate of phytate-phosphorus; T2 – diet with low rate of phytate-phosphorus with phytase (500 FTU/kg); T3 – diet with high rate of phytate-phosphorus; T4 – diet with high rate of phytate-phosphorus with phytase (500 FTU/kg), as showed in Table I. The commercial phytase used was produced from *Aspergillus ficuum* (3-phytase (EC 3.1.3.8)).

Phytase level in each meal was analyzed according to ISO (2009) and the rate of PP was calculated following De Boever et al. (1994) for determining the percentage of PP based on the ratio of the molecular weight of the six phosphorus molecules and the molecular weight of the whole phytate molecule.

Performance

Performance was evaluated from 11 to 21 and from 22 to 32 days of age, when birds were weighed to obtain average body weight (BW) and weight gain (WG). Feed provided and leftovers were also weighed at each stage to determine the feed intake (FI) and feed conversion (FC), obtained through the FI/WG ratio.

Collection of samples

When broilers were 22 and 32 days old, two birds per experimental unit were separated at random, weighed and euthanized by cervical dislocation (CEBEA 2013). The left tibia, a section of the skinless breast muscle (*Pectoralis major*) and serum were collected for analysis.

Bone's physical characteristics

Bone resistance was assessed by a texture analyzer[1]. Bones were placed on two stands 40 mm apart. Rath et al. (1999) describes that bone strength at break is represented by the force value and is related to factors such as bone mineral size and composition; bone stiffness is the relationship between strength and distance (bone size); bone flexibility is the relationship between bone resistance and stiffness (resistance/stiffness).

Bone's mineral deposition

After physical analysis, bones were oven-dried at 105 °C (Zenebon et al. 2008) and placed in a muffle furnace at 600 °C for ash determination. Then, mineral material was calculated (CBAA 2009) and calcium (Ca) and phosphorus (P) were obtained according to AOAC (2006).

Breast muscle's mineral deposition

After thawing, the section of the skinless breast muscle (*Pectoralis major*) was cut in cubes, placed in an identified aluminum tray and dehydrated in a lyophilizer[2] for 48 hours for obtaining the lyophilized dry matter (DMLyo). The sample was then ground for determining Ca, P and Na, following AOAC (2006). Dry matter (DM) was obtained in a common oven at 105 °C (Zenebon et al, 2008) and the mineral matter (MM) was determined by burning samples in a muffle at 600 °C for 24 hours (CBAA 2009).

Blood's mineral concentration

Two broilers from each experimental unit were euthanized by cutting their jugular vein for the collection of blood and measure the blood´s

[1] TA XT Plus Texture Analyzer ©Texture Technologies Corporation) with 3-Point Bending Rig (HDP/3PB and HDP/90) and software Exponent (Stable Micro Systems).

[2] JJ Scientific LJI-030.

mineral levels. Analyses were done from the serum by blood centrifugation. Commercial kits Ebram®, Labtest® and CELM® were used to analyze Ca, P and sodium (Na), respectively, following manufacturer's instructions.

Statistical analysis

Data were submitted to analysis of variance and the averages compared through F test at 5% significance, with procedure *MIXED SAS*™ (2008). Fixed effects of diet and phytase, the interaction between factors and the block´s random effect were tested according to statistical model: $Y_{ijk}=\mu+B_i+D_j+F_k+DF_{jk}+\varepsilon_{ijk}$, in which Y_{ijk} = value observed in the i^{th} block of the j^{th} content of PP of the k^{th} level of phytase; μ = general mean; B_i = block effect; i = 1, 2,...,8; D_j = effect of PP content; j = high, low; F_k = effect of phytase level; k =0.500; DF_{jk} = effect of the interaction between PP content and phytase level; ε_{ijk} = random error associated to each observation.

RESULTS AND DISCUSSION

Table II shows the results for performance. No interaction was observed among the analyzed factors for any of the variables measured ($p>0.05$). However, when effects were taken separately, broilers fed diet with low PP from 11 – 21 days had higher FI ($p=0.0003$), BW, WG and better FCR in comparison to broilers fed diet with high PP rate ($p<0.0001$).

During the growing phase (22 to 32 days), broilers fed diet with high PP rate had a higher

Table II. Feed intake (FI), body weight (BW), weight gain (WG) and feed conversion ratio (FCR) of male Cobb 500 broilers, fed diets with low and high of phytate-phosphorus (PP), with and without phytase (mean ± standard error).

Period (days old)	Variables	Diet	Phytase		*P-value* for main effect and interaction		
		PP	0	500	Main effect: Diet	Main effect: Phytase	Interaction
11 to 21[1]	FI (g/ave)	Low	743.46± 6.51	745.94±11.19	0.0003	0.6644	0.3888
		High	724.25± 2.92	716.81± 5.15			
	BW (g/bird)	Low	816.02±16.20	802.66±13.38	<0.0001	0.5616	0.4527
		High	756.98± 8.44	758.71± 8.69			
	WG (g/bird)	Low	554.77±11.29	543.39±12.18	<0.0001	0.5483	0.4909
		High	495.26± 5.92	496.05± 6.16			
	FCR (g/g)	Low	1.343±0.020	1.375±0.013	<0.0001	0.6499	0.1360
		High	1.463±0.013	1.446±0.016			
22 to 32[2]	FI (g/bird)	Low	1395.17± 15.29	1390.27± 20.23	0.0319	0.9924	0.6266
		High	1413.45± 17.91	1418.55± 19.41			
	BW (g/bird)	Low	1659.12± 23.14	1633.17± 31.56	0.0771	0.3948	0.6572
		High	1613.69± 23.43	1605.45± 20.59			
	WG (g/bird)	Low	843.04±11.22	830.51±22.89	0.3221	0.4542	0.9323
		High	856.74±17.69	846.75±12.69			
	FCR (g/g)	Low	1.656±0.013	1.679±0.031	0.8992	0.3056	0.9807
		High	1.652±0.020	1.677±0.025			

[1] Means of eight replications with 12 broilers per experimental unit.
[2] Means of eight replications with 10 broilers per experimental unit.

FI when compared with broilers fed diet with low PP rate (p=0.0319). The diet had a high fiber content (3.89%) due to wheat bran which may have increased the broilers' feed intake.

The supplementation of phytase to the broilers' diet has shown positive effects on the animals' performance (Technical Information 2003). However, in the current study these results were not found, even when a feed with high PP rate was included (p<0.005).

Angel et al. (2002) present some reasons why phytase may not work in some cases. For example, a large reduction in the efficacy of phytase when the PP complexes are present, regardless of the source of phytase and the fact that PP complexes are soluble in the pH of the proventriculus and gizzard, but insoluble in the pH of the small intestine. Although the inclusion of phytase or wheat bran in current assay did not provide better results, performance data were within the expectations for the broilers' strain.

Liu et al. (2014) emphasize that the PP predominantly lies in aleurone layer, due to its fibrous texture, and phytase is restricted to access the substrate and hinders the degradation of PP. Moreover, Kleyn (2013) stresses that phytase affects PP's anti-nutritional qualities due to myo-inositol hexaphosphate (InsP6), which may have a positive effect on the broilers' performance even though they cannot be measured. This kind of results was founded by Zeller et al. (2015) which used supplementary phytase that resulted in 92% InsP6 hydrolysis. This lead to higher P net absorption, confirmed by higher BW gain of the broilers evaluated.

Significant interaction in bone stiffness (p=0.0437) may be observed in 22-day-old broilers (Table III). It was probably due to the addition of phytase in the diet with low PP which provided significant bone stiffness (p=0.0059). A significant difference in bone stiffness in 32-day-old broilers with regard to the main diet effect (p=0.0242) was observed. In fact, broilers fed a diet with high PP had stiffer bones.

Table III. Bone resistance, flexibility and stiffness of 22 and 32-day-old male Cobb 500 broilers, fed diets with low and high phytate-phosphorus (PP), with and without phytase (mean ± standard error).

Period	Variables[1]	Diet	Phytase		*P-value* for main effect and interaction		
		PP	0	500	Main effect		Interaction
					Diet	Phytase	
22-days old	Bone resistance (kgf)	Low	18.69± 0.91	19.89± 0.30	0.0014	0.5268	0.2802
		High	16.92± 0.88	16.60± 0.43			
	Bone flexibility (kgf.mm)	Low	32.42± 2.12	33.43± 1.37	0.0209	0.8444	0.4512
		High	29.35± 1.93	27.63± 1.59			
	Bone stiffness (N/mm)	Low	5.89± 0.33 ^{bA}	7.05± 0.13 ^{aA}	0.0059	0.3188	0.0437
		High	5.56± 0.32 ^{aA}	5.15± 0.55 ^{aB}			
32-days old	Bone resistance (kgf)	Low	29.67± 1.17	30.67± 1.05	0.9497	0.2795	0.8859
		High	29.46± 1.56	30.75± 1.08			
	Bone flexibility (kgf.mm)	Low	54.25± 3.68	51.75± 3.50	0.8347	0.7800	0.6556
		High	53.43± 5.00	54.00± 2.90			
	Bone stiffness (N/mm)	Low	7.64± 0.90	8.22± 1.07	0.0242	0.8368	0.3964
		High	9.44± 0.38	9.08± 0.61			

[1]Means of eight replications with two broilers per experimental unit.
^{ab}Means followed by different small letters on the same line differ by F-test (p<0.05).
^{AB}Means followed by different capital letters in the same column differ by F-test (p<0.05).

Specialized literature provides results on bone resistance and the tibia´s mineral composition, but stiffness and flexibility are different variables that determine bone quality (Catalá-Gregori et al. 2006, Fukayama et al. 2008, Oliveira et al. 2008, Selle et al. 2009b, Jiang et al. 2013, Olukosi & Fru-Nji 2014), in which stiffer the bone, the greater chance for it to break (Currey 2003). In this case, it can be affirmed that bones with more mineralization due to low PP and the presence of phytase are more susceptible to break.

Bone resistance (p=0.0014) and flexibility (p=0.0209) were each one different due to diet in 22-day-old broilers. Diets with low PP provided better results and thus greater bone quality.

In current analysis, phytase, as its main effect, did not improve the bone traits evaluated (p>0.05). However, Fukayama et al. (2008) tested diets with a greater amount of phytic acid and different phytase inclusion levels and reported that the inclusion of up to 750 FTU of phytase/kg in the diet improved bone resistance and thus bone mineralization.

No significant interaction between the factors evaluated for mineral deposition in broilers' bone was observed (p>0.05). However, the diet´s effect on mineral matter (p=0.0002) and phosphorus (p=0.0121) was significant in 22-day-old broilers (Table IV), in which broilers fed diet with low PP had a greater deposition of minerals.

According to Rath et al. (2000), high fiber and PP levels in the diets may affect mineral absorption, especially Ca and P, with more breakable bones. These factors may decrease when exogenous enzymes are included. Enzyme activity was not reported in current study since phytase was not efficient to provide greater mineral deposition (p>0.05). However, studies show that diets with low PP were rather more efficient, corroborating with Rutherfurd et al. (2012) and Amerah et al. (2014) who reported the efficiency of phytase to increase the availability of P in diets with corn and soybean meal, or rather, low rates of PP.

Table V shows results on mineral deposition in the breast muscle. Although there was no

Table IV. Mineral matter (MM), calcium (Ca), phosphorus (P) in the tibia of 22 and 32-day-old male Cobb 500 broilers fed diets with low and high of phytate-phosphorus (PP), with and without phytase (mean ± standard error).

Period	Variables[1]	Diet	Phytase		*P-value* for main effect and interaction		
					Main effect		Interaction
		PP	0	500	Diet	Phytase	
22-days old	MM (%)	Low	47.01± 0.62	47.42± 0.36	0.0002	0.4537	0.1182
		High	45.68± 0.28	44.55± 0.54			
	Ca (%)	Low	15.63± 0.32	15.93± 0.14	0.2030	0.6661	0.0979
		High	15.73± 0.17	15.22± 0.28			
	P (%)	Low	8.298±0.118	8.464±0.087	0.0121	0.8607	0.2076
		High	8.135±0.125	8.008±0.116			
32-days old	MM (%)	Low	42.36± 0.50	44.08± 0.59	0.0540	0.0552	0.4577
		High	41.56± 0.73	42.35± 0.63			
	Ca (%)	Low	15.18± 0.26	15.68± 0.23	0.0811	0.1942	0.6183
		High	14.82± 0.31	15.05± 0.28			
	P (%)	Low	7.991±0.154	8.074±0.202	0.1123	0.4077	0.7158
		High	7.636±0.152	7.849±0.188			

[1]Means of eight replications with two broilers per experimental unit.

significant interaction between diet and phytase (p>0.05), the diet differed in mineral matter (p=0.0378) and sodium (p=0.0243) in 32-day-old broilers, as its main effect. Broilers fed a diet with high PP had a higher concentration of mineral matter, except for the sodium variable that behaved in the opposite way.

Results for the variable mineral deposition in the breast muscle are inconsistent and cannot be compared. Although no research has been undertaken on the subject, Shastak & Rodehutscord (2013) underscore the importance of alternatives to determine quantitatively the availability of phosphorus and thus tissue evaluation becomes optional. Silva & Pascoal (2014) stated that the obtention of mineral amount in the animals' body is an excellent parameter, although there are several disadvantages in the method since sampling problems may provide a variation of the body's estimated mineral rate.

Table VI shows results of the serum levels of Ca, P and Na evaluated in 22 and 32-day-old broilers. There is an interaction between PP rate and phytase for serum Ca in 32-day-old broilers (p=0.0438). Broilers fed diets with low PP and phytase had higher Ca level in the blood (p=0.0008), and no difference was observed between with and without supplemented phytase in the diet with high PP rate (p>0.05). No significant difference was reported between diets lacking phytase was well (p>0.05).

Table V. Lyophilized dry matter (DMLyo), mineral matter (MM), calcium (Ca), phosphorus (P), sodium (Na) in the breast muscle of 22 and 32-day-old male Cobb 500 broilers fed diets with low and high phytate-phosphorus (PP), with and without phytase (mean ± standard error).

Period	Variables[1]	Diet	Phytase		*P-value* for main effect and interaction		
		PP	0	500	Main effect		Interaction
					Diet	Phytase	
22-days old	MSLyo (%)	Low	26.08± 0.07	25.86± 0.13	0.1039	0.2051	0.6307
		High	25.81± 0.13	25.71± 0.16			
	MM (%)	Low	6.100±0.148	6.106±0.174	0.5168	0.6753	0.6428
		High	6.266±0.181	6.134±0.082			
	Ca (%)	Low	0.013±0.002	0.013±0.001	0.2505	0.4907	0.9439
		High	0.012±0.001	0.011±0.000			
	P (%)	Low	1.113±0.015	1.120±0.011	0.7505	0.9174	0.6683
		High	1.123±0.013	1.118±0.011			
	Na (%)	Low	0.144±0.003	0.146±0.004	0.5714	0.9278	0.5506
		High	0.144±0.003	0.143±0.004			
32-days old	MSLyo (%)	Low	26.54± 0.04	26.09± 0.16	0.1077	0.1047	0.0664
		High	26.51± 0.13	26.54± 0.14			
	MM (%)	Low	6.058±0.140	5.794±0.182	0.0378	0.2958	0.3825
		High	6.236±0.096	6.212±0.169			
	Ca (%)	Low	0.011±0.000	0.011±0.000	0.8692	0.0836	0.5687
		High	0.011±0.000	0.012±0.001			
	P (%)	Low	1.075±0.012	1.079±0.006	0.7118	0.0801	0.1917
		High	1.058±0.010	1.089±0.008			
	Na (%)	Low	0.142±0.003	0.150±0.004	0.0243	0.1498	0.6008
		High	0.135±0.003	0.139±0.006			

[1]**Means of eight replications with two broilers per experimental unit.**

Ghasemi et al. (2006) reported similar results about serum's mineral and showed that the supplementation of phytase in broilers' diet released complexed P and Ca in PP and enhanced the availability of Ca that should be absorbed by the broilers. They also emphasized that broilers had the best response due to the adjustment of non-phytic phosphorus (nPP) in the diet.

Diets with low PP and phytase in current analysis had a greater Ca concentration in the serum, with the use of Ca by the tissues that required it, as results of current study revealed when bone and breast muscle were analyzed. Knowing the negative effect of Ca on PP digestibility combined with increased use of efficient exogenous phytases, some authors suggest decreasing Ca addition in poultry diets (Selle et al. 2009a, Proszkowiec-Weglarz & Angel 2013).

Cromwell (1999) underscores that due to homeostatic mechanisms that control serum Ca and P, the concentration of the minerals in the blood does not indicate diet adjustment. Therefore, percentage of ash in the bone, weight of ash in the bones and resistance to breakage are more sensitive indexes than serum concentrations, since the broilers demand higher amounts of Ca and P due to bone formation.

CONCLUSION

The inclusion of phytase in diets with low and high PP failed to improve performance, bone resistance and flexibility, mineral deposits in the tibia and breast muscle, but increased bone stiffness after 22 days of age. It also provided a higher serum calcium rate in broilers fed low PP up to 32 days of age.

Table VI. Calcium (Ca), phosphorus (P) and sodium (Na) of blood serum of 22 and 32-day old male Cobb 500 broilers fed diets with low and high of phytate-phosphorus (PP), with and without phytase (mean ± standard error).

Period	Variables[1]	Diet	Phytase		*P-value* for main effect and interaction		
					Main effect		Interaction
		PP	0	500	Diet	Phytase	
22-days old	Ca (mg/dL)	Low	9.13± 0.32	8.83± 0.32	0.7848	0.2567	0.8300
		High	9.29± 0.34	8.85± 0.28			
	P (mg/dL)	Low	9.13± 0.33	9.08± 0.18	0.2309	0.9236	0.7530
		High	9.34± 0.10	9.43± 0.23			
	Na (mEq/L)	Low	137.88± 0.68	137.44± 0.59	0.8332	0.8332	0.4269
		High	137.13± 1.59	137.88± 0.59			
32-days old	Ca (mg/dL)	Low	9.60± 0.09 bA	aA	0.0008	0.1225	0.0438
		High	9.48± 0.10 aA	9.44± 0.06 aB			
	P (mg/dL)	Low	6.79± 0.18	7.16± 0.15	0.8504	0.5231	0.1235
		High	7.08± 0.21	6.93± 0.09			
	Na (mEq/L)	Low	139.00± 0.37	137.69± 0.49	0.1062	0.0825	0.3226
		High	139.31± 0.65	138.94± 0.65			

[1]Means of eight replications with two broilers per experimental unit.
[ab]Means followed by different small letters in the same row differ by F-test ($p<0.05$).
[AB]Means followed by different capital letters in the same column differ by F-test ($p<0.05$).

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