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Different phytase levels and energy densities in broiler diets on performance, nutrient digestibility, and bone integrity from 28 to 35 days of age

[*Diferentes níveis de fitase e de densidades energéticas em dietas de frangos de corte sobre desempenho, digestibilidade de nutrientes e integridade óssea de 28 a 35 dias de idade*]

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ABSTRACT

The study was carried out with the objective of evaluating the effects of using phytase levels at different energy densities in the diet of broilers, from 28 to 35 days of age. The experimental diets contained increasing levels of apparent metabolizable energy corrected for nitrogen balance and different levels of phytase. Growth performance variables, nutrient digestibility, as well as variables related to bone integrity were examined. Diets containing 500 FTU/kg in combination with 3150kcal.kg^{-1} of AMEn resulted in better growth performance. Supplementation with 500 and 1000 FTU/kg in the diets provide better coefficients of apparent and ileal digestibility of calcium and phosphorus, but negatively influence the deposition of calcium, phosphorus, dry matter, and ash in the tibia, in addition to adversely affecting the breakage and area of these bones in broilers. Bone length and the levels of magnesium and zinc present in the tibias were not influenced by the energy and phytase levels of the diets. There is no interaction between the different energetic densities and the phytase doses in the variables analyzed in the studied period. Increasing the energy density of diets resulted in improved apparent and ileal digestibility for most nutrients.

Keywords: additives, enzymes, phytate, phosphorus, tibia

RESUMO

O estudo foi realizado com o objetivo de avaliar os efeitos da utilização de níveis de fitase em diferentes densidades energéticas na dieta de frangos de corte, no período de 28 a 35 dias de idade. As dietas experimentais contiveram níveis crescentes de energia metabolizável aparente corrigida para balanço de nitrogênio e diferentes níveis de fitase. Foram avaliadas variáveis de desempenho zootécnico, digestibilidade de nutrientes e variáveis relacionadas à integridade óssea. Dietas contendo 500 FTU/kg de fitase em combinação com 3150kcal.kg^{-1} de EMAn resultam em melhor desempenho zootécnico. A suplementação com 500 e 1000 FTU/kg nas dietas propicia melhores coeficientes de digestibilidade aparente e ileal do cálcio e do fósforo, mas influencia negativamente na deposição de cálcio, fósforo, matéria seca e cinzas das tíbias, além de afetar adversamente a quebra e a área desses ossos em frangos de corte. O comprimento do osso e os níveis de magnésio e zinco presentes nas tíbias não são influenciados pelos níveis energéticos e de fitase das dietas. Não há interação entre as diferentes densidades energéticas e as doses de fitase nas variáveis analisadas no período estudado. O aumento da densidade energética das dietas resulta em melhora na digestibilidade aparente e ileal para a maioria dos nutrientes.

Palavras-chave: aditivos, enzimas, fitato, fósforo, tíbia

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INTRODUCTION

The success of modern industrial poultry farming stems from the development and improvement of genetic improvement, ambience, new diet formulations - mainly based on the context of the optimization of its ingredients - and the use of additives. Feeds for broilers are formulated almost exclusively with ingredients of plant origin, which mostly contain anti-nutritional compounds, as these animals do not produce enzymes capable of digesting these compounds in their digestive systems. From the nutrition point of view, the technical feasibility of exogenous enzymes can be seen as an important milestone, as it allows better use of nutrients. Among these nutrients, minerals stand out, since they are involved in almost all metabolic pathways of the animal organism, exerting vital physiological functions. Among the minerals required by broilers, phosphorus and calcium are quantitatively the most important, not only because they are necessary for an optimal growth rate with a consequent reduction in slaughter age, but also for bone mineralization. Thus, a deficiency or excess of these minerals in the diet would make it impossible to express maximum animal performance.

Phytate (Inositol Hexa-Phosphate), found in cereal grains and oilseeds used in diets, is one of the main anti-nutritional factors that complexes with phosphorus, making it unavailable to the animal. A potential chelating power with proteins is also attributed to phytate, in addition to metabolizable energy and other minerals contained in food. However, this phytic phosphorus will only be available to the animal in the presence of the enzyme phytase, but the production of endogenous phytase by birds is almost null and, therefore, exogenous phytase administered via feed has been used as a source of phosphorus supplementation. Nutritionally, the use of exogenous enzymes, such as phytase, for example, allows nutrients to be better used by the animal, so there would be an increase in the use of phosphorus, amino acids and energy, reducing the final cost of feed formulation (Tejedor *et al*., 2001), in addition to a reduction in environmental pollution, due to the lower excretion of phosphorus and other minerals not used by these birds (Donato *et al*., 2011).

In this way, exogenous phytase sources have been successfully used by the industry. In recent years, the concept of overdose, which is the use of higher doses of phytase (above 500 FTU/kg), has gained increasing attention and interest, not only because of the greater release of P, but also because it leaves less residual phytate and myo-inositol generation with lipotropic effects (Hamdi *et al*., 2018). This method of using phytase does not follow the application of a dose dependent matrix and basically focuses on optimizing animal performance. The current recommended phytase dosage for most non-ruminant animals is approximately 500 FTU/kg. However, benefits in terms of performance, bone strength, fat digestibility and antioxidant status were found with diets containing higher levels of phytase (1000 to 2000 FTU/kg). In addition, numerous studies seek to establish more adequate nutritional values for each stage of poultry creation.

Therefore, the work was carried out if the aim of to evaluate the effects of using phytase levels in different energy densities of the diet, measuring the reflexes on bird performance, nutrient digestibility and bone integrity specifically in the period from 28 to 35 days of age, period this is considered strategic because it is the one with high feed consumption and due to the scarcity of studies on the subject at this stage of production.

MATERIAL AND METHODS

The work was carried out at Embrapa Suínos e Aves – National Research Center for Swine and Poultry, in Concórdia/Santa Catarina. In a metabolism room, 576 one-day-old male *Cobb500* broilers from a commercial hatchery located in the city of Concórdia/Santa Catarina were housed. Metal metabolic cages measuring 0.90 x 0.90 x 0.25 m (length x width x height) were used, equipped with a trough-type feeder and nipple-type drinker, with food and water being provided ad libitum. The environment had controlled temperature, relative air humidity and lighting, according to the strain manual, with measurements and records being performed daily (*datalogger*). The birds were immunized in egg against Gumboro, Marek and Avian Pox, and in the hatchery against Infectious Bronchitis. The project was filed under number 014/2015 and approved by the Ethics Committee for the Use of Animals of Embrapa Suínos e Aves.

Diets based on corn and soybean meal were formulated to meet the nutritional requirements of birds (broilers male - average performance) as recommended by Rostagno *et al*. (2011) **(**Table 1**)**. The feed was produced in the form of pellets. For the phase from 1 to 14 days, the diets were ground after pelleting and from 15 to 35 days, the diets were supplied in the form of whole pellets (4.75 mm).

Table 1. Composition of experimental diets

Ingredients	Experimental diets											
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Corn	57.148	57.148	57.148	54.893	54.893	54.893	52.639	52.639	52.639	50.384	50.384	50.384
Soybean Meal	37.554	37.554	37.554	37.903	37.903	37.903	38.251	38.251	38.251	38.600	38.600	38.600
Soybean Oil	0.898	0.898	0.898	2.808	2.808	2.808	4.717	4.717	4.717	6.627	6.627	6.627
Dicalcium phosphate	0.199	0.199	0.199	0.201	0.201	0.201	0.204	0.204	0.204	0.206	0.206	0.206
Limestone	0.808	0.808	0.808	0.805	0.805	0.805	0.802	0.802	0.802	0.800	0.800	0.800
Salt	0.479	0.479	0.479	0.480	0.480	0.480	0.480	0.480	0.480	0.481	0.481	0.481
Vitamin Premix[1]	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Mineral Premix[2]	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050
DL-Methionine	0.262	0.262	0.262	0.265	0.265	0.265	0.267	0.267	0.267	0.270	0.270	0.270
L-Lysine	0.195	0.195	0.195	0.188	0.188	0.188	0.182	0.182	0.182	0.175	0.175	0.175
L-Threonine	0.074	0.074	0.074	0.073	0.073	0.073	0.073	0.073	0.073	0.073	0.073	0.073
Anticoccidial	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020
Antioxidant	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Adsorbent	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200
Kaolin	1.500	1.495	1.490	1.500	1.495	1.490	1.500	1.495	1.490	1.500	1.495	1.490
Phytase[3]	0.000	0.005	0.010	0.000	0.005	0.010	0.000	0.005	0.010	0.000	0.005	0.010
Chromic Oxide	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[1]Warranty levels per kg of product: Vitamin A: 9000000,000 IU, Vitamin D3: 2500000.00 IU, Vitamin E: 20000.00 IU, 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.
[2]Warranty levels per kg of product: Copper: 20g, Iron: 100g, Manganese: 160g, Cobalt: 2000.00mg, Iodine: 2000.00mg, Zinc: 100g.
[3]Commercial enzyme, produced from the fermentation of *Aspergillus oryzae*.

The treatments consisted of diets with increasing levels of apparent metabolizable energy corrected for nitrogen balance - AMEn (2950, 3050, 3150 and 3250 kcal.kg-1) pelleted and containing different levels of phytase (without phytase, 500 and 1000 FTU/kg) **(**Table 2**)**. The experimental diets were administered only in the period between 28 and 35 days of age of the birds.

Table 2. Composition of treatments

	Treatments											
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
AMEn ($kcal.kg^{-1}$)	2950	2950	2950	3050	3050	3050	3150	3150	3150	3250	3250	3250
Phytase (FTU/kg)	0	500	1000	0	500	1000	0	500	1000	0	500	1000

The phytase enzyme was added on top in the diets (according to the treatments), in the form of powder, along with the other ingredients, during the feed mixing procedure. The phytase used was a commercial enzyme, produced from the fermentation of *Aspergillus oryzae*. In addition, chromic oxide was used as a fecal indicator to determine the digestibility coefficients, being mixed with the other ingredients at a concentration of 0.5%.

Variables related to performance, digestibility and bone integrity of the animals were analyzed in this study.

The zootechnical performance variables were evaluated during the experimental period (from 28 to 35 days of age) where the birds were weighed to obtain the Average Body Weight (BW) and Average Weight Gain (AWG). The feed provided and the leftovers were weighed daily during this period to determine the Average Feed Intake (AFI) and Feed Conversion (FC), from the AFI/AWG.

The evaluation of nutrient digestibility was performed through total apparent digestibility and ileal digestibility. For total apparent digestibility, excreta were collected from 28 to 35 days of age of the birds. This collection was performed once a day, in the morning, and each collection was packed in a properly identified plastic bag, relative to each experimental unit, weighed and later stored in a freezer. At the end of the entire period of collections, homogenization of the collections related to each experimental unit was carried out, for subsequent removal of an aliquot for drying (48h at 55ºC in a forced air circulation oven) and analysis. The determinations of gross energy in calorimetric bomb, dry matter (105ºC), calcium, phosphorus and nitrogen followed the methodologies of routine analysis of the Laboratory of Physical-Chemical Analysis following protocols proposed by the AOAC, (Official…, 2000). Based on these chemical analysis information, the apparent digestibility coefficients of these components were calculated. Seeking to meet the methodological proposal proposed by Rodehutscord (2013), at 35 days of age, ileal digesta was collected to determine the digestibility coefficient of nutrients. After slaughter, the ileum was exposed through an abdominal incision and its contents were collected in a properly identified plastic container and later, the digestas were frozen for dehydration in a lyophilizer for 48 hours. Then, they were ground to determine the contents of dry matter (Zenebon *et al*., 2008), calcium, phosphorus, and nitrogen (Official…, 2000) in the diets and in the ileal digesta. With the laboratory results, the ileal apparent digestibility coefficients (ADC) of DM and minerals were determined using the formulas: Ileal indigestibility factor (IF) = (Diet AIC) / (Ileal AIC). Subsequently: Nutrient digestibility (%) = % of the nutrient in the diet - (% of the nutrient in the ileal digesta x FI) / % of the nutrient in the diet.

Also, at 35 days of age, two birds per experimental unit were randomly separated, weighed, and sacrificed by cervical dislocation. From them, the two tibias were removed from which the bone integrity was determined through the following variables: breaking strength, length, area, ash content, dry matter, magnesium, zinc, calcium, and phosphorus. Break strength, length and area tests were conducted using the Texture Analyzer TAXT Plus Texture Analyzer ©Texture Technologies Corporation with 3-Point Bending Rig probe (HDP/3PB and HDP/90) and Exponent software (Stable Micro Systems). The bones were positioned under two supports with a space of 40 mm between them. The breaking strength is represented by the strength value and is related to factors such as: size and mineral composition of the bone. The relationship between strength and length (bone size) represents the stiffness of the bone. Measures of strength and stiffness are related to bone stress (fracture) and tension. Stress represents the resistance to deformation, while tension represents the percentage of deformation (Einhorn, 1996; Zioupos and Currey, 1998). After the physical analysis, the tibias were dried in ovens at 105ºC (Zenebon *et al*., 2008) and submitted to a muffle furnace to obtain ash, dry matter and to determine the content of magnesium, zinc, calcium and phosphorus in the bone, according to the AOAC methodology (Official…, 2000), being carried out at the institution's laboratory.

The experimental design used was randomized blocks, considering the weight of the birds at 28 days as the initial weight. The animals were distributed in 12 treatments, 8 replicates with 6 birds each corresponding to an experimental unit.

The statistical methodology used for the zootechnical performance variables was the Analysis of Variance, through the MIXED procedure of the SAS^{TM} statistical program (System…, 2012). Block fixed effects for AMEn, phytase and the AMEn×Phytase interaction were tested. The comparison between the means was performed using the Student's t test, protected by the global F test ($p \leq 0.05$). For the variables of digestibility and bone integrity, Analysis of Variance was used, using the “R” statistical package (R Core Team, 2015). The “LSM-least squares means” procedure was used to compare the treatments, and the means were compared using the Tukey test. Block fixed effects for AMEn, phytase and AMEn×phytase interaction were tested. A probability value of P<0.05 was described as statistically significant.

CASUISTRY

The set of findings that constitutes and justifies the originality of the casuistry of the present work is in the fact that the period of realization took place during the high consumption of birds (28-35 days), with great impact on the industry and with scarcity of data in the current bibliography. In addition, the results found demonstrate that there were responses that are not commonly found in the literature, mainly on the effects of high doses of phytase on the tibia of broilers, making the work relevant for the development of research on the subject.

RESULTS AND DISCUSSION

It is possible to observe that there was a statistical difference for the variables of average body weight, average feed intake, average weight gain and feed conversion **(**Table 3**)**. The average body weight variable was affected both by the energy level of the diet and by the absence of phytase, therefore, there was no interaction between energy and phytase. There was an effect of the phytase level only in the diets with the highest AMEn (3250 kcal.kg-1) with the best response for the level of 1000 FTU/kg. However, the best results for average weight gain in the period were observed in treatments with lower energy level (2950 kcal.kg-1) and higher level of phytase inclusion (1000 FTU/kg) and in treatments with energy level of 3150 kcal .kg-1 and 500 FTU/kg. These same treatments had the highest average feed intake in the studied period. It was noted that with a level of 500 FTU/kg there was a gradual improvement in feed conversion as the level of AMEn increased, and the best conversions were between 3150 and 3250 kcal.kg-1.

Table 3. Means, standard errors and descriptive levels of probability of the F test for the performance variables of broilers, receiving diets with increasing levels of AMEn and phytase, from 28 to 35 days

Variable	AMEn ($kcal.kg^{-1}$)	Fhytase (FTU/kg) 0	500	1000	Pr > F
ABW	2950	^{A}2409.58±10.97	2406.25±39.39	2458.33±32.97	0.5262
	3050	^{A}2421.25±35.94	2385.00±38.45	2400.83±39.00	0.7782
	3150	^{AB}2361.50±33.95	2424.58±17.71	2332.08±31.63	0.1893
	3250	^{B}2287.71±47.02^{b}	2311.25±54.13^{b}	2413.92±28.68^{a}	0.0374
	Pr > F	0.0454	0.1395	0.1094	
AFI	2950	^{A}1136.98±24.24^{b}	^{A}1199.75±18.5^{ab}	^{A}1270.54±24.77^{a}	0.0067
	3050	^{A}1111.83±26.88	^{A}1130.09±40.19	^{B}1113.33±20.62	0.8844
	3150	^{B}1024.67±31.98^{b}	^{A}1141.46±19.75^{a}	^{B}1044.46±26.91^{b}	0.0121
	3250	^{B}1005.50±34.21	^{B}1018.46±43.63	^{B}1088.17±19.10	0.1006
	Pr > F	0.0033	0.0004	<0.0001	
AWG	2950	757.92±21.77^{b}	789.17±25.10^{b}	^{A}873.33±24.82^{a}	0.0167
	3050	786.67±26.61	759.58±34.96	^{B}765.83±16.78	0.7844
	3150	721.50±29.86^{b}	805.42±20.35^{a}	^{B}690.83±29.54^{b}	0.0175
	3250	693.13±33.47	731.67±44.95	^{B}755.17±11.74	0.3101
	Pr > F	0.1160	0.2843	0.0003	
FC	2950	1.503±0.022	^{A}1.527±0.034	1.458±0.016	0.2102
	3050	1.417±0.018	^{A}1.496±0.039	1.455±0.017	0.1428
	3150	1.425±0.021^{b}	^{B}1.420±0.019^{b}	1.521±0.031^{a}	0.0203
	3250	1.460±0.036	^{B}1.406±0.032	1.442±0.027	0.3762
	Pr > F	0.1246	0.0065	0.2043	

ABW= Average body weight at 35 days; AFI= average feed intake; AWG= average weight gain from 28 to 35 days; FC= feed conversion.

Means followed by distinct capital letters in the columns and lowercase letters in the lines differ significantly by the *Student's t* test (p≤0.05).

The difference found for the feed consumption variable in this study can be explained by the different energy densities of the diets, with AMEn having a regulatory effect on food consumption that ends up reflecting in the other variables studied. The growth promoting effect can be partially attributed to the increase in feed intake and better use of phosphorus and inositol. In addition, it is important to emphasize that due to the life span of the animals in the present study (28 to 35 days of age), it was not possible to observe more expressive and conclusive results for the performance variables. According to Sousa *et al.* (2015) in the first days of life, broilers show higher proportional indices of body growth, which may favor results.

In general, specialized literature has demonstrated the positive effect of the use of phytase on the performance of birds at levels ranging from 250 FTU/kg to 2000 FTU/kg of diet. Walk *et al.* (2013) hypothesized that improvements in feed efficiency are associated with the almost complete destruction of phytate and the removal of its anti-nutritional effects, promoting more efficient digestion. Sousa *et al.* (2015) conducting a study to determine the effect of phytase in the diet on performance found that the diet that was supplemented with phytase performed better, with broilers that were fed a diet supplemented with phytase showed 4.40, 11.04 and 7.14% improvement in feed intake, weight gain and feed conversion, respectively.

Other authors also showed benefits in the use of this enzyme. Shirley and Edwards (2003) comment that in diets for broilers, with different levels (0 to 1500 FTU/kg) of phytase there is a significant increase in the body weight of the birds. Sohail and Roland (1999) obtained results where phytase treatment increased the body weight of chickens at 21 days of age. Onyango *et al.* (2005) reported an increase in body weight gain with phytase supplementation at 500 and 1000 FTU/kg. As Cowieson *et al.* (2006) also found improvements in body weight gain in broilers.

Pirgozliev *et al.* (2011) describe an increase in feed intake as the phytase level increased from 250, 500 and 2500 FTU/kg, which in turn increased body weight gain. Shirley and Edwards (2003) reported that graded levels of phytase (0, 93.75, 187.5, 375, 750, 1500 FTU/kg) led to increased feed intake. Pirgozliev *et al.* (2011) also observed an improvement in the feed conversion of diets with phytase supplementation at 500 FTU/kg compared to the diet without phytase, corroborating the results found in this work.

On the other hand, however, some authors diverge from these realities. Chung *et al.* (2013) reported that there was no effect ($p>0.05$) on feed intake and weight gain of broilers fed diets supplemented with phytase (1500 and 3000 FTU/kg). Cowieson *et al.* (2011) when adding different levels of phytase also did not notice any effect on the performance of broilers.

According to Liu *et al.* (2014), phytate levels found in corn and soybean meal-based diets can have a negative effect on growth performance and efficiency. This is supported by studies demonstrating that phytate present in the diet reduces amino acid digestibility and endogenous enzymatic activity in broilers and interferes with mineral digestibility through chelation with cations (Cowieson *et al.*, 2006). However, phytase supplementation does not always improve the metabolizable energy of the diet, it usually improves feed intake and consequently the weight gain of broilers (Pirgozliev *et al.*, 2011).

Microbial phytase can consistently increase the energy metabolizable capacity of broiler diets, and as shown by Pirgozliev *et al.* (2011) promote an impact on the net energy content of the diet. The authors suggest that the positive effect of phytase on energy utilization stems from the accumulation of higher digestibility of protein, fat and starch. In addition, endogenous proteins, such as mucin, have their need for synthesis minimized (a process that requires energy).

In this way, the differentiation of the results found in the literature regarding the levels used of the phytase enzyme may occur due to numerous factors, among them: cultures of microorganisms used for the production of the enzyme; enzyme stability and activity on phytate; and concentration of phytic acid in the diet ingredients (Fukayama *et al.*, 2008; Laurentiz *et al.*, 2009).

Many studies have already reported that phytase enzyme supplementation in diets for broilers has positive effects on nutrient digestibility (Cowieson and Ravindran, 2007, Woyengo *et al*., 2010, Karimi *et al*., 2013). In general, in the present study, apparent and ileal digestibility showed very similar behaviors (Table 4). In the apparent digestibility, the diets with 500 and 1000 FTU/kg showed a higher coefficient of digestibility of calcium and phosphorus, without significantly affecting gross energy, dry matter and nitrogen. The ileal digestibility, in turn, showed that the supplementation of 1000 FTU/kg was the dose with the highest digestibility for calcium and phosphorus. However, the diet without enzyme supplementation showed the highest ileal digestibility for gross energy, dry matter and ash. Nitrogen was the only nutrient not affected by any phytase dose for the studied digestibility. On the other hand, the energy densities positively influenced both the apparent digestibility and the ileal digestibility of almost all nutrients, so that as the energy level of the diet gradually increases, the digestibility will be higher, highlighting the doses of 3150 and 3250 kcal. kg-1 as the best compared to the others. Interestingly, phosphorus is the exception, because, for ileal digestibility, energy densities were not able to positively influence the response, with no significant difference, despite a tendency to improve.

Table 4. Apparent and ileal digestibility coefficient of gross energy, dry matter, calcium, phosphorus, nitrogen and ash for broilers at 35 days of age fed diets containing different levels of phytase and energy density

	Apparent digestibility coefficient					Ileal digestibility coefficient				
		DM	Ca	P	N	GE	DM	Ca	P	N
FTU/kg										
0	62.2	53.5	26.5^{b}	35.1 ^{b}	32.2	64.54^{a}	60.58^{a}	41.4^{b}	46.6 ^{c}	55.0
500	62.4	52.4	37.7^{a}	41.0 ^{a}	32.8	64.19^{ab}	59.82^{b}	51.2^{a}	58.2 ^{b}	55.1
1000	62.3	52.2	38.1^{a}	43.5 ^{a}	30.4	63.93^{b}	59.60^{b}	51.0^{a}	60.4 ^{a}	55.0
EPM	0.551	0.673	1.28	1.41	1.07	0.1188	0.1143	0.996	0.503	0.212
P	NS	NS	<0.001	<0.001	NS	<0.01	<0.001	<0.001	<0.001	NS
kcal.kg^{1}										
2950	58.7^{b}	49.7^{b}	29.2^{b}	35.2^{b}	25.6^{b}	62.89^{c}	59.09^{c}	43.7^{b}	53.9	53.9^{c}
3050	62.7^{a}	53.2^{a}	31.5^{b}	39.5^{b}	32.3^{a}	64.18^{b}	60.03^{b}	47.4^{ab}	55.7	55.0^{b}
3150	64.0^{a}	54.2^{a}	38.3^{a}	45.6^{a}	35.5^{a}	64.52^{b}	60.18^{b}	50.3^{a}	55.7	55.2a^{b}
3250	63.9^{a}	53.7^{a}	37.5^{a}	39.1^{b}	33.8^{a}	65.27^{a}	60.71^{a}	50.1^{a}	54.9	56.0^{a}
EPM	0.636	0.777	1.48	1.63	1.24	0.1372	0.1319	1.15	0.581	0.245
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	NS	<0.001

GE= gross energy; DM= Dry Matter; Ca= Calcium; P= phosphorus; N= nitrogen.
Means followed by distinct lowercase letters in the columns differ significantly by the *tukey* test ($p \leq 0.05$)

Similarly, Ravindran *et al*. (2006) who worked with inclusions of 500 and 1000 FTU/kg in diets based on corn and soybean meal, obtained a linear improvement in P digestibility. Like, Olukosi *et al*. (2013) observed an increase in P digestibility in diets supplemented with phytase (500 to 1000 FTU/kg). On the other hand, Roberson and Edwards (1994) found that phytase supplementation in diets based on corn and soybean meal did not affect the retention of this mineral. According to Sebastian *et al*. (1998), the role of phytase on the availability of P and other nutrients is linked to factors such as: age of the animal, calcium and phosphorus levels in the feed, vitamin D3, sources of dietary ingredients and food processing.

Shirley and Edwards Jr. (2003) found that supplementation of 1200 FTU/kg of diet improved P retention by 157%. However, it is not a rule to admit that the higher the levels of the enzyme, the greater the availability of nutrients, since the interrelationship between the multivalent cations supplied in a diet can repress the action of phytase (Pointllart *et al*., 1984).

A possible explanation for the results found in this work is because with advancing age of the bird, the digestion process improves due to the maturation of the organs that make up the digestive system and the increase in the production of digestive enzymes such as lipase, amylase and proteases (Nitsan *et al*., 1991). Furthermore, it is known that the availability of minerals is highly sensitive to variations in the Ca:P ratio, therefore, the higher Ca:P ratios present in diets during the growth phase may explain the different coefficients of apparent and ileal digestibility of nutrients in this phase. creation phase.

Contrary to the data obtained, Gallardo *et al*. (2017), working with the inclusion of 500 FTU/kg, obtained better N digestibility compared to other treatments. Like Cowieson and Ravindran (2007) working with phytase, they obtained an increase in N digestibility. However, similarly to this study, Zhang (1999) evaluated corn and soybean meal-based diets supplemented with 600 FTU/kg of diet and found no significant differences in ileal digestibility in N levels in broilers at 42 days of age.

Ravindran *et al*. (2006) working with 0, 500, 750 and 1,000 FTU/kg, found positive results in the values of metabolizable energy (AME), indicating that the negative influence of phytic acid can be overcome by the fusion of phytase supplementation. The positive effects of phytase on the AME of corn and soybean diets were reported by Camden *et al*. (2001), who found an improvement of 35.82 kcal/kg DM in corn and soybean diets supplemented with 500 FTU/kg. Cowieson and Ravindran (2007) supplementing phytase reported the increase in energy use that was related to reduced energy flux and degradation of fibrous complexes.

Regarding the growth phase of the animals in this study, the inefficiency of supplementation on the digestibility of some of the analyzed nutrients can be attributed to the fact that there is a smaller effect of exogenous enzymes in birds at an advanced age (from 28 to 35 days), due to the physiological system being more improved in relation to young birds, requiring even higher doses of phytase to be applied so that the greatest effect of the enzyme can be expressed (Campbell and Bedford, 1992).

According to Sohail and Roland (1999), bone characteristics are the most sensitive parameters to evaluate the effect of phytase in detriment to performance characteristics.

In the present study, it was possible to observe that there was a significant difference (P<0.05) for the effects of different doses of phytase on the variables breaking strength and tibia area (Table 5), as well as for the levels of dry matter, ash, calcium, and phosphorus deposited in the bones of birds (Table 6). However, the treatments did not cause significant effects on the bone length variables, on the levels of magnesium and zinc present in the tibias. There was also no effect caused by different energy densities and interaction between energy densities and phytase levels in the variables analyzed (Tables 5 and 6).

Table 5. Breakage resistance, length and area of the tibiae of broilers fed diets containing different energy densities and phytase levels in the 28 to 35 day phase

Phytase Enzyme Effects (FTU/kg)			
0	33.41 ± 4.89[a]	4.85 ± 1.87	39359.38 ± 8397.25[a]
500	30.03 ± 5.50[b]	4.89 ± 2.18	33984.50 ± 8173.75[b]
1000	30.31 ± 4.01[b]	4.29 ± 1.47	35619.94 ± 7349.07[ab]
Linear	P=0.0285	P=0.377	P=0.0363
Effects of Metabolizable Energy (kcal.kg^{-1})			
2950	31.64 ± 4.25	4.63 ± 1.69	38272.43 ± 6772.68
3050	32.06 ± 5.15	4.59 ± 1.81	36089.68 ± 10070.51
3150	30.67 ± 4.63	4.19 ± 1.25	34092.19 ± 7323.28
3250	30.45 ± 5.99	5.26 ± 2.44	36566.04 ± 8160.24
Linear	NS	NS	NS
Quadratic	NS	NS	NS
P Value			
Phytase	P=0.0150	NS	P=0.0253
AMEn	NS	NS	NS
Phytase X AMEn	NS	NS	NS

Means followed by distinct lowercase letters in the columns differ significantly by the *tukey* test (p≤0.05)

Table 6. Levels of Dry Matter, Calcium, Magnesium, Phosphorus, Zinc and Ash in the tibia of broilers fed diets containing different energy densities and phytase levels in the 28 to 35 day phase

	Variables				
	DM (%)	Ca (mg/Kg)	Mg (mg/Kg)	P (mg/Kg)	Zn (mg/Kg)
	Effects of Phytase Enzyme (FTU/kg)				
0	50.07±1.55[a]	73873.27±3944.03[a]	1565.76±119.49	33676.30±1861.46[a]	63.26±5.48
500	48.79±2.50[b]	70631.66±4625.08[b]	1568.43±124.46	32327.75±1698.60[b]	61.53±6.041
1000	48.14±1.57[b]	69516.16±5188.15[b]	1525.25±147.73	31679.00±2392.66[b]	61.81±6.89
Linear	P=0.02	P<0.001	P=0.644	P=0.132	P=0.422
	Effects of Metabolizable Energy (kcal.kg-1)				
2950	48.24±1.53	71643.00±4511.79	1558.56±122.76	32704.61±1864.33	62.06±6.24
3050	48.99±1.30	72369.67±5213.37	1548.50±132.27	32908.17±2272.15	63.57±6.41
3150	49.02±2.18	71799.57±5348.02	1573.82±123.68	32794.52±1902.01	61.97±5.73
3250	48.63±2.81	69369.88±5348.02	1531.75±149.70	31759.54±2436.28	61.09±6.35
Linear	NS	NS	NS	NS	NS
Quadratic	NS	NS	NS	NS	NS
	P Value				
Phytase	<0.001	<0.001	NS	<0.001	NS
AMEn	NS	NS	NS	NS	NS
Phytase X AMEn	NS	NS	NS	NS	NS

DM= Dry Matter; Ca=Calcium; Mg= Magnesium; P= Phosphorus; Zn=Zinc
Means followed by distinct lowercase letters in the columns differ significantly by the *tukey* test (p≤0.05)

Differently from what is possible to verify in the great majority of the research on the subject, in the present work, the increasing levels of phytase in the diet had a negative effect in the mineral deposition (calcium and phosphorus), ash levels, dry matter and consequently in the indices of breaking strength and tibia area. In other words, the control diet, without the addition of phytase, presented a better level of bone mineralization when compared to the diets with phytase at the levels of 500 and 1000 FTU/kg of ration. There may be several reasons that explain these results, including the age of the birds used in the experiment, considering that over time the animals may have higher levels of endogenous phytase enzyme, in addition to greater adaptability of the gastrointestinal tract. This fact is suggested by Bedford and Partridge (2010) where they state that any enzyme will work better in young chicks that do not have a fully developed enzyme system. In the same line of reasoning Tiwari *et al*. (2010) reported the best response in young birds, as well as Bertechini (2012) clearly demonstrated the age relationship when the effectiveness of the phytase enzyme was significantly lower or null in broilers over 21 days of age. Simply put, enzymes tend to be more effective in young birds, as birds mature and overall digestibility improves, exogenous enzyme effectiveness decreases. Of course, any enzyme will work best when levels of endogenous enzymes are at their lowest, that is, in the young chick. Subsequently, the effectiveness of the enzyme can be expected to decrease as the gastrointestinal tract develops (Kleyn, 2013).

Although tibia bone mineralization was lower in diets with phytase supplementation, this does not mean that the enzyme did not act in the diets, as the release of phosphorus present in phytate probably caused an imbalance in the calcium:phosphorus ratio, with a compensatory reaction, mobilizing calcium and phosphorus from bones to ensure homeostasis. Possibly the justification for explaining the data found is due to the catalytic activity of phytase, which can be inhibited by its own final product, inorganic phosphorus, indicating that increasing dietary concentrations of available phosphorus can result in a decrease in phytate hydrolysis, by phytase (Kleyn, 2013). Fukayama *et al*. (2008) also reported negative effects of the addition of phytase on the phosphorus deposition in the tibias, evaluating the phosphorus deposition in the tibia of broilers at 21 days, found that the addition of 1000 FTU/kg had a negative influence on the concentration of this mineral, being that at this level the amount deposited in the bone was significantly lower than that of birds fed a diet without the addition of phytase.

Microbial phytase induces the release of phosphorus and calcium with the potential to impact effective cationic-anionic balance. If, in fact, phytase does release more calcium than phosphorus, this would generate a net increase in dietary cationic levels, which would be harmful. If the amount of phosphorus used in diets is too generous, this will be limiting and consequently there will be little or no response to phytase. Therefore, interactions between calcium, phosphorus, other minerals and phytase can occur (Rousseau *et al*., 2012). Phosphorus is generally more completely absorbed than calcium, although the exact mechanism by which absorption occurs is not fully understood. The amount of phosphorus absorbed is dependent on the level of phosphorus in the diet, the source of phosphorus, the amount of calcium, the calcium:phosphorus ratio, intestinal pH, and the antagonistic effects of other minerals such as zinc and copper. Furthermore, the use of the phytase enzyme has a dramatic impact on the calcium and phosphorus requirements of birds (Kleyn, 2013). As calcium and phosphorus are the minerals in greater proportion in bone, the greater availability of these minerals by the addition of phytase should reflect an improvement in the percentage of ash and in the resistance to bone breakage (Laurentiz *et al*., 2009).

Similar to this study, Walk *et al*. (2014) concluded that birds that received diets with high levels of phytase in the ration were related to the highest value of available phosphorus, which may have inhibited the use of minerals such as calcium, zinc, magnesium and iron in the intestine, requiring the bone mineral mobilization of calcium and phosphorus. According to the authors, phytase may indirectly affect protein digestibility and mineral availability, which may or may not favor intestinal absorption.

Saima *et al*. (2009) and Walk *et al*. (2014), also obtained similar results where the data revealed that the application of the enzyme showed a significant difference ($P<0.05$) for the parameters of characteristics of the tibia, mineral ash (zinc, calcium, and phosphorus) in broiler chickens. up to 21 days of age in all phytase enzyme supplementation treatments compared to the no-enzyme control treatment. Brenes *et al*., 2003 reported that the magnesium content in the tibia was not affected by the addition of phytase, corroborating the results found.

However, Cabahug *et al*. (1999) and Singh and Khatta (2003) demonstrated that phytase supplementation improved tibial weight and bone ash content. Rutherfurd *et al*. (2012) reported that phytase supplementation of 1000 and 2000 FTU/kg in the diet increased mineral concentration and tibial density by 35% and 24%, respectively, as did Guo *et al*. (2009) also observed that tibial weight was significantly higher in the phytase-supplemented group. Furthermore, Rousseau *et al*. (2012) also reported that tibial diameter was improved by supplementation with 500 FTU/kg phytase in broiler diets, but without improvement in bone length. The same authors observed that phytase supplementation in diets increased tibial ash and total phosphorus retention in bones, and this increase in tibial ash can be considered a good indicator of bone mineralization. Probably the levels of minerals analyzed in the present study were already capable of supplying the needs of animals in diets without phytase, suggesting that better results would be found with the use of phytase in reformulated diets.

These results can be explained by the greater digestibility of calcium and phosphorus in diets containing phytase, thus resulting in a higher circulating concentration of these minerals in the blood, and, consequently, may have caused a decrease in parathyroid hormone release. This hormone participates in the complex of hormones responsible for stimulating 1-α-hydroxylase, which converts 25-OH-D3 into calcitriol, the active form of vitamin D, responsible for the absorption of calcium and phosphorus in the intestine, as well as bone mineralization and demineralization. (Drezner and Harrelson, 1979). According to Wöhrle *et al*. (2011), 1-α-hydroxylase is mainly regulated by the serum levels of parathyroid hormone and phosphorus, being stimulated by the first and suppressed by the second. It is expressed in the cells of the proximal renal tubules, where most of the calcitriol required for systemic metabolism is synthesized. Consequently, the absence of adequate levels of vitamin D3 can cause mineralization failures and/or excess osteoclastic demineralization with structural and functional impairment to the bone, as bone density is a primary determinant of bone strength and may be associated with the risk of fracture (Combs Jr. and McClung, 2017).

CONCLUSION

Diets containing 500 FTU/kg of phytase in combination with 3150 kcal.kg-1 of AMEn result in better zootechnical performance.

Supplementation with 500 and 1000 FTU/kg in the diets provide better coefficients of apparent and ileal digestibility of calcium and phosphorus, but negatively influence the deposition of calcium, phosphorus, dry matter, and ash in the tibia, in addition to adversely affecting the breakage and area of these bones in broilers.

Bone length and the levels of magnesium and zinc present in the tibia are not influenced by energy and phytase levels in the diets. There is no interaction between the different energetic densities and the phytase doses in the variables analyzed in the period from 28 to 35 days of age.

Increasing the energy density of diets results in improved apparent and ileal digestibility for most nutrients.

REFERENCES

BEDFORD, M.R.; PARTRIDGE, G.G. *Enzymes in farm animal nutrition*. 2.ed. London: Cabi, 2010. 319p.

BERTECHINI, A.G. *Nutrição de monogástricos*. Lavras: UFLA, 2012. 373p.

BRENES, A.; VIVEROS, A.; ARIJA, I. *et al*. The effect of citric acid and microbial phytase on mineral utilization in broiler chicks. *Anim. Feed Sci. Technol*., v.110, p.201-219, 2003.

CABAHUG, S.; RAVINDRAN, V.; SELLE, P.H.; BRYDEN, W. L. Response of broilers chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus contents. I. Effects on bird performance and toe ash. *Br. Poult. Sci.*, v.40, p.660-666, 1999.

CAMDEN, B.J.; MOREL, P.C.H.; THOMAS, D.V.; RAVINDRAN, V.; BEDFORD, M.R. Effectiveness of exogenous microbial phytase in improving the bioavailabilities of phosphorus and other nutrients in maize-soya-bean meal diets for broilers. *Anim. Sci.*, v.73, p.289-297, 2001.

CAMPBELL, G.L.; BEDFORD, M.R. Enzymes applications for monogastric feeds: a review. *Can. J. Anim. Sci*., v.72, p.449-466, 1992

CHUNG, T.K.; RUTHERFURD, S.M.; THOMAS, D.V.; MOUGHAN, P.J. Effect of two microbial phytases on mineral availability and ret ention and bone min- eral density i low-phosphorus diets for broilers. *Br. Poult. Sci*., v.5, p.362-373, 2013.

COMBS Jr., G.F.; McCLUNG, J. P. *The vitamins* (fundamental aspects in nutrition and health). 5.ed. London: Academic Press, 2017. 628p.

COWIESON, A.J.; HRUBY, M.; PIERSON, E.E.M. Evolving enzyme technology: impact on commercial poultry nutrition. *Nutr. Res. Rev*., v.19, p.90-103, 2006.

COWIESON, A.J.; RAVINDRAN, V. Effect of phytic acid and microbial phytase on the flow and amino acid composition of endogenous protein at the terminal ileum of growing broiler chickens. *Br. J. Nutr*., v.98, p.745-752, 2007.

COWIESON, A.J.; WILCOCK, P.; BEDFORD, M.R. Super-dosing effects of phytase in poultry and other monogastrics. *World's Poult. Sci*. J., v.67, p.225-236, 2011.

DONATO, D.C.Z.; ALBUQUERQUE, R.; GARCIA, P.D.S.R.; BALIEIRO, J.C.C. Desempenho de frangos de corte alimentados com rações contendo diferentes níveis de cálcio suplementadas com fitase. *Rev. Bras. Zootec*., v.40, p.2161-2166, 2011.

DREZNER, M.K.; HARRELSON, J.M. Newer knowledge of vitamin D and its metabolites in health and disease. *Clin. Orthop. Relat. Res*., v.139, p.206-231, 1979.

EINHORN, T.A. Biomechanics of bone. In: BILEZIKIAN, J.P.; RAISZ, L.G.; RODAN, G.A. *Principles of bone biology*. San Diego: Academic Press, 1996. p.25-37.

FUKAYAMA, E.H.; SAKOMURA, N.K.; DOURADO, L.R.B. *et al*. Efeito da suplementação de fitase sobre o desempenho e a digestibilidade dos nutrientes em frangos de corte. Rev. Bras. Zootec., v.37, p.629-635, 2008.

GALLARDO, C.; DADALT, J.C.; KIARIE, E.; TRINDADE NETO, M.A. Effects of multicarbohydrase and phytase on standardized ileal digestibility of amino acids and apparent metabolizable energy in canola meal fed to broiler chicks. *Poult. Sci*., v.96, p.3305-3313, 2017.

GUO, Y.; SHI, Y.; LI, F. *et al*. Effects of sodium gluconate and phytase on performance and bone characteristics in broiler chickers. *Anim. Feed Sci.Technol*., v.150, p.270-282, 2009.

HAMDI, M.; PÉREZ, J.F.; LÉTOURNEAU-MONTMINY, M.P. *et al*. The effects of microbial phytases and dietary calcium and phosphorus levels on the productive performance and bone mineralization of broilers. *Anim. Feed Sci.Technol*., v.243, p.41-51, 2018.

KARIMI, A.; MIN, Y.; LU, C. Assessment of potential enhancing effects of a carbohydrase mixture on Phytase efficacy in male broiler chicks fed phosphorusdeficient diets from 1 to 18 days of age. *Poult. Sci*., V.92, p.192-198, 2013.

KLEYN, R. Enzymes in poultry nutrition. In: _____. *Chicken nutrition*: a guide for nutritionists and poultry professional. Leicestershire: Context Publications, 2013. p.251-272.

LAURENTIZ, A.C.; JUNQUEIRA, O.M.; FILARDI, R.S. *et al*. Desempenho, composição da cama, das tíbias, o fígado e das excretas de frangos de corte alimentados com rações contendo fitase e baixos níveis de fósforo. *Rev. Bras. Zootec*., v.38, p.1938-1947, 2009.

LIU, S.Y.; CADOGAN, D.J.; PÉRON, A.; TRUONG, H.H.; SELLE, P.H. Effects of Phytase supplementation on growth performance, nutrient utilization and digestive dynamics in broilers chickens offered maize-, sorghum- and wheat-based diets. *Anim. Feed Sci. Technol*., v.197, p.164-175, 2014.

NITSAN, Z.; EN-AVRAHAM, G.; ZOREF, Z.; NIR, I. Growth and development of the digestive organs and some enzymes in broiler chicks after hatching. *Br. Poult. Sci*., v.32, p.515-523, 1991.

OFFICIAL methods of analysis. 16.ed. Method 972.15. Arlington: AOAC, 2000.

OLUKOSI, O.A.; KONG, C.; FRU-NJI, F.; AJUWON, K.M.; ADEOLA, O. Assessment of a bacterial 6-phytase in the diets of broiler chickens. *Poult. Sci*., v.92, p.2101-2108, 2013.

ONYANGO, E.M.; BEDFORD, M.R.; ADEOLA, O. Efficacy of an evolved Escherichia coli phytase in diets of broiler chicks. *Poult. Sci*., v.84, p.248-255, 2005.

PIRGOZLIEV, V.; BEDFORD, M.R.; ACAMOVIC, T.; MARES, P.; ALLYMEHR, M. The effects of supplementary bacterial phytase on dietary energy and total tract amino acid digestibility when fed to young chickens. *Br. Poult. Sci*., v.52, p.245-254. 2011.

POINTLLART, A.; FONTAINE, N.; THOMASSET, M. Phytate phosphorus utilization and intestinal phosphatases in pigs fed low phosphorus: wheat or corn diets. *Nutr. Rep. Int*., v.29, p.473-483, 1984.

R CORE TEAM. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing, 2015.

RAVINDRAN, V.; MOREL, P.C.H.; PARTRIDGE, G.G.; HRUBY, M.; SANDS, J.S. Influence of an E. coli-derived phytase on nutrient utilization in broiler starter fed diets containing varying concentrations of phytic acid. *Poult. Sci*., v.85, p.82-89, 2006.

ROBERSON, K.D.; EDWARDS JR, H.M. Effects of 1,25-dihydroxycholecalciferol and phytase on zinc utilization in broiler chicks. *Poult. Sci*., v.73, p.1312-1326, 1994.

RODEHUTSCORD, M. Determination of phosphorus availability in poultry. *World's Poult. Sci. Journ*., v. 69, p. 697-698, 2013.

ROSTAGNO, H.S.; ALBINO, L.F.T.; HANNAS, M.I. *et al*. *Tabelas brasileiras para aves e suínos*: composição de alimentos e exigências nutricionais. Viçosa: UFV, 2011. 186p.

ROUSSEAU, X.; LÉTOURNEAU-MONTMINY, M.P.; MÊME, N. *et al*. Phosphorus utilization in finishing broiler chickens: Effects of dietary calcium and microbial phytase. *Poult. Sci*., v.91, p.2829-2837, 2012.

RUTHERFURD, S.M.; CHUNG, T.K.; THOMAS, D.V.; ZOU, M.L.; MOUGHAN, P.J. Effect of a novel phytase on growth performance, apparent metabolizable energy, and the availability of minerals and amino acids in a low-phosphorus corn-soybean meal diet for broilers. *Poult. Sci*., v.91, p.1118-1127, 2012.

SAIMA, M.Z.U.; KHAN, M.A.; JABBAR, M.; QADEER, M.A. Efficacy of microbial phytase at different levels on growth performance and mineral availability in broiler chicken. *J. Anim. Plant Sci*., v.19, p.58-62, 2009.

SEBASTIAN, S.; TOUCHBURN, S.P.; CHAVEZ, E.R. Implications of phytic acid and supplemental microbial phytase in poultry nutrition: a rewiew. *World's Poult. Sci. J*., v.54, p.27-47, 1998.

SHIRLEY, R.B.; EDWARDS JR, H.M. Graded levels of phytase past industry standards improves broilers performance. *Poult. Sci*., v.82, p.671-680, 2003.

SINGH, P.K.; KHATTA, V.K. Effect of phytase supplementation on the performance of broiler chickens fed wheat based diets. *Indian J. Anim. Nutr*., v.20, p.57-62, 2003.

SOHAIL, S.S.; ROLAND SR, D.A. Influence of supplemental phytase on performance of broilers four to six weeks of age. *Poult. Sci*., v.78, p.550-555, 1999.

SOUSA, J.P.L.; ALBINO, L.F.T.; VAZ, R.G.M.V. *et al.* The effect of dietary phytase on broiler performance and digestive, bone and blood biochemistry characteristics. *Rev. Bras. Ciênc. Avícola*, v.17, n.1, 2015.

SYSTEM for microsoft windows. Release 9.4, Cary: SAS Institute Inc, 2002-2012. (CD-ROM).

TEJEDOR, A.A.; ALBINO, L.F.T.; ROSTAGNO, H.S.; VIEITES, F.M. Efeito da adição da enzima fitase sobre o desempenho e a digestibilidade ileal de nutrientes. *Rev. Bras. Zootec*., v.30, p.802-808, 2001.

TIWARI, S.P.; GENDLEY, M.K.; PATHAK, A.K.; GUPTA, R. Influence of an enzyme cocktail and phytase individually or in combination in Ven Cobb broiler chickens. *Br. Poult. Sci*., v.51, p.92-100, 2010.

WALK, C.L.; BEDFORD, M.R.; SANTOS T.T. *et al*. Extra-phosphoric effects of superdoses of a novel microbial phytase. *Poult. Sci*., v.92, p.719-725, 2013.

WALK, C.L.; SANTOS, T.T.; BEDFORD, M.R. Influence of superdoses of a novel microbial phytase on growth performance, tibia ash, and gizzard phytate and inositol in young broilers. *Poult. Sci*., v.93, p.1172-1177, 2014.

WÖHRLE, S.; BONNY, O.; BELUCH, N. *et al.* FGF receptors control vitamin D and phosphate homeostasis by mediating renal FGF23 signaling and regulating FGF23 expression in bone. *J. Bone Miner. Res.*, v.10, p.2486-2497, 2011.

WOYENGO, T.A.; KIARIE, E.; NYACHOTI, C.M. Energy and amino acid utilization in expeller-extracted canola meal fed to growing pigs. *J. Anim. Sci*., v.88, p.1433-1441, 2010.

ZENEBON, O.; PASCUET, N.S.; TIGLEA, P. (Coords.). *Métodos físico-químicos para análise de alimentos*. 4.ed. São Paulo: Instituto Adolfo Lutz, 2008. 1020p.

ZHANG, X. Effect of Natuphos phytase supplementation to fed on performance and ileal digestibility of protein and amino acids of broilers. *Poult. Sci.*, v.78, p.1567-1572, 1999.

ZIOUPOS, P.; CURREY, J.D. Changes in stiffness, strength, and toughness of human cortical bone with age. *Bone*, v.22, p.57-66, 1998.