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Digestibility, Determination of Metabolizable Energy and Bone Mineralization of Broilers Fed with Nutritionally Valued Phytase

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ABSTRACT

The objective of this study was to evaluate the effect of using exoenzyme phytase in broiler's diets on digestibility of nutrients, feed energy and tibia bone mineralization. A completely randomized design was used, with the following treatments: sorghum with dicalcium phosphate (SDP), corn with dicalcium phosphate (CDP), sorghum with meat and bone meal (SMBM), sorghum with valued phytase (SVP) and sorghum with phytase without valued (SPWV). For digestibility analysis, eighty 15 day old broilers were used, a total of 1400 male Hubbard Flex chickens, which were submitted to total excreta collection to obtain the percentages of food digestibility, crude protein, ether extract, apparent metabolizable energy, calcium and phosphorus while for tibias mineralization. Six birds per treatment were used, where determination of mineral matter, calcium and phosphorus were performed. Metabolizable energy (ME) and apparent metabolizable energy corrected for nitrogen (AMEn) of the feed were also calculated. Data were subjected to variation analysis and the average compared by 5% Tukey test. There was no difference between treatments for the digestibility at 15-20 day old as well as for the feed energy values, but the diets with phytase had higher phosphorous percentage values for tibia bone mineralization, demonstrating that exogenous phytase enzyme is able to hydrolyze phytate origininated from plant and release the phosphorus for assimilation by animals, acting as a substitute for phosphorus plant sources.

INTRODUCTION

Several studies regarding modern poultry are developed in the areas of genetics, management and nutrition; aimed at increasing productivity and reducing production costs through the maximum development of the animal´s genetic potential.

Adequated nutrition is based on the principle that the animal is receiving a suitable amount of nutrients such as proteins, carbohydrates, lipids, vitamins and particularly minerals for participating of all biochemical body processes (Sechinato *et al.,* 2006). Supplementation of macro minerals in poultry feed is frequently modified according to the genetic improvement as well as new knowledge of the physical and chemical characteristics of the sources that influence the bioavailability of these macro minerals for the animals (Bertechini & Fassani, 2001).

Among the minerals required by poultry, phosphorus and calcium are the most important, not only because they are required for optimal growth rate, but also for bone mineralization. Phosphorus participates of metabolic processes and nutrients absorption, besides being the most expensive mineral in the feed´s final cost (Gomes *et al.*, 2004).

The search for solutions to correctly fit phosphorus and calcium needs in the animal´s body is constant, especially due to their fast growth and the consequent reduction in their slaughter age. Thus, it emphasizes the importance of using best quality feeds and the use of additives that enable broilers to take advantage of the nutrients in feeds (Brandão *et al.*, 2007).

In diets formulated for poultry, the available phosphorus supply by vegetable sources is not sufficient to meet the appropriate nutritional requirements for animal performance and bone mineralization. Thus, there is a need for supplementation with phosphorus sources in the inorganic form, highlighting the differences in the values of bioavailability between these sources (Rostagno *et al.*, 2005).

The production of endogenous phytase enzyme by birds is almost null and P complexed in form of phytate, which is considered the largest reserve of this element in plants, becomes unavailable for the animals, that is why the exogenous phytase administered via feed has been used as a phosphorus supplementation source (Conte *et al.*, 2002), acting in the hydrolysis of phytate present in grains and releasing P for animal´s assimilation.

Nutritionally, using 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, as well as amino acids and energy, reducing the feed formulation´s final cost (Tejedor *et al.*, 2001).

Therefore, the objective of this study was to evaluate the effect of using exoenzyme phytase in diets for broilers regarding the nutrient´s digestibility, feed energy and tibia bone mineralization.

MATERIAL AND METHODS

The experiment was performed at the Experimental Farm Gloria - Poultry Research Farm in the Federal University of Uberlândia, in Uberlândia - MG, from September to October 2011 in accordance with ethical standards and approved by the Ethics Committee on Animal Use - CEUA-UFU, under research protocol number 077/11.

The birds were raised in a 60x10 meters barn, covered with metal structure and fibro-cement tiles, concreted floor, sided with masonry short walls and with four square centimeters wire mesh. The barn was internally equipped with 80 pens, each one measuring 1.90 x 1.50 meters, fans and water sprinklers to control the temperature, internal and external avian curtains. Each pen was composed of a tubular feeder of 20 kg and a pendular drinker, and for four pens there was an infrared hood. Rice shell was used as the litter material.

To perform this study, a performance research provided 1400 male chicks which were housed at one day old (average initial weight 43g) from Hubbard Flex lineage. These birds were from a multiplier company from Uberlândia, State of Minas Gerais, and vaccinated against Marek’s disease and Gumboro at the hatchery.

A feeding program with four phases was used: pre-starter (1-7 days), starter (8-21 days), fattening (22-35 days) and slaughter (36-42 days). The diets were formulated with energy and nutrient levels based on Rostagno´s *et al.* (2005) recommendations and according to each treatment (Tables 1, 2 and 3), they were calculated from bromatological analysis, performed at the Animal Nutrition Laboratory of the Faculty of Veterinary Medicine at UFU - LAMRA. During the experimental period the birds received feed and drinking water *ad libitum*.

The birds handling practices in the experiment period followed the model recommended by the Experimental Farm to ensure proper ambience to every stage of life, fresh and clean water supply, and feed *ad libitum*.

The birds were reared in three pens in order to develop the digestibility test. Fourty male chicks were allocated in each pen, fed with control diet (sorghum based feed / soybean meal + dicalcium phosphate) under 12 days, when four birds from each pen were transferred to test cages, with similar weights previously selected with an average weight of the three pens (± 5% of variation). Thus, the design was randomized composed of five treatments: SDP (feed sorghum / soybean meal + dicalcium phosphate), CDP (corn based feed / soybean meal + dicalcium phosphate), SMBM (sorghum based feed / soybean meal + meat and bone meal), SVP (sorghum based feed / soybean meal + valued phytase) and SPWV (sorghum based feed / soybean meal + phytase without enrichment) and four replications. The corn feed was used as a performance control and as a comparative effect with sorghum. The valued phytase differs from the non valued phytase because in the first, to achieve the same energy level, the ingredients are altered compared to the control diet (SDP), because the inclusion by the enzyme action is expected, whereas in the second, the enzyme is added without altering the ingredients (Junqueira *et al.*, 2010).

Table 1 – Ingredients, percentage composition and calculated nutritional values of sorghum based feed (A) and corn based (B) with inorganic phosphate feed for broilers in the pre-starter phase (1-7 days), starter (8-21 days), fattening (22 to 35 days) and slaughter (36 to 42 days).

Ingredients (%)	Pre-starter		Starter		Fattening		Slaughter	
	A	B	A	B	A	B	A	B
Sorghum 8,6% CP	54,33	-	56,63	-	58,71	-	61,65	-
Corn grain 8,0% CP	-	56,42	-	58,74	-	61,59	-	64,96
Soybean meal 46,5 CP	37,21	37,33	34,39	34,58	31,17	31,06	28,55	28,19
Soybean oil	4,12	2,00	5,11	2,92	6,53	3,86	6,72	3,87
Dicalcium phosphate	1,94	1,84	1,58	1,47	1,34	1,23	1,10	0,98
Limestone	0,77	0,83	0,82	0,88	0,78	0,85	0,71	0,78
Salt	0,46	0,44	0,44	0,42	0,42	0,40	0,41	0,39
L-lysine HCL	0,32	0,31	0,27	0,26	0,27	0,26	0,26	0,25
DL-Methionine	0,21	0,19	0,16	0,13	0,17	0,15	0,21	0,19
L-Threonine	0,12	0,12	0,09	0,09	0,08	0,08	0,06	0,07
Px FC – Agroceres	0,50[1]	0,50[1]	0,50[1]	0,50[1]	0,50[2]	0,50[2]	0,30[3]	0,30[3]
TOTAL	100	100	100	100	100	100	100	100
Calculated nutritional composition (%)								
Crude Protein	22,50	22,40	21,28	21,20	20,08	19,80	19,12	18,75
Calcium	0,92	0,92	0,84	0,84	0,76	0,76	0,66	0,66
Available phosphorus	0,47	0,47	0,40	0,40	0,35	0,35	0,31	0,31
Potassium	0,86	0,85	0,81	0,81	0,76	0,75	0,72	0,71
Sodium	0,22	0,22	0,21	0,21	0,20	0,20	0,19	0,19
Chlorine	0,28	0,31	0,27	0,30	0,26	0,29	0,25	0,28
Linoleic acid	3,11	1,77	3,66	2,27	4,43	2,77	4,54	2,78
Digestible lysine	1,32	1,32	1,21	1,21	1,13	1,13	1,06	1,06
Digestible methionine	0,67	0,66	0,60	0,59	0,57	0,56	0,53	0,51
Methionine + cystine	0,95	0,95	0,87	0,87	0,91	0,82	0,77	0,77
Digestible threonine	0,86	0,86	0,79	0,79	0,73	0,73	0,69	0,69
Digestible Tryptophan	0,25	0,24	0,24	0,23	0,22	0,21	0,21	0,19
Digestible Arginine	1,40	1,41	1,31	1,33	1,22	1,23	1,14	1,14
AME (Mcal kg^{-1})	2,96	2,96	3,05	3,05	3,15	3,15	3,20	3,20

*AME – Apparent metabolizable energy

[1]VitA 1.600.000,00 IU kg^{-1}, VitB1 600,000 mg kg^{-1}, VitB12 2.000,00 mcg kg^{-1}, VitB2 800,00mg kg^{-1}, VitB6 400,000 mg kg^{-1}, VitD3 400.000,00 IU kg^{-1}, VitE 3.000,00mg kg^{-1}, VitK 400mg kg^{-1}, Zn 12,600g kg^{-1}, Cu 1260,0000 mg kg^{-1}, Selenium 80,00mg kg^{-1}, Fe 10,5g kg^{-1}, I 252,00mg kg^{-1}, Mn12,6g kg^{-1} Folic acid 140,0000mg kg^{-1}, Pantothenic acid 1600,00mg kg^{-1}, Bacitracin of Zn exact 11,000g kg^{-1}, Biotin 12,000mg kg^{-1}, Colina 70,00g kg^{-1}, Met 336,600g kg^{-1}, Sodium monensin exact 22,00g kg^{-1}, Niacin 6000,00mg kg^{-1}. [2]VitA 1.280.000,00 IU kg^{-1}, VitB1 400,000 mg kg^{-1}, VitB12 1.600,00 mcg kg^{-1}, VitB2 720,00mg kg^{-1}, VitB6 320,000 mg kg^{-1}, VitD3 350.000,00 IU kg^{-1}, VitE 2.400,00mg kg^{-1}, VitK 300mg kg^{-1}, Cu 1200,0000 mg kg^{-1}, Fe 10,0g kg^{-1} , I 240,00mg kg^{-1}, Mn12,0g kg^{-1}, Selenium 60,00mg kg^{-1}, Zn 12,000g kg^{-1}, Folic acid 100,0000mg kg^{-1}, Pantothenic acid 1600,00mg kg^{-1}, Biotin 6,000mg kg^{-1}, Colina 50,00g kg^{-1}, , Halquinol exact 6000,00 mg kg^{-1}, Met 267,300g kg^{-1}, Niacin 4800,00mg kg^{-1}, Salinomycin exact 13,200 g kg^{-1}.

[3]VitA 1.300.260,00 IU kg^{-1}, VitB1 166,000 mg kg^{-1}, VitB12 1.667,00 mcg kg^{-1}, VitB2 666,800mg kg^{-1}, VitB6 200,000 mg kg^{-1}, VitD3 400.000,00 IU kg^{-1}, VitE 2.167,10mg kg^{-1}, VitK 333,400mg kg^{-1}, Cu 2000,0000 mg kg^{-1}, Fe 16,60g kg^{-1}, I 400,00mg kg^{-1}, Mn 20,0g kg^{-1}, Selenium 60,680mg kg^{-1} , Zn 20,000g kg^{-1}, Folic acid 100,0000mg kg^{-1}, Pantothenic acid 1333,00mg kg^{-1}, Biotin 6,670mg kg^{-1}, Colina 50,00g kg^{-1}, Met 230,000g kg^{-1}, Niacin 4000,00mg kg^{-1}, Virginiamycin exact 3.666,00 mg kg^{-1}.

For the digestibility analysis, the total collection method of excreta in broilers was used with starting and ending time, determined by the appearance of tagged excreta (1% Fe_2O_3 - Diets with Iron Oxide added). The data was collected in the period of 12 to 20 days (initial), consisting of three days of adaptation of the experimental diets and the new environment; and five days for excreta collection. Along with this analysis, an assay was performed to determine the value of the feed´s Apparent Metabolizable Energy (AME) and Apparent Metabolizable Energy corrected for nitrogen (AMEn). The total collection of excreta from trays of each cage were taken out twice a day, caring about removing feathers and other foreign

Table 2 – Ingredients, percentage composition and calculated nutritional values of sorghum based feed and meat and bone based meal for broilers in pre-starter phase (1-7 days), starter (8-21 days), fattening (22 to 35 days) and slaughter (36 to 42 days).

Ingredients (%)	Pre-starter	Starter	Fattening	Slaughter
Sorghum 8,6% CP	56,75	58,59	60,38	63,03
Soybean meal 46,5 CP	34,29	32,01	29,15	26,89
Soybean oil	3,31	4,45	5,98	6,27
Meat and bone meal	3,93	3,19	2,71	2,23
Limestone	0,10	0,28	0,32	0,33
Salt	0,41	0,39	0,38	0,38
L-lysine HCL	0,35	0,30	0,29	0,28
DL-Methionine	0,21	0,16	0,19	0,21
L-Threonine	0,13	0,10	0,09	0,07
Px FC – Agroceres	0,50[1]	0,50[1]	0,50[2]	0,30[3]
TOTAL	100	100	100	100
Calculated nutritional composition (%)				
Crude protein	22,862	21,577	20,334	19,332
Calcium	0,920	0,841	0,758	0,663
Available phosphorus	0,470	0,401	0,354	0,309
Potassium	0,840	0,799	0,748	0,712
Sodium	0,220	0,210	0,200	0,195
Chlorine	0,272	0,261	0,251	0,246
Linoleic acid	2,694	3,315	4,141	4,309
Digestible Lysine	1,324	1,217	1,131	1,060
Digestible methionine	0,672	0,607	0,575	0,534
Methionine+cystine	0,953	0,876	0,826	0,774
Digestible threonine	0,861	0,791	0,735	0,689
Digestible tryptophan	0,246	0,233	0,219	0,208
Digestible arginine	1,400	1,315	1,221	1,145
AME (Mcal kg^{-1})	2,9600	3,0500	3,1500	3,2000

[*]AME – Apparent metabolizable energy

[1], [2] and [3]: idem Table 1.

bodies in the tray, and then placed in labeled plastic bags, weighed for excreta quantification in grams and frozen for storage and later analysis.

At the laboratory (LAMRA), excreta were defrosted and homogenized. Samples were taken and pre-dried in forced ventilation oven at 56 °C for 72 hours and then ground in a knife mill to perform the analyzes of dry matter, ether extract, crude protein, crude fiber, ash, calcium, phosphorus according to the methodology proposed by the Brazilian Compendium of Animal Nutrition (Brasil, 2005) and gross energy by bomb calorimeter IKA-Werke C2000 basic. The feed digestibility was calculated as follows:

Digestibility = ((ingested nutrient quantity - Excreted) / ingested nutrient Quantity) * 100

From the excreta gross energy, the values of Apparent Metabolizable Energy (AME) and Apparent Metabolizable Energy corrected for nitrogen (AMEn) were determined.

At 42 days of age, the birds were slaughtered according to official procedures; six males were chosen with weights into the treatment average for analysis of the tibia´s mineralization, which were removed, cleaned and sectioned by the epiphysis of the left tibias. The diaphysis of these tibias were dried in forced ventilation oven at 56 °C for 72 hours and then placed in containers with petroleum ether for fat extraction. Subsequently, they were placed in an oven at 600ºC for 4 hours to determine the mineral matter content (% MM), and from the ash, the calcium (Ca%) and phosphorus (P%) content was determined, following the Brazilian Compendium of Animal Nutrition (Brasil, 2005) methodology.

All data were submitted to variances homogeneity checking, residuals normality and then analysis of variance was performed and the averages compared by Tukey test at 5%.

Table 3 – Ingredients, percentage composition and calculated nutritional values of sorghum based feed and phytase with energy and amino acids enrichment (A); and phytase without energy enrichment and amino acids (B) for broilers in the pre-starter phase (1-7 days), starter (8-21 days), fattening (22 to 35 days) and slaughter (36 to 42 days).

Ingredients (%)	Pre-starter		Starter		Fattening		Slaughter	
	A	B	A	B	A	B	A	B
Sorghum 8,6% CP	55,58	54,62	57,88	56,92	60,08	59,57	63,02	62,52
Soybean meal 46,5 CP	37,09	37,18	34,27	34,36	31,03	31,08	28,41	28,46
Soybean oil	3,46	4,02	4,45	5,012	5,84	6,25	6,03	6,44
Dicalcium phosphate	1,44	1,44	1,07	1,08	0,78	0,78	0,54	0,55
Limestone	0,83	1,09	0,89	1,14	0,84	0,84	0,77	0,77
Salt	0,46	0,46	0,44	0,44	0,42	0,42	0,41	0,41
L-lysine HCL	0,31	0,32	0,26	0,27	0,25	0,27	0,24	0,26
DL-Methionine	0,20	0,21	0,15	0,15	0,17	0,17	0,20	0,21
L-Threonine	0,10	0,12	0,07	0,08	0,06	0,07	0,05	0,06
Px FC – Agroceres	0,50[1]	0,50[1]	0,50[1]	0,50[1]	0,50[2]	0,50[2]	0,30[3]	0,30[3]
Microtech 500	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01
TOTAL	100	100	100	100	100	100	100	100
Calculated nutritional composition (%)								
Crude Protein	22,74	22,51	21,53	21,29	20,34	20,11	19,39	19,16
Calcium	0,92	0,92	0,84	0,84	0,76	0,76	0,66	0,66
Available phosphorus	0,47	0,47	0,40	0,40	0,35	0,35	0,31	0,31
Potassium	0,86	0,86	0,81	0,81	0,76	0,76	0,72	0,72
Sodium	0,22	0,22	0,21	0,21	0,20	0,20	0,19	0,19
Chlorine	0,28	0,28	0,27	0,27	0,26	0,26	0,25	0,25
Linoleic acid	2,77	3,06	3,31	3,61	4,68	4,28	4,18	4,40
Digestible lysine	1,32	1,32	1,21	1,21	1,13	1,13	1,06	1,06
Digestible methionine	0,67	0,67	0,60	0,60	0,57	0,57	0,53	0,53
Methionine + cystine	0,95	0,95	0,87	0,87	0,82	0,82	0,77	0,77
Digestible threonine	0,86	0,86	0,79	0,79	0,73	0,73	0,69	0,69
Digestible Tryptophan	0,25	0,25	0,24	0,24	0,22	0,22	0,21	0,21
Digestible Arginine	1,40	1,40	1,31	1,31	1,22	1,22	1,14	1,14
AME (Mcal kg^{-1})	2,96	2,96	3,05	3,05	3,15	3,15	3,20	3,20

*AME – Apparent metabolizable energy

[1], [2] and [3]: idem Table 1.

RESULTS AND DISCUSSION

From 15-20 days of age, there was no difference in feed digestibility, crude protein, ether extract, apparent metabolizable energy, calcium and phosphorus among the diets (Table 4), demonstrating that the phytase enzyme, when participated in the nutritional matrix feed, could provide the nutrients to the birds at the starter phase period, and there was no difference between the phosphorus sources used.

The digestibility results of this study from 15-20 days of age are according to Adeola & Sands (2003) ones, in the case of using phytase enzyme, since they did not observe improvement in amino acid´s and protein´s

Table 4 – Percentage of feed digestibility values (FD%), crude protein (CP%), ether extract (EE%), apparent metabolizable energy (AME%), Calcium (Ca%) and phosphorus (P%) of broilers at 15-20 days of age, fed diets with different phosphorus and phytase sources.

Treatment	FD%	CPD%	EED%	AMED%	CaD%	PD%
SDP	72,25	60,74	78,83	74,55	58,58	65,55
CDP	72,54	61,00	79,43	74,45	58,21	66,95
SMBM	72,29	64,31	84,43	77,12	61,95	70,18
SVP	72,49	61,07	81,80	74,59	57,85	65,61
SPWV	73,54	63,03	78,66	75,45	59,96	66,82
CV	3,435	6,250	5,111	2,953	7,016	5,067
Pvalue	0,4399	0,6691	0,2462	0,4145	0,6701	0,3039

SDP (sorghum based feed / soybean meal + dicalcium phosphate) CDP (corn based feed / soybean meal + dicalcium phosphate) SMBM (sorghum based feed / soybean meal + meat and bone meal), SVP (sorghum based feed / soybean meal + enriched phytase) and SPWV (sorghum based feed / soybean meal + phytase without enrichment).

digestibility using this enzyme. Similarly, Boling *et al*. (2000) reported that supplementation of diets with phytase did not improve ileal digestibility of amino acids in chicks.

In contrast, Tejedor *et al*. (2001) evaluated 10-24 day old chickens, and found better digestibility values of calcium, phosphorus, protein and apparent energy with the addition of phytase (500-750 uft kg^{-1} (unit of phytase per kg)) and a better utilization of protein, for example, by breaking the nutrient complex (protein-phytic acid) by the use of phytase breaking and releasing phytate protein and amino acids.

Ferlin (2006) and Viveiros *et al*. (2002) showed that the use of phytase increased the diet´s total digestibility, improving the metabolization of nutrients and reducing the amount of nitrogen and phosphorus in bird excreta, reducing then, the contamination in the environment, which was not seen in this study.

In case of phosphorus and energy, Fukayama *et al*. (2008) showed that the improvement in digestibility of these nutrients is only possible when the phytase supplementation is performed using levels from 750 uft kg^{-1}, whereas in our studies, the levels of enzyme used was 500 uft kg^{-1}.

Meat bone meal may be used as phosphorus source in the starter phase of rearing, once similar results to diets with only dicalcium phosphate were obtained; demonstrating as well as Traylor *et al*. (2005), that the presence of bone in the feed formulation makes it a good source of minerals, particularly phosphorus, in addition to being economically viable since this ingredient is generally produced in a large scale.

The metabolizable energy and apparent metabolizable energy corrected for nitrogen from 15-20 days of age did not differ among the treatments (Table 5), so the enzyme phytase was not able to provide energy increase as enunciated by Tejedor *et al*. (2001), who worked with different breed of broiler chickens (Avian Farm) from the one used in this study.

Lan *et al*. (2002), when analyzing the metabolizable energy content in the corn and soybean based feed supplemented with phytase enzyme (250-1000 uft kg^{-1}) for Avian chickens in the initial and final phases, found higher values of apparent metabolizable energy when compared to a diet with normal levels of phosphorus and without enzyme supplementation.

Dourado *et al*. (2007) found that phytase provided the greatest increase in true metabolizable energy corrected for nitrogen balance (TMEn) of corn compared with other enzymes in broilers diets, in which we observed improvement of 95 kcal kg-1 in TMEn adding phytase, while Viana *et al*. (2009) studying laying hens, reported that the addition of enriched phytase provides increased metabolism of energy and energy corrected by nitrogen balance.

In accordance with our studies, Lelis *et al*. (2010), working with Ross chickens from 16-25 days days of age, said that the diets supplemented with enriched phytase (250 and 500 uft kg-1) had similar values of apparent metabolizable energy corrected by nitrogen balance when compared to diets formulated only with inorganic phosphate.

After 42 days days of age, the treatment with phytase without enrichment showed highest deposition of calcium and phosphorus in the bone and did not differ by treatment with enriched phytase (Table 6), demonstrating that the enzyme was able to provide these birds with phosphorus, or the enzyme is able to hydrolyze phytate present in the grain and release phosphorus for the animal´s absorption.

Table 5 – Metabolizable Energy Values (AME) and Apparent Metabolizable Energy corrected for nitrogen (AMEn) of diets with different sources of phosphorus and phytase enzyme to broilers from 15-20 days of age.

Treatment	ME kcal/g MN	AMEn kcal/g MN
SDP	2,841	2,675
CDP	2,767	2,600
SMBM	2,939	2,758
SVP	2,808	2,638
SPWV	2,850	2,668
CV	3,318	3,139
p value	0,0728	0,0574

SDP (sorghum based feed / soybean meal + dicalcium phosphate) CDP (corn based feed / soybean meal + dicalcium phosphate) SMBM (sorghum based feed / soybean meal + meat and bone meal), SVP (sorghum based feed / soybean meal + enriched phytase) and SPWV (sorghum based feed / soybean meal + phytase without enrichment).

Table 6 – Percentage values of mineral matter (MM%), calcium (Ca%) and phosphorus (P%) of tibia of broilers at 42 days days of age.

Treatment	MM%	Ca%	P%
SDP	43,93	16,80b	10,18b
CDP	44,87	17,79b	7,89c
SMBM	45,96	18,90ab	8,75bc
SVP	45,94	19,39ab	10,32ab
SPWV	47,18	21,92a	12,04a
CV	6,216	13,69	17,755
Pvalue	0,3516	0,0030*	<0,0001*

SDP (sorghum based feed / soybean meal + dicalcium phosphate) CDP (corn based feed / soybean meal + dicalcium phosphate) SMBM (sorghum based feed / soybean meal + meat and bone meal), SVP (sorghum based feed / soybean meal + enriched phytase) and SPWV (sorghum based feed / soybean meal + phytase without enrichment).

According to Sohail & Roland (1999), the bone characteristics are the most sensitive parameters to evaluate the effect of phytase compared to performance characteristics. Taking in consideration that Ca and P are the most prevalent minerals in the bone structure, the increased availability of these minerals by adding phytase enzyme justifies the higher percentage of ash, Ca and P in the tibia (Fukayama *et al.*, 2008). The efficiency of phytase enzyme in hydrolyzing mineral-phytic-acid complex, leaving them free for absorption and contributing to bone mineralization is evidenced, justifying higher percentages of Ca and P in the treatments with phytase.

Oliveira *et al*. (2008), evaluating the tibia bone density in their different areas (middle and distal epiphyseal and diaphyseal), observed that reducing the levels of available phosphorus in diets for broilers also reduces the density of the tibia regions, while the inclusion of phytase in the diet increases the density of different regions, since there were higher phosphorus supplies for bone deposition, demonstrating that the use of this enzyme increases the utilization of the organic phosphorus which is in the form of phytate, reducing the cost of adding inorganic phosphorus to the diet as elucidated by Brandão *et al*. (2007).

The exogenous phytase enzyme, when included in the nutritional matrix feed, is able to hydrolyze phytate plant and release phosphorus to be digested by broilers, thereby, acting as a substitute for phosphorus plant sources.

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