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Dietary hybrid phytase and carbohydrases on nutrient digestibility and bone quality of broiler chickens

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Abstract – The objective of this work was to evaluate the effect of the use of hybrid phytase, alone or combined with carbohydrases, in poultry diets with nutritional reductions of calcium, available phosphorus, and metabolizable energy on the nutrient digestibility and bone quality of broiler chickens. A total of 1,875 broilers were distributed in five treatments in a completely randomized design, with 15 replicates of 25 chickens each. The treatments consisted of a positive control feed (T1) and of four negative controls (T2 to T5): T1, basal diet (BD) with corn and soybean; T2 and T3, BDs with reductions of 70 and 100 kcal kg^{-1} metabolizable energy, respectively, and both with reductions of 0.16% Ca and 0.15% available P; and T4 and T5, BDs with the same nutritional reductions, but supplemented with enzymes, i.e., T4 = T2 + 500 phytase units (FTU) per kilogram and T5 = T3 + 500 FTU kg^{-1} + 560 xylanase units (TXU) per kilogram + 250 glucanase units (TGU) per kilogram. The use of 500 FTU kg^{-1} hybrid phytase in pelleted corn-soybean meal diets allows a good digestive performance by broilers and replaces the nitrogen-corrected apparent metabolizable energy at 70 kcal kg^{-1}, as well as 0.16% Ca and 0.15% available P.

Index terms: calcium, energy, enzymes, phosphorus, zinc.

Fitase híbrida e carboidrases dietéticas sobre digestibilidade de nutrientes e qualidade de ossos em frangos de corte

Resumo – O objetivo deste trabalho foi avaliar a eficiência do uso de fitase híbrida, associada ou não com carboidrases, em dietas para frangos, com reduções nutricionais de cálcio, fósforo disponível e energia metabolizável, sobre a digestibilidade dos nutrientes e a qualidade óssea em frangos de corte. Um total de 1.875 frangos foi distribuído em cinco tratamentos, em delineamento inteiramente casualizado, com 15 repetições de 25 frangos cada uma. Os tratamentos consistiram de uma dieta controle positivo (T1) e de quatro controles negativos (T2 a T5): T1, dieta basal (DB) de milho e farelo de soja; T2 e T3, DBs com reduções de 70 e 100 kcal kg^{-1} de energia metabolizável, respectivamente, e ambas com reduções de 0,16% de Ca e 0,15% de fósforo disponível; e T4 e T5, DBs com as mesmas reduções nutricionais, mas com a adição de enzimas, ou seja, T4 = T2 + 500 unidades de fitase (FTU) por quilograma e T5 = T3 + 500 FTU kg^{-1} + 560 unidades de xilanase (TXU) por quilograma + 250 unidades de glucanase (TGU) por quilograma. O uso de 500 FTU kg^{-1} de fitase híbrida em rações peletizadas com milho e farelo de soja permite bom desempenho digestivo por frangos de corte e substitui a energia metabolizável aparente corrigida por nitrogênio a 70 kcal kg^{-1}, bem como 0,16% de Ca e 0,15% de P disponível.

Termos para indexação: cálcio, energia, enzimas, fósforo, zinco.

Introduction

Enzymes have been added over 20 years to poultry feed for different purposes (Choct, 2006). One of the main ones is to increase nutrient digestibility, which is highly advantageous because it allows reducing the inclusion of expensive ingredients in the composition of the feed, reducing its cost without compromising animal performance (Romero et al., 2014). In addition, dietary enzyme supplementation is important to reduce the amount of certain nutrients to limits specific for a sustainable production (Ravindran, 2014).

Among the known enzymes, phytase has been mainly used to cleave phytate-releasing phosphorous for absorption (Dersjant-Li et al., 2015). However, while releasing P, other nutrients – such as calcium, manganese, magnesium, iron, and zinc (Nissar et al., 2017), as well as proteins and carbohydrates (Slominski, 2011) – can also be released and absorbed. Therefore, when using phytase, not only P should be considered but all other nutrients, previously chelated by phytic acid (Woyengo & Nyachoti, 2011).

Additionally, exogenous enzymes can act synergistically, increasing nutrient bioavailability and animal performance (Woyengo & Nyachoti, 2011). These enzymes include the carbohydrases group that can hydrolyze non-starch polysaccharides, as shown by Yu & Chung (2004) and Meng & Slominski (2005), who reported that supplementing corn and soybean meal-based poultry diets with xylanase and β-glucanase increased metabolizable energy content. Carbohydrases can also promote an increase in phytase efficacy in degrading phytate (Woyengo & Nyachoti, 2011), leveraging phytase action.

The enzyme industry, as many others, has introduced novel products with an increased efficacy, stability during feed processing, and resistance to the adverse conditions of the bird gastrointestinal tract (Ravindran, 2013). Hybrid bacterial phytases, for example, have been reported to improve the digestibility of P, as well as of other nutrients linked to phytate (Torrallardona et al., 2017). However, although enzymes can act synergically, it is still not clear if this potential can be achieved by combining enzymes from different generations.

The objective of this work was to evaluate the effect of the use of hybrid phytase, alone or combined with carbohydrases, in poultry diets with nutritional reductions of calcium, available phosphorus, and metabolizable energy on the nutrient digestibility and bone quality of broiler chickens.

Materials and Methods

The experiment was conducted at the experimental poultry sector of Embrapa Suínos e Aves, located in the municipality of Concórdia, in the state of Santa Catarina, Brazil. The project was approved by the ethics committee for animal use of Embrapa, under number 006-2017. The experimental period lasted 28 days.

A total of 1,875 Cobb 500 male broilers, with 28 days of age, were housed in 1.65×1.70 m floor pens equipped with tubular feeders and five nipple drinkers per pen. Poultry house temperature was controlled by a central heating system and ventilators. Bedding consisted of pine wood shavings. The birds had an initial body weight of 46.9±3.7 g and were distributed in a completely randomized design among five treatment groups, with 15 replicates of 25 birds each.

The experimental diets included a positive control (T1) and four negative controls (T2 to T5): T1, basal diet (BD) with corn and soybean, which met the typical nutritional requirements (Rostagno, 2011); T2 and T3, BDs with reductions of 70 and 100 kcal kg^{-1}, respectively, and both with reductions of 0.16% Ca and 0.15% available P; and T4 and T5, BDs with the same nutritional reductions but supplemented with enzymes, i.e., T4 = T2 + 500 phytase units (FTU) per kilogram and T5 = T3 + 500 FTU kg^{-1} + 560 xylanase units (TXU) per kilogram + 250 glucanase units (TGU) per kilogram (Table 1).

The used hybrid phytase was Natuphos E 10000 G (BASF, Ludwigshafen, Germany), which is granulated and a microbially derived myo-inositol-hexakisphosphate beta-phosphohydrolase (EC 3.1.3.26), exhibiting a phytase activity of 10,000 FTU g^{-1}. FTU is defined as the amount of enzymes that releases 1.0 μmol per minute of inorganic P, in the form of 0.0051 mol L^{-1} sodium phytate, at pH 5.5 and 37°C (Basf, 2017). According to the manufacturer, that molecule was created by hybridization with three bacteria, which confers it a higher resistance to acidic conditions, endogenous proteases, and pelleting temperatures up to 95°C.

The carbohydrase used was Natugrain TS (BASF, Ludwigshafen, Germany), cleaving bonds commonly targeted by endo-1,4-beta-xylanase (EC 3.2.1.8) and endo-1,4-beta-glucanase (EC 3.2.1.4). This product is produced from *Aspergillus niger*. According to the manufacturer, TXU is defined as the amount of enzymes that releases 5.0 µmol per minute of reducing sugars, measured as equivalent xylase, from a 0.01 g mL^{-1} solution of arabinoxylan at pH 3.5 and 40°C. Similarly, TGU is defined as the amount of enzymes that releases 1.0 µmol per minute of reducing sugars, measured as equivalent glucose, from a 0.00714 g mL^{-1} solution of beta-glucan, at pH 3.5 and 40°C.

The feeding program consisted of three phases: pre-starter, from 1–7 days; starter, 8–21 days; and grower, 22–28 days. The feed was pelleted at 80°C for 20 s and its consumption was ad libitum. Feed composition and the nutritional profiles of the experimental diets are presented in Table 2.

The enzymatic activity (expressed per kilogram of feed) of each diet was evaluated by Laboratório CBO (Valinhos, São Paulo, SP, Brazil). In T4, recovery was measured considering the following values in each phase: 420 FTU in pre-starter, 440 FTU in starter, and 510 FTU in grower. In T5, these values were: 440 FTU, 529 TXU, and 322 TGU in pre-starter; 480 FTU, 579 TXU, and 290 TGU in starter; and 470 FTU, 540 TXU, and 321 TGU in grower.

For bone quality measurements, three birds from each experimental unit (average body weight ±5% of the pen mean) were slaughtered at 28 days of age for ileal content and tibia collection.

Nutrient digestibility was determined using the ileal sampling method, by adding 0.5% chromium (III) oxide (Cr_2O_3) to all experimental diets as an undigestible indicator during 7 days (from the twenty-first to the twenty-eighth day). Immediately after slaughter, the ileum of each animal was exposed by abdominal incision: a 20 cm segment, ending 4 cm before the ileocecal junction was removed, and the contents of the segments were collected, weighed, and frozen for further analysis. Later, the frozen contents were lyophilized (freeze-dried at -50°C and -80 kPa for 72 hours), weighed, and homogenized, being ground with the A11 Basic analytical mill (IKA, Staufen, Germany) and analyzed at the Physicochemical Laboratory of Embrapa, located in Concórdia, in the state of Santa Catarina, Brazil.

Samples of the experimental feeds were also assessed at the same laboratory, in order to determine: dry matter (DM), by method 012/IV of Instituto Adolfo Lutz (Zenebon et al., 2008), using the SX12DTME drying oven (Prolab, São Paulo, SP, Brazil); mineral matter (MM), by method 36 of Compêndio Brasileiro de Alimentação Animal (2009), using the SSFM67L muffle furnace (Prolab, São Paulo, SP, Brazil); Ca, with the NovAA 800 flame atomic absorption spectrometer (AnalytikJena GmbH, Überlingen, Germany); P, by method 958.01 of Association of Official Analytical Chemists (Latimer, 2012); crude protein (CP), using the FP 628 Dumas protein analyzer (LECO, St. Joseph, MI, USA); and gross energy, with the AC500 automatic calorimeter (LECO, St. Joseph, MI, USA).

In addition, Cr_2O_3 was measured using inductively coupled plasma optical emission spectrometry, and indigestibility factor (IF) values were calculated for each sample, as follows:

(IF) = Marker in the feed (%DM) / Marker in ileal content (%DM)

Table 1. Experimental diets (treatments) fed to Cobb 500 male broiler chickens.

Nutritional reduction	Treatment				
	T1	T2	T3	T4	T5
Energy (kcal kg^{-1})		70	100	70	100
Calcium (%)	PC[(1)]	0.16	0.16	0.16	0.16
Available phosphorous (%)		0.15	0.15	0.15	0.15
	Enzyme supplementation				
Phytase (FTU kg^{-1})	0	0	0	500	500
Xylanase (TXU kg^{-1})	0	0	0	0	560
Glucanase (TGU kg^{-1})	0	0	0	0	250

[(1)]Positive control. Values according to the recommendations of the Brazilian tables for poultry and swine (Rostagno, 2011) for the regular performance of male broilers, in three growing phases: pre-starter (1–7 days), 2,925 kcal kg^{-1}, 0.92% Ca, and 0.47% available P; starter (8–21 days), 2,980 kcal kg^{-1}, 0.86% Ca, and 0.38% available P; and grower (22–28 days), 3,050 kcal kg^{-1}, 0.75% Ca, and 0.33% available P.

Using the analytical results, digestibility coefficients (DC) were obtained for DM, MM, Ca, P, CP, and nitrogen-corrected apparent metabolizable energy (AMEn), as described by Sakomura & Rostagno (2007):

DC (%) = 100 - [IF × (excreted nutrient / nutrient in the feed) × 100]

AMEn (kcal kg^{-1} DM) = FGE - [(DGE × IF) + 8.22 × NB]

where NB is N balance, representing N in the diet (excreted N× IF); and FGE and DGE correspond to feed gross energy and digesta gross energy, respectively.

For the bone quality analysis, the right and left tibias were extracted from three selected animals from

Table 2. Composition and nutritional profiles of poultry experimental diets containing hybrid phytase and carbohydrases.

Ingredient (%)	Pre-starter (1–7 days)			Starter (8–21 days)			Grower (22–28 days)		
	T1	T2/T4	T3/T5	T1	T2/T4	T3/T5	T1	T2/T4	T3/T5
Corn	48.440	48.440	48.440	52.380	52.380	52.380	50.727	50.727	50.727
Soybean meal (44% crude protein)	44.050	44.050	44.050	40.570	40.570	40.570	40.660	40.650	40.650
Soybean oil	3.240	2.450	2.105	3.385	2.565	2.236	4.456	3.663	3.321
Kaolin	0.045	1.540	1.885	0.040	1.570	1.884	0.526	1.991	2.323
Chromic oxide	-	-	-	-	-	-	0.526	0.526	0.526
Dicalcium phosphate(1)	1.780	0.970	0.970	1.330	0.520	0.520	1.069	0.255	0.255
Limestone(2)	0.970	1.070	1.070	1.120	1.220	1.220	1.010	1.112	1.112
Salt	0.530	0.530	0.530	0.500	0.500	0.500	0.480	0.480	0.480
DL-methionine	0.300	0.300	0.300	0.210	0.210	0.210	0.151	0.151	0.151
L-lysine	0.170	0.170	0.170	0.060	0.060	0.060	0.000	0.000	0.000
Toxin binder(3)	0.185	0.185	0.185	0.185	0.185	0.185	0.185	0.185	0.185
L-threonine	0.080	0.080	0.080	0.010	0.010	0.010	0.000	0.000	0.000
Vitamin premix(4)	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Mineral premix(5)	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050
Monensin sodium (Coban)	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050
Butylated hydroxytoluene	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
500 FTU kg^{-1} phytase(6)	-	T4	T5	-	T4	T5	-	T4	T5
Xylanase + glucanase(7)	-	-	T5	-	-	T5	-	-	T5
Total	100	100	100	100	100	100	100	100	100
				Nutritional profile (calculated)					
AMEn (kcal kg^{-1})	2,925(8)	2,855	2,825	2,980(8)	2,910	2,880	3,050(8)	2,980	2,950
Crude protein (%)	23.65	23.65	23.65	22.14	22.14	22.14	22.01	22.03	22.03
Fat (%)	5.72	4.94	4.60	5.95	5.17	4.83	6.99	6.20	5.86
Crude fiber (%)	3.17	3.17	3.17	3.05	3.05	3.05	3.05	3.05	3.05
Available phosphorous (%)	0.47(8)	0.32	0.32	0.38(8)	0.23	0.23	0.33(8)	0.18	0.18
Total phosphorous (%)	0.74	0.58	0.58	0.64	0.49	0.49	0.59	0.44	0.44
Calcium (%)	0.92(8)	0.76	0.76	0.86(8)	0.70	0.70	0.75(8)	0.59	0.59
Digestible lysine (%)	1.30	1.30	1.30	1.14	1.14	1.14	1.09	1.09/1.14	1.09/1.14
Digestible methionine cystine (%)	0.93	0.93	0.93	0.82	0.82	0.82	0.76	0.76/0.82	0.76/0.82
Digestible methionine (%)	0.59	0.59	0.59	0.49	0.49	0.49	0.43	0.43/0.49	0.43/0.49
Digestible threonine (%)	0.84	0.84	0.84	0.74	0.74	0.74	0.73	0.73/0.74	0.73/0.74
Digestible tryptophan (%)	0.26	0.26	0.26	0.24	0.24	0.24	0.25	0.25/0.24	0.25/0.24

(1)Quantities: 210 g kg^{-1} minimum calcium, 250 g kg^{-1} maximum calcium, and 180 g kg^{-1} phosphorus. (2)33% minimum quantity of calcium. (3)Mycotoxin binder with aluminosilicates, yeast wall, and activated carbon. (4)Composition of the product (guarantee levels per kilogram of product): 11,000,000 I.U. vitamin A, 4,000,000 I.U. vitamin D3, 55,000 I.U. vitamin E, 3,000 mg vitamin K3, 2,300 mg vitamin B1, 7,000 mg vitamin B2, 12 g pantothenic acid, 4,000 mg vitamin B6, 25,000 μg vitamin B12, 60 g nicotinic acid, 2,000 mg folic acid, 250 mg biotin, and 300 mg selenium. (5)Composition of the product (guarantee levels per kilogram of product): 100 g iron, 20 g copper, 130 g manganese, 130 g zinc, and 2,000 mg iodine. (6)Natuphos E 10000G (BASF, Ludwigshafen, Germany), 0.005 kg 100 kg^{-1} of the diet. (7)Natugrain TS (BASF, Ludwigshafen, Germany), 0.010 kg 100 kg^{-1} of the diet. (8)According to the recommendations of the Brazilian tables for poultry and swine (Rostagno, 2011) for the regular performance of male broilers.

each experimental unit and tested for breaking force (kgf), rigidity (kgf mm^{-1}), and flexibility (kg s^{-1}). Bone strength is typically measured as applied force per bone area. The ratio of force and distance (distance of probe movement until bone rupture) corresponds to bone rigidity (Świątkiewicz & Arczewska-Wlosek, 2012), whereas bone flexibility is represented by area and is measured as bone deformity as a function of applied force (Gopinger et al., 2017). For the analysis, the TA.XTPlus texture analyzer (Texture Technologies Corporation, Hamilton, MA, USA) was used, being connected to the Exponent software (Stable Micro Systems, Surrey, United Kingdom).

After the physical analysis, the bones were dried at 105°C to measure DM (Zenebon et al., 2008). The tibias were then subjected to total ash analysis (Compêndio..., 2009). Once this was completed, the six tibias from each experimental unit were pooled and ground, stored in plastic bags, and subsequently analyzed for Ca content, using atomic absorption, and for P and Zn contents, using flow injection analysis, as described by method 965.17 of AOAC (Cunniff, 1995).

The data were subjected to the analysis of variance. After the least-squares means procedure, means were compared by Tukey's test. All analyses were performed using the R statistical software (R Core Team, 2015), at 5% probability.

Results and Discussion

The experimental diets presented significantly different AMEn contents than expected due to the strategy used for their formulation, in which a nutritional matrix for enzymes was adopted (Table 3). The diets that were not supplemented with enzymes (T2 and T3) yielded lower AMEn values of 3,203 and 3,192 kcal kg^{-1}, respectively, when compared with that of 3,368 kcal kg^{-1} of the positive control (T1). However, the diets supplemented with enzymes (T4 and T5) showed a recovered AMEn, with an average of 3,273 and 3,216 kcal kg^{-1}, respectively, representing an increase of 2.16 and 0.76% for T4 versus T2 and for T5 versus T3, indicating improved energy availability as a consequence of enzyme supplementation.

The hybrid phytase was able to replace the applied energy reduction of 70 kcal kg^{-1} DM, indicating that phytase acts not only on the provision of P but also on that of other nutrients, which can be metabolized as energy by birds. Phytase, associated with carbohydrases, also increased AMEn in 24 kcal kg^{-1} DM; however, the energy reduction in these diets was greater, being of 100 kcal kg^{-1} DM. In the diets supplemented with enzymes, the reduced AMEn level was restored to one statistically equivalent to that of the positive control.

When consolidating data of 17 studies on different diets – most made of wheat and sorghum and a few of corn and soybean, with an average phytase inclusion rate of 662 units per kilogram (range from 400 to 1,200 units) –, Lei et al. (2013) observed that the amino acid response in poultry fed with enzymes was inconsistent and the energy response was less than 100 kcal kg^{-1} of diet (0.36 MJ kg^{-1}).

For the efficacy of enzymes during their supplementation, it should be taken into account that

Table 3. Values of nitrogen-corrected apparent metabolizable energy (AME_n) on a dry matter basis and of ileal digestibility coefficients of crude protein (CP), dry matter (DM), mineral matter (MM), calcium, and phosphorus in broilers fed diets containing a hybrid phytase associated with carbohydrases (mean ± standard deviation)(1).

Treatment(2)	AMEn (kcal kg^{-1} DM)	Ileal digestibility coefficients on a dry matter basis (%)				
		CP	DM	MM	Ca	P
T1	3,368.40±100.42a	85.02±1.65a	71.21±1.96a	41.20±3.18a	41.88±15.81	55.45±6.80c
T2	3,203.36±129.61b	81.79±2.23b	64.72±2.80c	31.55±1.91c	41.52±10.16	58.93±10.08c
T3	3,192.17±183.38b	81.63±2.93b	66.16±2.21bc	33.11±2.98c	49.96±13.56	62.05±9.10bc
T4	3,273.77±164.03ab	83.71±2.82ab	68.21±2.55b	37.73±2.72b	48.72±9.30	70.10±8.49ab
T5	3,216.62±161.65ab	83.12±2.78ab	65.74±3.83bc	37.13±4.52b	51.59±10.30	72.48±11.36a
Probability	0.01	<0.0001	<0.0001	<0.0001	0.07	<0.0001

(1)Means followed by equal letters, in the columns, do not differ from each other by Tukey's test, at 5% probability. (2)T1, positive control basal diet (BD) with corn and soybean meal; T2, BD with reduction of 70 kcal kg^{-1}, 0.16% Ca, and 0.15% available P; T3, BD with reduction of 100 kcal kg^{-1}, 0.16% Ca, and 0.15% available P; T4, T2 + 500 phytase units (FTU) per kilogram; and T5, T3 + 500 FTU kg^{-1} + 560 xylanase units per kilogram + 250 glucanase units per kilogram.

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corn-soybean meal-based diets contain a lower amount of non-starch polysaccharides such as arabinoxylans and β-glucans (Stefanello et al., 2016; Slominski, 2011). This is important since the presence of a substrate affects enzyme action, with more expressive results expected when carbohydrates are supplemented in diets with a greater amount of substrate, such as those containing wheat, rye, and barley (Zarghi, 2018). The results of the present study are indicative of the positive action of the use of carbohydrases in association with hybrid phytase in diets based on corn and soybean meal, which allowed a reduction of 100 kcal kg^{-1} in diet composition, representing an extra contribution of 30 kcal kg^{-1} AMEn. This confirms a previous study conducted with broiler chickens by Krabbe et al. (2014), who tested the Naturgrain TS enzyme package containing xylanase and glucanase and found a 32 kcal kg^{-1} increase in metabolizable energy in diets based on corn and soybean meal.

Despite the significant difference verified between treatments for DM digestibility, there was no evidence of enzyme action increasing nutrient digestibility. However, the improvement of the ileal apparent digestibility coefficients of CP, MM, and P as a consequence of enzyme supplementation was clearly observed (Table 3).

The T2 and T3 treatments, with nutritional reductions and without enzyme supplementation, negatively affected CP digestibility. When phytase or phytase + xylanase + glucanase were introduced in T4 and T5, respectively, the CP in the diets was restored, reaching digestibility coefficients equivalent to those of T1, the control.

For the MM digestibility coefficient, although the T4 and T5 treatments did not reach means equivalent to those of the positive control, there was an increase of 16.38% for T4 versus T2 and of 10.83% for T5 versus T3. For P digestibility, there was an even greater positive effect of enzyme supplementation, leading to high levels of 70.10 and 72.48%, respectively, for T4 and T5, in comparison with that of 55.93% for the control. In contrast, the digestibility of Ca was relatively unaffected, which could be explained by a high dietary Ca content. Recent data are indicative that Ca:P ratios are critical for these kinds of results (Bavaresco et al., 2020a).

Regarding mineral deposition in chicken tibias, the positive action of the enzymes was confirmed by the responses of MM and P (Table 4). Enzymatic supplementation increased mineral content in broiler bones by 4.09% for T4 versus T2 and by 4.87% for T5 versus T3. The deposition of P in the tibias was also improved by the use of enzymes, with an increase of 4.33% for T4 versus T2 and of 7.18% for T5 versus T3. In contrast, no significant effects were observed for Ca and Zn.

Dersjant-Li et al. (2015) concluded that the supplementation of broiler diets based on corn-soybean meal with 500 and 1,000 FTU kg^{-1} Buttiauxella phytase compensated for a reduction in nutrients (available P, amino acids, metabolizable energy, Na, and Ca) and maintained growth performance and bone ash over a 42 day period, when compared with diets with the recommended phytase levels of 500 and 1,000 FTU kg^{-1}. The experimental diets showed three Ca reduction levels equivalent to 0.13, 0.16, and 0.23% and to 0.16,

Table 4. Tibia mineral mater (MM), calcium, phosphorus, and zinc content of 28-day-old male Cobb 500 broilers (mean ± standard deviation)[(1)].

Treatment[(2)]	Mineral composition on a dry matter basis (%)			
	MM (%)	Ca (%)	P (%)	Zn (mg kg^{-1})
T1	45.71±1.17a	15.38±1.20	7.02±0.39ab	162.68±11.42
T2	43.15±1.36b	14.69±1.05	6.64±0.47ab	161.12±12.60
T3	43.00±1.29b	14.68±1.66	6.60±0.52b	163.61±8.33
T4	44.99±1.22a	15.24±1.46	6.94±0.48ab	167.42±9.18
T5	45.20±1.11a	15.21±1.05	7.11±0.53a	167.66±12.44
Probability	<0.0001	0.44	0.01	0.38

[(1)]Means followed by equal letters, in the columns, do not differ from each other by Tukey's test, at 5% probability. [(2)]T1, positive control basal diet (BD) with corn and soybean meal; T2, BD with reduction of 70 kcal kg^{-1}, 0.16% Ca, and 0.15% available P; T3, BD with reduction of 100 kcal kg^{-1}, 0.16% Ca, and 0.15% available P; T4, T2 + 500 phytase units (FTU) per kilogram; and T5, T3 + 500 FTU kg^{-1} + 560 xylanase units per kilogram + 250 glucanase units per kilogram.

0.19 and 0.23% in the treatments with 500 and 1,000 FTU kg^{-1} phytase, respectively, when compared with the positive control. The reductions in available P in these treatments were of 0.146 and 0.174%, respectively.

The bone strength, rigidity, and flexibility of the broilers fed nutritionally reduced diets were significantly decreased (Figure 1). However, supplementation with phytase alone or combined with enzymes improved all three, indicating nutritional matrix compensation for Ca and available P content.

Strategically, the reduction of 0.16% Ca and 0.15% available P was applied to the dietary nutritional profile, considering that phytase activity is also influenced by the contents of Ca and P. Higher dietary levels of Ca reduce phytase activity by increasing gastrointestinal pH (Dersjant-Li et al., 2015), a condition that favors the formation of complexes between phytate and metallic cations with large ionic rays (Oh et al., 2004), with Ca^{2+}, Zn^{2+}, Co^{2+}, Mn^{2+}, Mg^{2+}, Fe^{2+}, and Cu^{2+} being the most susceptible (Kornegay, 2001). The increase in P, which is the final product of the phytase catalytic reaction, reduces the hydrolysis of phytate due to the self-regulation of the enzymatic reaction, by identifying a greater availability of the final product and decreasing the action of the enzyme (Oh et al., 2004). The dietary supplementation with 500 FTU kg^{-1} hybrid phytase was efficient for P dephosphorylation from phytate, increasing the availability of this mineral for bone deposition, besides reducing the chelating capacity of phytate.

Phytase and carbohydrate supplementation allows nutritionists to reduce energy and P contents in diets (Francesch & Geraert, 2009), both known as expensive ingredients that impact production costs (Shirley & Edwards Jr., 2003). Bavaresco et al. (2020b), using the same enzymes (500 FTU kg^{-1} + 560 TXU kg^{-1} + 250 TGU kg^{-1}) and nutritional reductions (100 kcal kg^{-1} AMEn, 0.16% Ca, and 0.15% available P), reported a lower production cost when chickens were fed with diets formulated with an optimized nutritional matrix with enzymatic association. Based on the present study, the combination of a hybrid phytase with specific carbohydrases (xylanase and glucanase) increases the metabolizable energy content and also the CP and P digestibility of the diets formulated with a reduction of 100 kcal kg^{-1}, 0.16% Ca, and 0.15% available P, besides maintaining broiler bone quality by restoring mineral and P contents in the tibia, which results in greater bone strength, resistance, and flexibility.

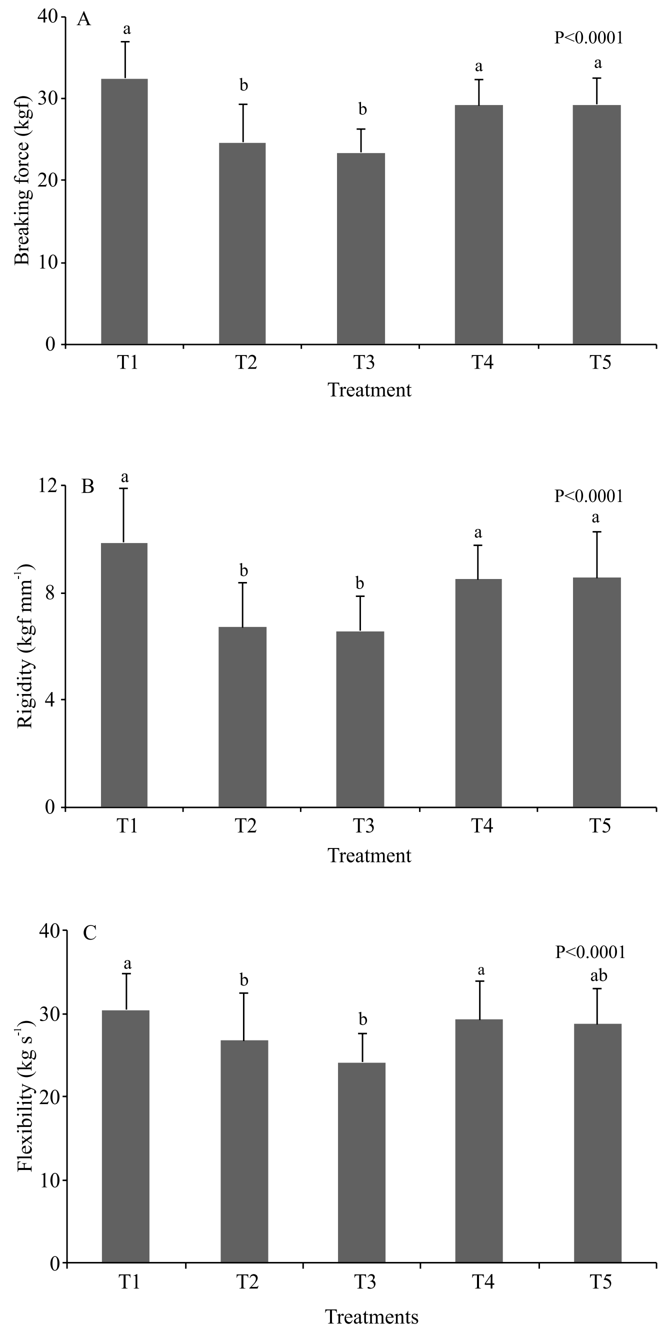

Figure 1. Tibia breaking force (A), rigidity (B), and flexibility (C) of 28-day-old male Cobb 500 broilers fed with a hybrid phytase associated with carbohydrases. Means followed by lowercase letters differ from each other by Tukey's test, at 5% probability. T1, positive control basal diet (BD) with corn and soybean meal; T2, BD with reduction of 70 kcal kg^{-1}, 0.16% Ca, and 0.15% available P; T3, BD with reduction of 100 kcal kg^{-1}, 0.16% Ca, and 0.15% available P; T4, T2 + 500 phytase units (FTU) per kilogram; and T5, T3 + 500 FTU kg^{-1} + 560 xylanase units per kilogram + 250 glucanase units per kilogram.

The experimental model was functional, confirmed by losses in nutrient digestibility and bone resistance due to the nutritional decrease used for the establishment of the experimental diets in the absence of enzyme supplementation. The addition of a 500 FTU kg^{-1} hybrid phytase, either alone or combined with carbohydrases, to poultry diets with nutritional reductions of 0.16% Ca, 0.15% available P, and 70 kcal kg^{-1} metabolizable energy is effective on ileal nutrient digestibility coefficients and broiler bone quality. However, there is still the need for further studies on the synergic effect of this phytase in association with carbohydrases.

Conclusions

1. The use of 500 phytase units (FTU) per kilogram of hybrid phytase in diets with pelleted corn-soybean meal allows a good digestive performance by broilers and replaces the nitrogen-corrected apparent metabolizable energy (AMEn) at 70 kcal kg^{-1}, as well as 0.16% Ca and 0.15% available P.

2. The association of 500 FTU kg^{-1} hybrid phytase and carbohydrases (560 xylanase units per kilogram + 250 glucanase units per kilogram) in diets with pelleted corn-soybean meal, when adopting an optimized nutritional matrix with 100 kcal kg^{-1} AMEn, 0.16% Ca, and 0.15% available P, is efficient for most studied parameters, including crude protein digestibility, phosphorus digestibility, bone mineral content, and resistance of broilers at 28 days of age.

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