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ANIMAL PRODUCTION

Phytase in diets with different phytate concentrations for broilers

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ABSTRACT: *This study evaluated the effect of including different doses of phytase in broiler diets with different levels of phytate and reduced levels of calcium (Ca) and available phosphorus (aP), on broilers performance, digestibility (CAID), and bone characteristics. One thousand four hundred and four Cobb®500 broilers were used, distributed in a complete randomized design, and a 2x3 factorial arrangement, with two phytate levels (4.25 kg/ton - MP and 6.0 kg/ton - HP) and three phytase levels (0, 750, and 1500 FTU/kg) totalizing six treatments with nine replicates each. The animals that received phytase in their diet showed higher average feed intake and weight gain, and higher bone weight, % mineral residue, % Ca, and % P, when compared to the treatment without phytase (P < 0.01). Animals that received a diet containing HP with the inclusion of 1500FTU/kg phytase had the best CAID of nutrients (P < 0.0001). Regarding phytate, animals that received the MP diet showed a higher CAID of dry matter, and higher %P in the tibia when compared to animals that received the HP diet. The addition of phytase to diets with reduced Ca and aP levels can improve the CAIDs and bone characteristics in the presence of high phytate levels. However, reducing Ca and aP levels in the diet without adding phytase impairs the performance of broilers and has no effect on their carcass and cuts yield.*

Key words: *Calcium, Digestibility, Enzyme, Antinutritional factor., Phosphorus.*

Fitase em dietas com diferentes concentrações de fitato para frangos de corte

RESUMO: *Este estudo teve como objetivo avaliar o efeito da inclusão de diferentes doses de fitase em rações para frangos de corte com diferentes níveis de fitato e níveis reduzidos de cálcio (Ca) e fósforo disponível (Pd) sobre seu desempenho. Foram utilizados 1.404 frangos machos Cobb®500, distribuídos em delineamento inteiramente casualizado, em esquema fatorial 2x3, com dois níveis de fitato (4,25 kg / ton - MP e 6,0 kg / ton - HP) e três níveis de fitase (0, 750 e 1500 FTU / kg), com nove repetições por tratamento. Foram avaliados os parâmetros de desempenho, e aos 21 dias de idade, quatro frangos por repetição foram abatidos para coleta do conteúdo ileal para análise do coeficiente de digestibilidade ileal aparente (CDIA), e foram removidas a tíbia direita de um frango por repetição para quantificar seu peso e porcentagem de resíduo mineral (% RM), Ca e P. Aos 42 dias de idade, uma ave por repetição foi abatida para medir o rendimento de carcaça e cortes. Os animais que receberam fitase na dieta apresentaram maior consumo médio de ração e ganho de peso médio, e maior peso ósseo, % RM, % Ca e % P, quando comparados ao tratamento sem fitase (P < 0,01). Os animais que receberam dieta contendo HP com inclusão de 1500FTU / kg de fitase apresentaram os melhores CDIAs de nutrientes (P < 0,0001). Em relação ao fitato, os animais que receberam a dieta MP apresentaram maior CDIA de matéria seca e maior %P na tíbia quando comparados aos animais que receberam a dieta HP. A adição de fitase a dietas com níveis reduzidos de Ca e Pd pode melhorar os CDIAs e as características ósseas na presença de níveis elevados de fitato. No entanto, a redução dos níveis de Ca e Pd na dieta sem adição de fitase prejudica o desempenho dos frangos de corte e não afeta o rendimento de carcaças e cortes.*

Palavras-chave: *cálcio, digestibilidade, enzima, fator antinutricional, fósforo.*

INTRODUCTION

Phytate is widely studied, so we can understand its effects when present in monogastric diets, as it is known as an anti-nutritive factor in broiler diets (SANTOS et al., 2014; GIBSON et al., 2018; INGELMANN et al., 2019). This molecule contains approximately 80% of the P present in plant seeds and has the ability to interact and complex with other dietary components, which reduces the ability to absorb nutrients such as Ca, zinc, iron, manganese, proteins, peptides, and amino acids (ABD EL-HACK et al., 2018). Besides, it increases the endogenous loss of these compounds and affects feed utilization by birds (COWIESON et al., 2008; GIBSON et al., 2018; ABBASI et al., 2019). One of the options

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to mitigate these effects is the use of exogenous enzymes, such as phytase.

Phytase is an enzyme that can hydrolyze phytate and, when present, can reduce its anti-nutritive effects, as well as releasing phytic P (ABBASI et al., 2019). Due to phytase's ability to hydrolyze phytate, this enzyme caused corn and soybean meal-based diets to become more economically viable, as P is the third most costly component in poultry feed formula, behind energy and amino acids, and other ingredients with a greater amount of phytic P can be included in the diet without harming the animals' performance (LAMID et al., 2018).

In addition to improving the use of the diet by animals, the use of exogenous phytase also allows a reduction in the use of inorganic P and a decrease in the excretion of P not absorbed in the environment (ABBASI et al., 2019).

The efficiency of phytase use depends on several factors, such as substrate concentration, pH, exposure time, age of the birds, dietary calcium and P levels, and temperature. Despite being thermostable, most phytases resist only up to 70 °C, and the pelleting process exceeds this temperature which can decrease enzyme activity (TAMIM et al., 2004; SANTOS et al., 2014; ABBASI et al., 2019). At some growing phases, the metabolism of calcium and P is mediated by the same hormones and the uptake of the two minerals in the intestine is linked. Also, during the first 2 weeks post hatching broilers were able to utilize P more efficiently than at an older age, (BABATUNDE et al., 2019a,b). The availability of Ca and P and their proportion in the diet influences their intestinal absorption, as an increase in intestinal availability and absorption of P negatively influences the metabolism of Ca and an overload of P will negatively impact their own metabolism. This seems to be especially true when calcium intake is moderate to low, as in those of grain-based, phosphate-rich diets (VÖTTERL et al., 2020).

It is important to continue with research involving phytases as there are a wide variety of types on the market, and if any are able to further reduce the amount of supplemental phosphorus and calcium in the diet, the poultry industry will certainly be interested. Considering the demand for more information related to phytate and phytase and the interactions between Ca and P during their absorption, this evaluated the inclusion of phytase at different doses in broiler diets with different levels of phytate and reduced levels of Ca and aP on their performance parameters, carcass yield, ileal digestibility, and bone composition.

MATERIALS AND METHODS

Animals and facilities

A total of 1404 male *Cobb® 500* (Cobb-Vantress, Inc.) broilers were housed in an experimental farm, in pens, from 1 to 42 days of age. The animals were distributed in a completely randomized design, in a 2x3 factorial arrangement, which totals six treatments and nine replications. Each pen (2.06 m²) contains 26 animals and was considered a replication. The room temperature control during the experimental period followed the management manual of the broiler breeding company and was done by the opening and closing of curtains. Feed and water were provided *ad libitum* in tubular feeders and *nipple* water drinkers throughout the experimental period.

Experimental diets

The diets had reduced levels of calcium (Ca) and available phosphorus (aP), -0.192 and -0.175 respectively, and 2 levels of phytate (medium, MP, and high, HP). Those were tested with three levels of phytase (0, 750, and 1500 FTU/kg phytase Quantum Blue, E. coli, AB Vista, Marlborough, UK - IUB: 3.1.3.26). FTU refers to phytase units and is defined as the amount of enzyme required to hydrolyze 1 μmol inorganic P per minute from 0.0051 mol/L sodium phytate at pH 5.5 and a temperature of 37 °C. The MP treatments were formulated based on corn and soybean meal and an average phytate level of 4.25 kg/ton feed, while the HP treatments were formulated based on corn, soybean meal, and defatted rice bran, and contained an average phytate level of 6.00 kg/ton feed (ROSTAGNO et al., 2011; table 1).

The starter phase diet was used for the digestibility trial and contained 2% of the inert compound celite (Celite, Celite Corp., Lompoc, CA), included as an indigestible internal marker for the digestibility calculations. All diets were provided in mash form and had an average geometric diameter (GMD) of 728μm.

Zootechnical performance

The birds were weighed on the housing day, and at 21, 35, and 42 days of age for weight gain (WG) evaluation. During this period, the volumes of feed provided and the leftovers in each experimental unit (pen) were also weighed to determine feed intake (FI). Feed conversion (FC) was calculated by the ratio of FI and WG. Mortality was checked daily by measuring the weight of the dead bird.

Ileal digestibility test and bone characteristics

All birds were fed with the starter diet (Table 1) until 21 days of age, in order to determine the apparent digestibility coefficient (CAID) of dry matter (DM), crude protein (CP), and gross energy (GE).

To collect ileal contents, four birds per replicate were slaughtered by cervical dislocation and eviscerated for removal of the ileal portion. The ileal portion was defined as the segment between 4cm after the Meckel's diverticulum and 4cm before the ileocecocolic junction. The ileal contents were manually removed by compression and packed in identified plastic pots. All samples were stored in a freezer at -5 °C for later analysis.

Next, the ileal content samples were thawed, homogenized, and dried in an oven at 55 °C, until they reached a constant weight, and then ground in a Willey mill to a particle size of 1mm. The already

Table 1 - Ingredients and calculated chemical composition of the experimental diet used in the initial, growth, and final periods for superior performance broilers.

	-------------Initial--------------		------------Growth-------------		--------------Final-----------	
Ingredients (g/kg)[1]	Medium Phytate	High Phytate	Medium Phytate	High Phytate	Medium Phytate	High Phytate
Corn	551.6	478.6	664	611.8	642.01	609.7
Soybean oil	35.3	44.9	10.3	17.2	31.41	38.29
Soybean meal 48%	362.2	356	299.6	295.1	274.24	269.77
Limestone	11.5	11.7	10.5	10.6	9.19	9.31
Monocalcium phosphate	6.6	6.1	3.2	2.8	1.44	1.08
Salt	4.1	4.1	3.7	3.7	3.82	3.8
Econase XT[2]	0.1	0.1	0.1	0.1	0.06	0.06
Vitamin and mineral premix[3]	3	3	3	3	3	3
L-Lysine HCl	2	2	3	3	2.12	2.09
DL-methionine 99%	3.2	3.2	2.7	2.7	2.57	2.57
L-Threonine	0.3	0.3	0.1	0.2	0.15	0.19
Celite	20	20	0	0	0	0
Raw Rice Bran	0	70	0	50	0	50
----------Nutritional composition----------						
*Crude protein %	22.45	21.86	21.02	20.89	18.94	19.12
*Crude fat %	6.23	6.88	4.01	4.91	6.03	6.93
*Crude fiber %	2.72	2.67	2.33	2.70	2.65	2.32
*Ashes %	5.91	6.65	2.02	2.87	5.15	5.56
**Calcium %	0.69	0.69	0.57	0.57	0.48	0.48
**Sodium %	0.18	0.18	0.17	0.17	0.17	0.17
**Available Phosphorus %	-	-	0.18	0.18	0.14	0.14
**Phytic phosphorus %	0.24	0.33	0.24	0.3	0.23	0.29
*Metabolisable energy MJ/kg	13.00	13.00	13.09	13.09	13.51	13.51
**Digestible lysine %	1.25	1.25	1.18	1.18	1.04	1.04
**Digestible methionine %	0.62	0.62	0.55	0.55	0.52	0.52
**Digestible Met+Cys %	0.92	0.92	0.83	0.83	0.78	0.78
**Digestible threonine %	0.78	0.78	0.7	0.7	0.65	0.65
**Digestible tryptophan %	0.25	0.25	0.22	0.22	0.2	0.2
**Digestible valine %	0.94	0.94	0.85	0.85	0.79	0.79

[1]750 and 1500 FTU/kg in the treatments with its inclusion (Quantum Blue, AB Vista, Marlborough, UK).
[2]Econase XT 25, AB Vista, Wiltshire, UK.
[3]Content per kilogram of diet A, 15000 IU; vit. D3, 5000 IU; vit. E, 100mg; vit. K, 5mg; folic acid, 3mg; nicotinic acid, 75mg; pantothenic acid, 25mg; riboflavin, 8mg; thiamine, 5mg; pyridoxine, 7mg; biotin, 300qg; choline, 400mg; vit. B12, 20qg; iodine, 2mg; selenium, 200qg; copper, 20mg; iron, 50mg; manganese, 120mg; zinc, 100mg.
Met+Cys: Methionine plus Cysteine.
*Determined values.
**Calculated. values.

dried samples of ileal content and experimental diets were oven-dried at 105 °C according to AOAC methodology (1995) to determine the CAID of dry matter (DM, method 934.01), crude protein content (CP, method 954.01), and for gross energy (GE), which was determined using an isoperibol bomb calorimeter using benzoic acid as the calibration standard (Model 1261, Parr Instrument Co., Moline, IL).

The acid-insoluble ash content (IAC) of the diet and ileal content samples was determined according to the methodology described by SCOTT & BOLDAJI (1997). The digestibility of the dietary fractions was determined using the IAC as an external indicator to calculate the indigestibility factor (IF): IF = diet IAC/IAC ileal content. The ileal digestibility coefficient (IDC) of the parameters DM, CP, and GE was calculated according to the equation: Parameter's IDC = (Parameter in diet) - (Parameter in ileal content × IF) / Parameter in diet.

The quantification of phytate was done by the colorimetric method, derived from the method described by HEUBNER & STANDLER (1914), which is based on the reaction between the ferric ion Fe^{3+} and the sulfosalicylic acid, known as Wade's reagent (WR), and corrections of the purified sample according to the methodology described by LATTA & ESKIN (1980).

For analysis of bone characteristics, were analyzed one bird for each replicate to remove the tibia of its right leg. For quantification of total ash, calcium and phosphorus, the tibias were removed and stripped by hand, cleaned, defatted, and submerged in ethyl ether for 3 days. After this period the bones were left in open air until all ether had evaporated, they were dried in an oven at 105 °C and weighed on scales accurate to 0.0001 g. After weighing, the tibias were calcined in a muffle furnace at 600 °C for 8 hours and then ground until they reached 1mm particle size for further analysis of the mineral residue (MR), calcium (Ca) and phosphorus (P) contents according to the AOAC (1995) methodology.

Carcass and cuts yield

At 42 days of age, one bird per experimental unit - totaling nine birds per treatment - was identified and weighed to evaluate carcass and cuts yield. The birds were identified with a numbered plastic seal on one of the legs. After slaughter, removal of feathers, head, feet, and viscera, the carcasses were washed and cooled in a chiller for 60 minutes at 2 °C. After this period, the birds were transferred to the cutting room, where breast with skin and bone, and thigh + drumstick were separated. Carcass yield was determined by the ratio between the live weight of each bird and the weight of its carcass, while the yields of thigh + drumstick and breast with skin and bone were determined by the ratio between the weight of the carcass and the parts.

Statistical analysis

Each pen was considered an experimental unit for performance data analysis (AWG, AFI, FC). Each animal was considered an experimental unit for the variables: carcass yield, cuts yield and bone characteristics. The pool of 4 animals was considered an experimental unit for the digestibility data (CAID of DM, CP, and GE). Initially, all data were analyzed for normality using the Shapiro-Wilk test and all parameters were found to be normal ($P > 0.05$). Then homoscedasticity analysis was performed using Bartlett's test. Subsequently, the data were submitted to analysis of variance (ANOVA) and the means were compared using Tukey's test at 5% significance level. All data were analyzed in the statistical software SAS (SAS Inst., Inc., Cary, NC, USA).

RESULTS

At 21, 35, and 42 days of age was observed higher AFI and AWG in animals that received 750 and 1500 FTU/kg of phytase in their diet compared to those that received diet without phytase ($P < 0.05$). However, the inclusion of phytase did not change FCR between treatments ($P > 0.05$). The level of phytate in the diet did not affect any of the performance parameters evaluated ($P > 0.05$; Table 2). The treatments also had no effect on the carcass, breast, and thigh and drumstick yield ($P > 0.05$; Table 3).

Regarding CAIDs, with the average phytate level, phytase at 1500 FTU/kg presented the lowest CAID for DM and GE, while phytase at 750 FTU/kg presented the highest CAID for CP ($P < 0.05$). With high phytate level, phytase at 750 and 1500 FTU/kg was efficient in increasing the CAID of DM, CP, and GE in relation to treatment without its inclusion ($P > 0.05$), and the two levels of phytase inclusion had no effect on these parameters ($P > 0.05$). With medium and high phytate levels, there was a difference in the CAID of DM, CP and GE with inclusion of phytase at 0 and at 1500 FTU/kg ($P < 0.05$). With phytase inclusion at 750 FTU/kg, phytate levels differed only for CAID of DM ($P < 0.05$; Table 4).

Regarding bone characteristics, there was no effect of the interaction between phytate levels and phytase inclusion upon them ($P > 0.05$). The animals that received diet containing phytase (750 and 1500

Table 2 - Average feed intake (AFI), average weight gain (AWG) and feed conversion ratio (FCR) of broilers fed two different phytate levels and three phytase levels in a diet with reduced calcium and phosphorus at 21, 35, and 42 days of age (Mean±Standard Error of the Mean (SEM)).

		-----------------AFI (g)-----------------			---------------AWG (g)----------------			-----------------FCR (g/g)-------------		
Phytate	Phytase FTU	21 days	35 days	42 days	21 days	35 days	42 days	21 days	35 days	42 days
Medium phytate	0	1032± 8.6	3057± 30.8	4201± 27.1	726± 11.4	1761± 25.4	2336± 30.4	1.42± 0.01	1.73± 0.01	1.79± 0.01
	750	1135± 18.0	3302± 41.6	4541± 43.9	794± 6.8	1906± 24.6	2543± 21.1	1.43± 0.01	1.73± 0.01	1.78± 0.01
	1500	1097± 8.0	3265± 26.7	4500± 36.3	777± 10.9	1921± 22.6	2571± 27.4	1.41± 0.02	1.70± 0.01	1.75± 0.01
High phytate	0	1062± 10.0	3136± 23.0	4328± 25.9	748± 8.7	1833± 16.1	2453± 19.8	1.42± 0.01	1.71± 0.01	1.76± 0.01
	750	1134± 14.7	3330± 31.7	4581± 28.6	791± 8.6	1912± 26.8	2581± 50.0	1.43± 0.01	1.74± 0.01	1.77± 0.01
	1500	1097± 21.0	3265± 40.4	4490± 41.4	772± 16.1	1912± 22.2	2532± 26.0	1.42± 0.01	1.71± 0.01	1.77± 0.01
------------------Independent Effects------------------										
------------------Phytate levels------------------										
Medium		1088± 6.9	3208± 19.1	4414± 23.2	766± 6.3	1862± 14.9	2483± 16.5	1.42± 0.01	1.72± 0.01	1.78± 0.01
High		1098± 10.3	3244± 19.9	4466± 19.6	770± 7.8	1886± 14.9	2522± 20.5	1.43± 0.01	1.72± 0.01	1.77± 0.01
------------------Phytase FTU------------------										
0		1047± 7.1b	3097± 20.2b	4264± 19.6b	737± 7.0b	1797± 15.0b	2395± 18.0b	1.421± 0.01	1.72± 0.01	1.78± 0.01
750		1135± 11.2a	3316± 25.8a	4561± 28.1a	792± 5.4a	1909± 17.9a	2562± 25.4a	1.433± 0.01	1.74± 0.01	1.78± 0.01
1500		1097± 10.6a	3265± 23.0a	4495± 26.6a	775± 9.5a	1917± 15.4a	2551± 18.6a	1.416± 0.01	1.70± 0.01	1.76± 0.01
CV%*		0.18	0.04	0.03	0.18	0.09	0.06	156.00	101.00	98.06
P Phytate		0.520	0.248	0.167	0.579	0.290	0.168	0.786	0.965	0.440
P Phytase		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.577	0.188	0.231
Phytate vs Phytase		0.618	0.552	0.329	0.360	0.281	0.084	0.940	0.543	0.085

Lowercase letters in 'independent effects' differ from each other by Tukey Test at 5% probability.
*Coefficient of Variation.
P: Phosphorus.

FTU/kg) had higher bone weight and higher contents of mineral residue, calcium, and phosphorus (% P), when compared to the treatment without phytase ($P < 0.01$). Animals fed the medium phytate diet had higher %P when compared to animals fed the high phytate diet ($P < 0.05$; Table 4).

DISCUSSION

It is possible to observe a clear effect of phytase on the availability of P in the diet, since in the treatments with supplementation of 750 or 1500 FTU/kg of phytase the performance and CAID results were superior to the treatment without its addition. This is in line with the findings of INGELMANN et al. (2019), who reported a 15% increase on the availability of phosphorus when evaluating the inclusion of phytase in the diet.

According to COWIESON et al. (2008), phytate is an anti-nutritive factor that can interfere with the absorption and digestion of minerals and nutrients, thus worsening birds' performance.

Table 3 - Carcass and part yields (%) of broilers fed with two different levels of phytate and three levels of phytase in diet with reduced calcium and phosphorus at 42 days of age (Mean±Standard Error of the Mean (SEM)).

Phytate	Phytase	Yield		
		Carcass	Chest	Thigh+drumstick
Medium phytate	0 FTU	78.70±0.38	37.02±0.77	28.34±0.59
	750 FTU	79.00±3.78	36.51±0.87	28.59±0.60
	1500 FTU	79.62±0.68	36.62±0.71	27.25±0.51
High phytate	0 FTU	78.59±0.37	37.07±0.53	28.30±3.12
	750 FTU	79.58±0.64	36.23±0.63	28.13±0.27
	1500 FTU	80.12±0.65	36.50±0.75	28.49±0.44
----------Independent Effects----------				
----------Phytate levels----------				
Medium		79.11±1.27	36.60±0.44	28.06±0.33
High		79.43±0.31	36.72±0.35	28.30±1.15
----------Phytase levels----------				
0 FTU		78.64±0.27	37.04±0.47	28.32±1.58
750 FTU		79.29±2.00	36.37±0.53	28.36±0.34
1500 FTU		79.87±0.46	36.56±0.50	27.87±0.37
CV%*		2.82	6.67	7.96
P Phytate		0.513	0.850	0.600
P Phytase		0.130	0.648	0.627
Phytate vs Phytase		0.818	0.976	0.297

*Coefficient of Variation.
P: phosphorus.

However, we did not observe differences between treatments, considering only the different levels of phytate in the diet on birds' performance, nor interaction effects between phytate levels and the inclusion of phytase. This differs from the data reported by SANTOS et al. (2014), who observed improvement in broiler performance when the level of phytate in the diet was reduced from 10.65 to 6.40 g/kg. These authors also found better performance in animals fed with diet with the highest level of phytate and inclusion of phytase, showing that there was an interaction effect between the two factors. However, these results agree with BABATUNDE et al. (2021), who also reported no correlation between the level of phytic P in the diet and the inclusion of phytase on performance parameters and bone characteristics of the animals.

When evaluating only the inclusion of phytase, we noticed a main influence of the reduction of P - that came from the lower use of phytate by the birds – on the reduction in AFI and AWG in treatments without phytase addition. This is because, according to MCDOWELL (1992), loss of appetite is one of the first clinical signs of P deficiency due to its participation in energy metabolism and ATP formation. This leads to a reduction in the weight gain of the birds, as occurred in this study.

There was no difference between treatments on Carcass and cuts yields, showing that probably the reduction of Ca and P levels does not influence the yield of prime cuts, as breast or thigh and drumstick. The same occurred in studies by ATIA et al. (2000) and AKTER (2016), who reported no difference between treatments when working with different levels of phytase and reduction of inorganic phosphorus for broilers from 1 to 42 days.

These results go against the expected according to a review published by COWIESON et al. (2011), who concluded that a high dose of phytase can increase birds' carcass yield. The authors explain; however, that contrary to what is commonly stated, birds have a high activity of endogenous effective phytase and that phytase doses are considered high starting at 2500 FTU/kg. Therefore, the results of the present study showed that the dose used was not sufficient to achieve a higher carcass and cuts yield.

Some studies have attempted to demonstrate the effects of phytase in improving the digestibility of other nutrients such as protein and starch (RAVINDRAN

Table 4 - Digestibility coefficient of dry matter (DM), crude protein (CP) and gross energy (GE) and bone characteristics (tibia weight, mineral residue (MR), calcium (Ca) and phosphorus (P) of broilers fed two different levels of phytate and three levels of phytase in diet with reduced calcium and phosphorus at 21 days of age (Mean±Standard Error of the Mean (SEM)).

		--------------Digestibility coefficient (%)--------------			------------------------Bone characteristics------------------------			
Phytate	Phytase	DM	CP	GE	Tibia weight (g)	% MR	% Ca	% P
Medium phytate	0 FTU	65.40±0.62Ae	76.58±0.43Be	70.68±0.80Ae	1.472±0.08	43.54±0.82	16.10±0.60	8.46±0.15
	750 FTU	65.51±0.75Am	79.25±0.41Am	70.31±0.70Am	1.670±0.07	47.26±0.56	17.76±0.40	9.31±0.16
	1500 FTU	61.47±0.84By	76.53±0.72By	66.63±0.83By	1.675±0.07	48.37±0.49	17.18±0.34	9.49±0.19
High phytate	0 FTU	57.46±0.96Bf	73.16±0.71Bf	64.91±0.81Bf	1.483±0.04	42.98±0.84	14.62±0.36	8.28±0.16
	750 FTU	62.93±0.77An	78.33±0.61Am	68.54±0.74Am	1.666±0.05	45.82±0.92	18.12±0.60	8.72±0.18
	1500 FTU	65.32±0.75Ax	79.62±0.62Ax	70.76±0.65Ax	1.706±0.07	46.98±0.44	16.91±0.66	8.97±0.12
---Independent Effects---								
---Phytate levels---								
Medium		64.13±0.68a	77.45±0.40	69.21±0.61	1.606±0.05	46.39±0.37	17.02±0.26	9.09±0.11
High		61.91±0.60b	77.04±0.39	68.07±0.48	1.618±0.03	45.26±0.44	16.55±0.35	8.66±0.09
---Phytase levels---								
0 FTU		61.43±0.60b	74.87±0.44b	67.80±0.64	1.477±0.05b	43.25±0.59b	15.36±0.37b	8.37±0.12
750 FTU		64.22±0.61a	78.79±0.38a	69.42±0.54	1.668±0.04a	46.54±0.55a	17.94±0.35a	9.01±0.14
1500 FTU		63.40±0.72am	78.07±0.60a	68.70±0.72	1.690±0.05a	47.67±0.36a	17.05±0.36a	9.23±0.13
CV%*		3.58	2.25	3.28	120.82	4.85	8.38	22.58
P Phytate		0.002	0.398	0.093	0.817	0.054	0.260	0.003
P Phytase		0.005	<0.0001	0.145	0.003	<0.0001	<0.0001	<0.0001
Phytate vs Phytase		<0.0001	<0.0001	<0.0001	0.965	0.782	0.184	0.448

Lowercase letters in 'independent effects' differ from each other by Tukey Test at 5% probability.
Letters A, B and C differ from each other in phytase levels within each phytate level; lowercase letters e and f differ in phytate levels within the 0 FTU phytase level; lowercase letters m and n differ in phytate levels within the 750 FTU phytase level; lowercase letters x and y differ in phytate levels within the 1500 FTU phytase level by Tukey Test at 5% probability.
*Coefficient of Variation.
P: phosphorus.

et al., 2001; OMAR and SABHA, 2009), with consequent improvement in animal performance and waste excretion to the environment. The study of RAVINDRAN et al. (2001) showed improved coefficients of apparent ileal digestibility (CAID) of dry matter and gross energy when phytase was included in diets with reduced Ca and P levels, matching treatments with standard Ca and P levels. These results are similar to those of OMAR & SABHA (2009), when comparing diets with conventional or reduced levels of Ca and P and addition of 1000, 2000 and 3000FTU/Kg of phytase observed that, regardless of the dose of phytase, CAIDs of dry matter and crude protein were equal to the positive control.

However, it was observed in the present study that the use of 1500 FTU/kg of phytase in diets containing HP outperformed the CAID of CP and GE when compared to the MP treatment. These results lead us to believe that the greater availability of substrate in the diet created conditions for the enzyme to act more efficiently, but this did not imply an improvement in performance results. Despite this, it can be understood that phytase mitigated the anti-nutritive effects of phytate. In addition, higher digestibility of the diet is important to decrease the emission of excreta into the environment, especially of P.

Regarding bone tissue data, SOUZA (2012) explains that this is the second tissue prioritized by the body, second only to nerve tissue and ahead of muscle and adipose tissue. Bone tissue is made up of approximately 70% minerals, among them Ca and P that are responsible for the formation of the mineral matrix of bone, that compose approximately 95% of this fraction (RATH et al., 2000), what was not observed in this trial, probably because of the body composition of the modern broilers that is constantly selected for a greater composition, as far as possible. GHOSH et al. (2016) also reported tibia mineral residue in a range between 46,6 and 48,5%. When there is a deficiency of any of these minerals there will invariably be motor and structural problems related to poor bone formation, which did not happen in this study. The addition of phytase was enough to keep the results of all bone parameters evaluated better than the treatments without phytase, which matches the digestibility results and shows that the inclusion of phytase is indeed able to raise dietary levels of Ca and P.

These results for bone characteristics are in agreement with those published by WALK & RAMA RAO (2020), who found higher tibia weight, percentage of ash and Ca in the diets with higher phytase inclusion when evaluating increasing doses of phytate and phytase in diets for broilers from 1 to 43 days, but the same was not observed by GHOSH et al. (2016), when working with manganese and phytase supplementation did not find improvement in tibia ash and composition, maybe because of the interaction between both additives.

CONCLUSION

Under the experimental conditions of this study, the addition of phytase to diets with reduced levels of Ca and P was able to improve feed intake, weight gain and apparent digestibility coefficients of dry matter, crude protein and gross energy in birds consuming diets with 6.0 kg/ton of phytate, and to improve bone characteristics of animals consuming diets with 4.25 or 6.0 kg/ton of phytate. There was no effect of phytase inclusion and phytate level in the diet on feed conversion ratio and carcass and cuts yield of broilers.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The experiment was approved by the ethics committee on animal use (CEUA) of the agricultural sciences sector of the Universidade Federal do Paraná (UFPR), Curitiba, Paraná, Brazil, under protocol number 120/2016.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest for this article. The founding sponsors had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, and in the decision to publish the results.

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AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved the final version.

REFERENCES

ABBASI, F. et al. Low digestibility of phytate phosphorus, their impacts on the environment, and phytase opportunity in the poultry industry. **Environmental Science and Pollution Research**, v.26, p.9469-9479, 2019. Available from: <https://doi.org/10.1007/s11356-018-4000-0>. Accessed: Nov. 23, 2021. doi: 10.1007/s11356-018-4000-0.

ABD EL-HACK, M. E. et al. The uses of microbial phytase as a feed additive in poultry nutrition – a review. **Annals of Animal Science**, v.18, p.639-658, 2018. Available from: <https://doi.org/10.2478/aoas-2018-0009>. Accessed: Mar. 28, 2022. doi: 10.2478/aoas-2018-0009.

AKTER, M. et al. Response of broiler chickens to different levels of calcium, non-phytate phosphorus and phytase. **British Poultry Science**, v.57, p.799-809, 2016. Available from: <https://doi.org/10.1080/00071668.2016.1216943>. Accessed: Nov. 23, 2021. doi: 10.1080/00071668.2016.1216943.

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS - AOAC. **Official methods of the association of the agricultural chemists**. 16.ed. Washington, D.C.: 1230p, 1995.

ATIA, F. A. et al. Effect of dietary phosphorus, calcium, and phytase on performance of growing turkeys. **Poultry Science**, v.79, p.231-239, 2000. Available from: <https://doi.org/10.1093/ps/79.2.231>. Accessed: Nov. 23, 2021. doi: 10.1093/ps/79.2.231.

BABATUNDE, O. O. et al. Influence of age and duration of feeding low-phosphorus diet on phytase efficacy in broiler chickens during the starter phase. **Poultry Science**, v.8, p.2588–2597, 2019a. Available from: <https://doi.org/10.1093/ps/79.2.231>. Accessed: Nov. 23, 2021. doi: 10.1093/ps/79.2.231.

BABATUNDE, O. O. et al. The impact of age and feeding length on phytase efficacy during the starter phase of broiler chickens. **Poultry Science**, v.98, p.6742–6750, 2019b. Available from: <https://doi.org/10.3382/ps/pez390>. Accessed: Nov. 24, 2021. doi: 10.3382/ps/pez390.

BABATUNDE, O. O. et al. Evaluation of the responses of broiler chickens to varying concentrations of phytate phosphorus and phytase. I. Starter phase (day 1-11 post hatching). **Poultry Science**, v.100, p.101396, 2021. Available from: <https://doi.org/10.1016/j.psj.2021.101396>. Accessed: Nov. 24, 2021. doi: 10.1016/j.psj.2021.101396.

COWIESON, A. J. et al. Influence of dietary phytic acid and source of microbial phy- tase on ileal endogenous amino acid flows in broiler chick- ens. **Poultry Science**, v.87, p.2287-2299, 2008. Available from: <https://doi.org/10.3382/ps.2008-00096>. Accessed: Nov. 24, 2021. doi: 10.3382/ps.2008-00096.

COWIESON, A. J. et al. Super-dosing effects of phytase in poultry and other monogastrics. **Poultry Science**, v.67, p.225-236, 2011. Available from: <https://www.researchgate.net/publication/231952899_Super-dosing_effects_of_phytase_in_poultry_and_other_monogastrics>. Accessed: Nov. 24, 2021. doi: 10.1017/S0043933911000250.

GHOSH, A. et al. Effects of supplementation of manganese with or without phytase on growth performance, carcass traits, muscle and tibia composition, and immunity in broiler chickens. **Livestock Science**, v.191, p.80–85, 2016. Available from: <https://doi.

org/10.1016/j.livsci.2016.07.014>. Accessed: Mar. 29, 2022. doi: 10.1016/j.livsci.2016.07.014.

GIBSON, R. S. et al. Implications of phytate in plant-based foods for iron and zinc bioavailability, setting dietary requirements, and formulating programs and policies. **Nutrition Reviews**, v.76, p.793-804, 2018. Available from: <https://doi.org/10.1093/nutrit/nuy028>. Accessed: Nov. 24, 2021. doi: 10.1093/nutrit/nuy028.

HEUBNER, W.; STADLER, H. Determination of phytin by titration. **Biochem Z**, v.64, p.422. 1914.

INGELMANN, C. J. et al. Phytate degradation and phosphorus digestibility in broilers and turkeys fed different corn sources with or without added phytase. **Poultry Science**, v.98, p.912-922, 2019. Available from: <https://doi.org/10.3382/ps/pey438>. Accessed: Nov. 24, 2021. doi: 10.3382/ps/pey438.

LAMID, M. et al. Characterization of phytase enzymes as feed additive for poultry and feed. **IOP Conference Series: Earth and Environmental Science**, v.137, p.1-4, 2018. Available from: <https://iopscience.iop.org/article/10.1088/1755-1315/137/1/012009>. Accessed: Nov. 24, 2021. doi: 10.1088/1755-1315/137/1/012009.

LATTA, M.; ESKIN, M. A. A simple and rapid colorimetric method for phytate determination. **Journal of Agricultural and Food Chemistry**, v.28, p.1313–1315, 1980. Available from: <https://doi.org/10.1021/jf60232a049>. Accessed: Nov. 24, 2021. doi: 10.1007/s00240-012-0473-3.

MCDOWELL, R. L. 1992. Calcium and phosphorus. Minerals in animal and human nutrition. **San Diego: Academic Press**, p.31-32.

RATH, N. C. et al. Factors regulating bone maturity and strength in poultry. **Poultry Science**, v.79, p.1024–1032, 2000. Available from: <https://doi.org/10.1093/ps/79.7.1024>. Accessed: Nov. 24, 2021. doi: 10.1093/ps/79.7.1024.

RAVINDRAN, V. et al. Microbial Phytase Improves Performance, Apparent Metabolizable Energy, and Ileal Amino Acid Digestibility of Broilers Fed a Lysine-Deficient Diet. **Poultry Science**, v.80, p.338-344, 2001. Available from: <https://doi.org/10.1093/ps/80.3.338>. Accessed: Nov. 24, 2021. doi: 10.1093/ps/80.3.338.

ROSTAGNO, H. S. et al. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. Tabelas Brasileiras para aves e suínos. **Composição Alimentar e Exigência Nutricional**. p.252, 2011.

SANTOS, T. T. et al. Influence of phytate level on broiler performance and the efficacy of 2 microbial phytases from 0 to 21 days of age. **Journal of Applied Poultry Research**, v.23, p.181-187, 2014. Available from: <http://dx.doi.org/10.3382/japr.2013-00842>. Accessed: Nov. 24, 2021. doi: 10.3382/japr.2013-00842.

SCOTT, T. A.; BOLDAJI, F. Comparison of inert markers [chromic oxide or insoluble ash (Celite)] for determiningapparentmetabolizable energy of wheat or barley-based broiler diets with or without enzymes. **Poultry Science**, v.76, p.594-598, 1997. Available from: <https://doi.org/10.1093/ps/76.4.594>. Accessed: Nov. 24, 2021. doi: 10.1093/ps/76.4.594.

SOUZA, A. F. G. O. Tecido ósseo em frangos de corte. **Nutritime**, v.9, p.1663-1679, 2012. Available from: <https://doi.org/10.18011/bioeng2014v8n2p97-103>. Accessed: Nov. 24, 2021. doi: 10.18011/bioeng2014v8n2p97-103.

TAMIM, N. M. et al. Influence of dietary calcium and phytase on phytate phosphorus hydrolysis in broiler chickens. **Poultry Science**, v.83, p.1358-1367, 2004. Available from: <https://doi.org/10.1093/ps/83.8.1358>. Accessed: Nov. 24, 2021. doi: 10.1093/ps/83.8.1358.

VÖTTERL, J. C. et al. Dietary Phytase and Lactic Acid-Treated Cereal Grains Differently Affected Calcium and Phosphorus Homeostasis from Intestinal Uptake to Systemic Metabolism in a Pig Model. **Nutrients**, v.12, p.1542-1554, 2020. Available from: <https://doi.org/10.3390/nu12051542>. Accessed: Nov. 24, 2021. doi: 10.3390/nu12051542.

WALK, C. L.; RAMA RAO, S. V. Dietary phytate has a greater anti-nutrient effect on feed conversion ratio compared to body weight gain and greater doses of phytase are required to alleviate this effect as evidenced by prediction equations on growth performance, bone ash and phytate degradation in broilers. **Poultry Science**, v.99, p.246-255, 2020. Available from: <http://dx.doi.org/10.3382/ps/pez469>. Accessed: Nov. 24, 2021. doi: 10.3382/ps/pez469.