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ANIMAL SCIENCE

Phosphorus release capacity in different dietary commercial phytases through performance and bone characteristics assessment of broiler chickens

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Abstract: A trial was conducted to evaluate phosphorus release capacity in different commercial phytases throught performance and bone characteristics of broilers. A total of 2,400-day-old male Cobb 500® chicks were assigned in a completely randomized design with 12 dietary treatments ((1 to 5: with increasing levels of non-phytate phosphorus (NPP) (0.20; 0.25; 0.30; 0.35 and 0.40%) without phytase); and 6 to 12: with 0.20% supplemented with different commercial phytases), 8 replicates and 25 birds per pen. There was a linear increase (P<0.05) in the feed intake and body weight gain and a linear improvement (P<0.05) in the feed conversion ratio of broilers fed increasing NPP. The tested phytases did not release similar amounts of phytic phosphorus. Based on body weight gain response, 500 FTU/kg of diet releases a minimum and maximum of 0.074 to 0.152%, 0.062 to 0.157 and 0.059 to 0.169% of PP among the studied phytases in the periods from 1 to 21, 1 to 35 and 1 to 42 days, respectively. In conclusion, the commercial phytases released different concentrations of phytate phosphorus from the diets. Thus, is necessary to have a better method to evaluate phytase activity in order to avoid subnormal levels of available P in diets.

Key words: phosphorus, poultry nutrition, phytate, phytase activity.

INTRODUCTION

Studies in poultry nutrition have been developed to estimate the adequate nutritional requirements focused on improving performance and reducing production costs. Thus, cost-saving opportunities should always be accompanied by the concern of maintaining adequate poultry nutrition. In this context, many studies have been done on phosphorus (P) because it is the third costliest nutrient in the diet. The studies focused their on finding alternatives that can contribute to the reduction of P costs by improving the P utilization from phytate present in vegetable ingredients. For this purpose, phytase enzyme is remains main tool for optimizing organic P utilization and costs reduction of the diets for broilers (Dessimoni et al. 2019, 2020). In addition to P, other minerals chelated with phytic acid can be released by the action of phytase which otherwise cannot be utilized by poultry (Rao et al. 1999, Selle & Ravindran 2007, Bedford et al. 2016).

Currently, various phytases are available on the market for use; however, it is unclear whether they have the same efficacy in hydrolysis and release of the P present in phytic ingredients, mainly corn and soybean meal, which dominate the compositions of broiler diets worldwide. In addition, there are doubts about the mode of expression in the activity of this enzyme.

Engelen et al. (1994) initially proposed the FTU method, wherein 1 µmol inorganic phosphate is released in 1 min at a temperature of 37 °C and pH 5.5. However, due to the differences in structure, composition and the optimum pH for the catalytic action of phytase, the available commercial phytases may differ in the amount of P released when they are supplemented in broiler diets (Konietzny & Greiner 2004, Shaw et al. 2010).

The use of diets with low P levels has been proposed for efficacy studies of new phytases (Aureli et al. 2011). However, there is a variation in the efficacy of phytases on phytate released P within the different doses and type of diets (Kebreab et al. 2012). In addition to the nutritional aspects, it is necessary to take into account the sustainability factor that combines production with the reduction of the negative impact of P on environmental pollution (Carter & Kim 2013). The excreted P can contribute to eutrophication processes and nitrification of rivers and lakes (Boling et al. 2000, Carpenter 2005, McGrath et al. 2005). The use of phytases can decrease the excretion of P and other minerals, contributing to the environmental susceptibility of production of broiler meat.

Therefore, the aim of this study was to evaluate seven different commercial phytases associated with low P in the diets based on the standard curve approach and using performance and bone characteristics as response criteria for broilers raised in different age periods.

MATERIALS AND METHODS

Birds and experimental design

The study was performed according to ethical principles for animal experimentation established by the Brazilian College of Animal Experimentation (Cobea 1991) and complying with the current legislation. All experimental procedures were approved by the Ethics Committee on Animal Use (protocol No. 017/19). The trial was conducted at the Center for Research in Poultry Technology (CPTA), in a partnership with the Federal University of Lavras - UFLA, Lavras, Brazil.

A total of 2,400-day-old male Cobb-500® chicks acquired from a commercial hatchery and properly vaccinated against Marek's disease were allocated into 96 experimental units at a density of 15 birds/m^2 in each floor pen. Heating was provided by wood furnace with a digital temperature control and kept according to the management guidelines (Cobb Guidelines 2015). The light program used in the experimental period was 23L:1D from one to ten days, and 16L:8D from 11 to 42 days of age.

A completely randomized design was used, consisting of 12 treatments, eight replicates and 25 birds per pen (2 x 1.5 m). The diets 1 to 5 were formulated to provide basal diets with increasing non-phytate phosphorus (NPP) levels of 0.20, 0.25, 0.30, 0.35 and 0.40% NPP, respectively; diets 6 to 12 were formulated from basal diets containing 0.20% of NPP plus one of the seven different commercial phytases identified by letters (from A to G). The phytases were supplemented at the dose recommended by the manufacturer to provide 500 FTU/kg. The total experimental period was 42 days.

Experimental diets

Diets were formulated to meet the requirements of the broiler, except for NPP, for each growing phases (starter: 1 to 21, grower: 22 to 35, and finisher: 36 to 42 days). The diets were based on corn and soybean meal, supplemented with minerals and vitamins, and formulated according to the nutritional recommendations of Bertechini (2013) for starter, grower, and finisher diets (Table I).

Table I. Diets composition (g/kg as feed basis) and nutritional levels of the experimental diets used within the age periods from 1 to 21, 22 to 35 and 36 to 42 days.

	Starter (1 to 21 days)					Growth (22 to 35 days)					Finish (36 to 42 days)				
Ingredient (g/kg)[1]	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Corn	591.6	589.6	587.5	585.3	583.2	632.8	630.6	628.5	626.3	624.2	624.9	622.8	620.7	618.6	616.4
Soybean meal	344.8	345.2	345.6	346.0	346.4	295.1	295.5	295.8	296.2	296.6	286.3	286.7	287.1	287.4	287.8
Soybean oil	26.6	27.3	28.0	28.7	29.4	35.7	36.5	37.2	37.9	38.6	52.6	53.3	54.0	54.7	55.4
Dicalcium phosphate	3.9	6.6	9.3	12.0	14.8	4.3	7.1	9.8	12.5	15.2	4.5	7.2	9.9	12.6	15.4
Limestone	18.8	17.2	15.5	13.8	12.1	18.8	17.1	15.5	13.8	12.1	18.8	17.1	15.4	13.8	12.1
Salt	3.6	3.6	3.6	3.6	3.7	3.6	3.7	3.7	3.7	3.7	3.8	3.8	3.8	3.8	3.8
Mineral-vitamin supplement[2]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
DL-Methionine 99%	2.3	2.3	2.3	2.3	2.3	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2
L-Lysine.HCl 78%	1.3	1.3	1.3	1.3	1.3	1.6	1.6	1.5	1.5	1.5	1.2	1.2	1.2	1.2	1.2
L-Threonine 98%	2.1	2.1	2.1	2.1	2.1	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Choline Chloride 60%	0.5	0.5	0.5	0.5	0.5	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Sodium bicarbonate	1.3	1.3	1.3	1.3	1.3	1.0	0.9	0.9	0.9	0.9	0.7	0.7	0.7	0.7	0.7
Halquinol 60%	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Maduramycin 1%	0.6	0.6	0.6	0.6	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Salinomycin 12%	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Kaolin	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Energy and nutrient calculated															
Metabolizable energy (MJ/kg)	12.77	12.77	12.77	12.77	12.77	13.19	13.19	13.19	13.19	13.19	13.61	13.61	13.61	13.61	13.61
Crude protein (g/kg)	210	210	210	210	210	190	190	190	190	190	185	185	185	185	185
Ash (g/kg)	57.5	58.5	59.5	60.5	61.6	54.9	55.9	56.9	57.9	59.0	54.3	55.3	56.3	57.3	58.3
Calcium (g/kg)	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
Non-phytate phosphorus (g/kg)	2.0	2.5	3.0	3.5	4.0	2.0	2.5	3.0	3.5	4.0	2.0	2.5	3.0	3.5	4.0
Digestible methionine (g/kg)	5.1	5.1	5.1	5.1	5.1	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8
Digestible methionine+cystine (g/kg)	8.0	8.0	8.0	8.0	8.0	7.5	7.5	7.5	7.5	7.5	7.4	7.4	7.4	7.4	7.4
Digestible lysine (g/kg)	11.0	11.0	11.0	11.0	11.0	10.0	10.0	10.0	10.0	10.0	9.5	9.5	9.5	9.5	9.5
Digestible threonine (g/kg)	8.9	8.9	8.9	8.9	8.9	7.4	7.4	7.4	7.4	7.4	7.2	7.2	7.2	7.2	7.2
Sodium (g/kg)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	1.9	1.9	1.9	1.9	1.9
Na+K-Cl (meq/kg)	230	230	230	230	230	200	200	200	200	200	194	194	194	194	194

[1]Feeds with crescent levels of non-phytate phosphorus (1=0.20, 2=0.25, 3=0.30, 4=0.35 and 5=0.40 % NPP); The seven different commercial phytases was added to diet number 1 with 0.20% NPP.
[2]Supplied per kg of diet: vitamin A – 12,000 IU (all-trans retinol); vitamin D3- 2,500 IU; vitamin E -30 IU (dl-α-tocopheryl acetate); vitamin B1 - 2 mg; vitamin B6 - 3 mg; calcium pantothenate - 10 mg; biotin - 0.07 mg; vitamin k3 - 3 mg; folic acid - 1 mg; nicotinic acid - 35 mg; vit. B12 - 15 µg; selenium - 0.250 mg; BHT - 5 mg; manganese - 80 mg; iron – 50 mg; zinc 50 mg; copper - 10 mg; cobalt – 1 mg; iodine - 1 mg.

Commercial phytases

The phytases studied were obtained from the Brazilian market and were produced with different types of microorganisms. All phytases from this study were rated as class 6-phytases. Phytase A is fungal. Phytases B, C, D, E, F and G are bacterial. The enzyme activity was determined by Engelen et al. (1994) method and added in sufficient quantity to provide exactly 500 FTU/kg of feed. Thus, the dietary enzyme level of each phytase was different, to present the same phytase activity per kg of feed. Phytase product was extracted in 0.25 mol L^{-1} of acetate buffer containing 0.05% Triton X-100 (w/v) and 0.05% bovine serum albumin (w/v) at pH 5.0 under horizontal stirring for 30 min. After extraction, the sample was centrifuged (2000 *g* for 10 min at 4 °C) and the supernatant collected. Determination of the activity in the supernatant was based on the effect of phytase on the sodium phytate substrate 7.5 mmol L^{-1} ($C_6H_6O_{24}P_6Na_{12}$ - Sigma-Aldrich) in buffered medium (acetate buffer 0.25 mol L^{-1}, pH 5.0) at 37°C and pH 2.0 to 9.0 for four different incubation times to determine its pH curve. Initially, the following buffers were used: HCl-KCl (pH 2.0); glycine-HCl (pH 2.0 and 3.0); citrate (pH 3.0, 4.0, 5.0, and 6.0); maleate (pH 6.0 and 7.0); and Tris-HCl (pH 7.0, 8.0, and 9.0) (Naves et al. 2012). Reaction was stopped by mixing three solutions (21.67% nitric acid, 0.081mol L^{-1} ammonium molybdate, and 0.02 mol L^{-1} ammonium vanadate) in proportions of 2:1:1, respectively, forming a yellow product with a maximum absorption wave length of 415 nm. Potassium phosphate was used as a standard (Engelen et al. 1994).

Performance

The birds and feeds were weighed by pen at day 1, 21, 35 and 42 to evaluate feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) of broilers in phases from 1 to 21, 1 to 35, and 1 to 42 days of age. At 42 days of age, the broilers in each pen were individually weighed to obtain the standard deviation (SD) and the mean value in each pen to calculate the coefficient of variation (CV), which was used as a measure for uniformity. At 21 and 42 days, one bird per replicate were sacrificed (by cervical dislocation) to collect the left tibia for the quantification of ash, calcium (Ca) and total phosphorus (tP).

Chemical analysis of feed and bones

All diets and tibias were analyzed for dry matter, ash, calcium and phosphorus by AOAC (2005) methods. Dry matter (method no. 930.15, AOAC 2005) was determined by drying samples at 105 °C for 16 h using a forced-draft oven (Tecnal Equipamentos para Laboratório, TE-394/2, São Paulo, Brazil). The fat-free tibia (method no. 942.05) were dried at 90 °C, weighed and the ash obtained by incineration at 550 °C for 8 h using a muffle furnace (Sanchis Fornos Industriais, Porto Alegre, RS, Brazil). Calcium and phosphorus in bone ash were measured using the flame atomic absorption method in the Perkin Elmer 5000 atomic absorption spectroscope (method no. 968.08D). Crude protein content of the diets was determined by the combustion method using a CNS-2000 carbon, nitrogen, and sulphur analyzer (LECO® Corporation, St. Joseph, MI, USA).

Phytate phosphorus release

The phytate phosphorus (PP) release by phytases was calculated on a linear base response over performance (FI, BWG, and FCR) and the tibia ash, concerning the treatments with dietary levels of 0.20% to 0.40% of NPP, with no phytase included, following the method described by Han et al. (2009). Example: BWG to phytase A from 1 to 42 days = 2.422 kg (2.422 = 1.227 + 4.026X) where X = 0.297 and the PP release from phytase = 0.297 – 0.200 = 0.097%.

Statistical analysis

All data were subjected to one-way ANOVA analysis and the linear models obtained using the PROC GLM procedures in the Statistical Analysis System (SAS Institute Inc., Cary, NC, 2002, Version 9.00). The assumption of normality was verified by the tests of Cramer-Von Mises. The Duncan's test was used to compare the means obtained by each commercial phytase supplementation. The Dunnett's test was used to compare the responses obtained with the supplementation of each commercial phytase in comparison to the response obtained with the highest NPP level (0.40%). The results were considered significant when the *P* value were less or equal to 0.05.

Calculation of phytate phosphorus release

The NPP levels (from 0.20 to 0.40%) were regressed against the performance responses (FI, BWG, FCR and the tibia ash). The PP release was calculated by substituting Y in the linear equation by the response obtained with the phytase supplementation, where the value of the PP estimate is based on the NPP equivalent in the diets without phytase, following the method described by Han et al. (2009).

RESULTS

Overall performance

There was a linear effect ($P<0.05$) of NPP levels studied (0.20% to 0.40%) on all performance responses measured in broilers chickens (Table II). The FCR decreased linearly ($P<0.05$) with increasing NPP levels. At 21 days, the broilers fed diets 6, 8, 9, 10 and 12 to 12 presented a reduced FI in comparison to the broilers fed diet with the highest level of NPP (0.40%).

The performance of broilers in the accumulated phase from 1 to 35 days (Table II) was influenced by the treatments in a similar way as in the previous phase. Linear increases ($P<0.05$) in FI and BWG and improvements in FCR occurred with elevated NPP levels in the diets. Linear increases ($P<0.05$) in FI and BWG and a linear improvement ($P<0.05$) in FCR were observed considering the period from 1 to 42 days (Table IV). Phytase F alone could recover the FI and BWG; however, the phytases E, F, and G could recover the FCR.

Bone ash, calcium and phosphorus

The results for ash, total phosphorus (tP), and calcium (Ca) in tibia at 21 and 42 days (Table III) were different ($P<0.05$) between treatments.

At 21 days of age, broilers supplemented with phytase had no difference in tibia ash and tP, showing that the differences observed in performance between all phytases was not reflected in those two bone characteristics. At 42 days of age, the results showed no difference between broilers supplemented with phytase for any bone parameter ($P>0.05$), except for tP from tibia bone of broilers with enzyme D compared to broilers in treatment 5 (0.40% NPP without phytase).

Linear equation and phytic phosphorus release

The response of broilers receiving feeds with different NPP levels (Table IV) showed a linear pattern ($P<0.05$) for performance variables of FI and BWG (1 to 21 days); FI, BWG and FCR (1 to 35 days); and FI and FCR from 1 to 42 days. However, the results for tibia ash had a linear relation ($P<0.05$) only at 21 days.

In the periods from 1 to 35 and 1 to 42 days, all performance responses were used to calculate the equivalence of PP for each phytase (Table V). On average, the PP released by phytases was respectively 0.087, 0.095 and 0.146 for FI, BWG and FCR for 1 to 35 days and 0.091, 0.110, 0.175 and 0.152 for FI, BWG, FCR and tibia ash, respectively for 1 to 42 days.

Table II. Summarized results for body weight (BW), feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR) of broilers in the age periods from 1 to 21, 1 to 35, and 1 to 42 days.

Diets	Treatment[1]	1 to 21 days			1 to 35 days			1 to 42 days			
		FI (kg)	BWG (kg)	FCR (kg:kg)	FI (kg)	BWG (kg)	FCR (kg:kg)	FI (kg)	BWG (kg)	FCR (kg:kg)	CV of BW (%)
1	0.20% NPP	0.72	0.49	1.45	2.81	1.49	1.89	3.79	2.01	1.89	13.97
2	0.25% NPP	0.92	0.65	1.43	2.87	1.63	1.76	3.82	2.17	1.76	17.11
3	0.30% NPP	1.01	0.71	1.43	3.39	1.92	1.77	4.46	2.52	1.77	12.33
4	0.35% NPP	1.13	0.79	1.43	3.63	2.13	1.70	4.79	2.75	1.75	11.85
5	0.40% NPP	1.17	0.83	1.40	3.63	2.18	1.67	4.74	2.73	1.74	11.18
6	0.20% NPP+A	0.97^{cd*}	0.66^{d*}	1.46	3.30^{cd*}	1.82^{d*}	1.82^{bc*}	4.35^{cd*}	2.42^{c*}	1.80^{bc}	14.64
7	0.20% NPP+B	1.06^{ab*}	0.72^{b*}	1.47	3.45^{ab*}	1.95^{b*}	1.77^{b*}	4.54^{b*}	2.55^{b*}	1.78^{ab}	14.93
8	0.20% NPP+C	1.01^{c*}	0.69^{c*}	1.46	3.36^{bc*}	1.86^{cd*}	1.80^{bc*}	4.46^{bc*}	2.48^{bc*}	1.80^{bc}	14.11
9	0.20% NPP+D	0.95^{d*}	0.65^{d*}	1.46	3.19^{de*}	1.83^{e*}	1.85^{c*}	4.17^{e*}	2.72^{d*}	$1.84c^{*}$	17.39
10	0.20% NPP+E	0.99^{cd*}	0.70^{c*}	1.43	3.13^{e*}	1.84^{cd*}	1.70^{a}	4.24^{de*}	2.45^{bc*}	1.73^{a}	12.86
11	0.20% NPP+F	1.10^{a*}	0.78^{a*}	1.41	3.54^{a}	2.08^{a*}	1.70^{a}	4.69^{a}	2.71^{a}	1.73^{ab}	12.19
12	0.20% NPP+G	1.02^{bc*}	0.71^{bc*}	1.44	3.38^{bc*}	1.91^{bc*}	1.77^{b*}	4.47^{bc*}	2.52^{b*}	1.77^{ab}	12.89
P-value		<0.0001	<0.0001	0.552	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0286
SEM		0.012	0.008	0.005	0.030	0.020	0.010	0.030	0.020	0.010	0.346

[1] NPP = non-phytate phosphorus; CV (%) = coefficient of variation; A = Fungal phytase; B, C, D, E, F and G = bacterial phytases. The phytases represents seven different commercial phytases.
[a–e]Means within a column with different superscripts differ significantly (*P*<0.05) by Duncan test.
*Means 6 to 12 differ from treatment 5 (P<0.05) by Dunnett test.

Table III. Summarized results for ash, calcium (Ca) and total phosphorus (tP) from tibia bone of broilers at 21 and 42 days of age.

Diets	Treatment[1]	21 days			42 days		
		Ash	tP	Ca	Ash	tP	Ca
			(%)			(%)	
1	0.20%NPP	44.90	9.48	17.14	49.97	8.37	17.39
2	0.25%NPP	45.49	9.66	18.19	50.93	8.82	18.42
3	0.30%NPP	45.76	9.72	18.37	52.01	8.88	18.75
4	0.35%NPP	46.13	9.88	18.44	52.05	8.93	18.74
5	0.40%NPP	46.62	9.96	18.53	52.22	9.20	18.91
6	0.20% NPP+A	45.31	9.52	17.47ab*	50.92	8.82	18.52
7	0.20% NPP+B	44.92	9.97	17.25b*	51.72	8.83	18.55
8	0.20% NPP+C	45.27	9.58	17.02b*	51.05	8.51	18.42
9	0.20% NPP+D	45.18*	9.14*	17.25b*	51.12	8.40*	18.07
10	0.20% NPP+E	45.54	9.69	18.07ab	51.23	8.71	18.57
11	0.20% NPP+F	45.81	10.12	18.28a	52.17	8.96	18.68
12	0.20% NPP+G	45.79	9.93	18.09ab	51.02	8.79	18.74
P-value		0.269	0.101	0.020	0.955	0.405	0.800
SEM		0.152	0.100	0.060	0.270	0.090	0.060

[1]NPP is non-phytate phosphorus; A = Fungal phytase; B, C, D, E, F and G = bacterial phytases. The phytases represents seven different commercial phytases.
[a–b]Means within a column with different superscripts differ significantly (*P*<0.05) by Duncan test.
*Means differ from treatment 5 (*P* < 0.05) by Dunnett test.

Table IV. Linear equations obtained for feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR) and tibia ash of broilers fed increasing non-phytate phosphorus levels in the age periods from 1 to 21, 1 to 35 and 1 to 42 days[1].

Evaluated Period	Parameter	Linear equation	R^2	P-value <
1 to 21 days	FI	Y = 0.32 + 2.22X	0.95	0.01
	BWG	Y = 0.20 + 1.64X	0.94	0.01
	tibia ash	Y = 42.82 + 9.60X	0.95	0.01
1 to 35 days	FI	Y = 1.83 + 4.80X	0.89	0.02
	BWG	Y = 0.74 + 3.76X	0.96	0.01
	FCR	Y = 2.06 - 1.00X	0.97	0.02
1 to 42 days	FI	Y = 2.60 + 5.74X	0.87	0.02
	BWG	Y = 1.22 + 4.04X	0.92	0.01
	FCR	Y = 1.97 - 0.62X	0.64	0.11
	tibia ash	Y = 48.56 + 10.24X	0.71	0.07

[1]The responses obtained with the increasing NPP levels (0.20, 0.25, 0.30, 0.35 and 0.40% of NPP) were used for fitting the linear models.

Table V. Estimated phytate phosphorus (PP) release of different commercial phytases supplemented to broilers considering feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR) and tibia ash evaluated in different periods[1].

	1 to 21days		1 to 35 days			1 to 42 days			21 days	42 days
Phytase[2]	FI (% PP)	BWG (% PP)	FI (% PP)	BWG (% PP)	FCR (% PP)	FI (% PP)	BWG (% PP)	FCR (% PP)	tibia ash (% PP)	tibia ash (% PP)
A	0.093*	0.082	0.108	0.086	0.042	0.105	0.097	0.076	0.059	0.060
B	0.133	0.118	0.139	0.121	0.087	0.138	0.128	0.104	0.048	0.109
C	0.107	0.098	0.119	0.098	0.054	0.124	0.112	0.079	0.054	0.043
D	0.081	0.074	0.083	0.062	0.059	0.074	0.059	0.049	0.046	0.050
E	0.102	0.102	0.071	0.092	0.156	0.086	0.103	0.182	0.082	0.061
F	0.149	0.152	0.158	0.157	0.156	0.165	0.169	0.184	0.111	0.152
G	0.113	0.107	0.124	0.110	0.084	0.126	0.121	0.112	0.109	0.040

[1]Linear equations obtained for each variable (Table IV) were used to estimate the phytate phosphorus release for all seven phytases. A = Fungal phytase; B, C, D, E, F and G = bacterial phytases. The phytases represents seven different commercial phytases.
* Example: A Phytase FI 1-21d Y = 0.32 +2.22 X (Table IV) – Y = 0.97, X = 0.293, PP release = 0.293 – 0.200 = 0.093%.
[2]All phytase were added to feed in concentration of 500 FTU/kg after analyzed enzyme.

DISCUSSION

Among the evaluated enzymes, the phytases B and F showed similar FI (P>0.05) where the broilers in both treatments showed an increase in this response (P<0.05) in contrast to the other supplemented groups, which suggests an increase in the availability of P. Recently, some authors also described a reduced FI when the animals are submitted to diet with a low available P content (Sebastian et al. 1996, Yi et al. 1996, Singh et al. 2003, Shaw et al. 2010, Lalpanmawia et al. 2014). For BWG measures, there was a linear increase (P<0.05) following the NPP levels (0.20%, 0.25%, 0.30%, 0.35% and

0.40%), which can be explained by an increase in FI. The differences among the phytases were confirmed by Duncan's test, where enzyme F showed a better result at 21 days of age (P<0.05) in comparison with other phytases. These results indicate differences in P release by broilers supplemented with different commercial phytases. Shang et al. (2015) observed a recovery in BWG and FCR in male chicks from 5 to 21 days when 500 FTU of phytase/kg of feed was supplied in a negative control diet containing 0.25% of available P. Dessimoni et al. (2019) also observed that supplementation of phytase in 500 FTU in the negative control diet with 0.26% available P and a reduction of 100 kcal/kg of metabolizable energy, improves BWG and FCR of broilers in phase 15 to 21 days. In the present study, using 500 FTU of phytase/kg of feed in the P deficient diets (0.20% NPP) resulted in different amounts of P released from different commercial phytases. The FCR results showed a different pattern from those observed for FI and BWG, where broilers supplemented with phytase for 21 days showed a similar FCR (P>0.05). In general, the results obtained for the NPP levels and the phytase supplementation are in agreement with several previous studies (Onyango et al. 2005, Pillai et al. 2006, Han et al. 2009, Tang et al. 2012, Lalpanmawia et al. 2014, Shang et al. 2015). Except for FCR, the supplementation of the phytases in the diets containing 0.20% NPP did not provided the same response obtained with the control diet containing 0.40% NPP, which is recommended for the evaluated phase. The same response for FCR was observed by Lalpanmawia et al. (2014) which did not observe any difference in FCR of phytase-supplemented broilers in comparison to broilers fed a diet containing 0.32% NPP in the first three weeks. The same authors also observed differences in the broilers' performance when supplemented with different phytases, where a commercial phytase recovered the FI and BWG in comparison with the control group (0.45% NPP), but broilers supplemented with a laboratory-produced phytase did not demonstrate performance recovery in the first three weeks.

Han et al. (2009) also observed positive effects on performance using levels of NPP ranging from 0.13% to 0.35% in broilers from 22 to 42 days. For FI, only treatment 11 supplemented with phytase F showed similar results (P>0.05) in comparison with a higher level of NPP. This pattern was expected, because there is a reduction in the P requirement as the broilers advance in age, and the P released by phytase may be sufficient to recover the broilers' performance. However, the broilers supplemented with phytase did not have the same BWG as those receiving a control diet with a higher level of NPP (0.40%) (P<0.05). Thus, the P release by phytase supplementation did not meet the broilers' P requirements for better BWG. In the case of FCR, only treatments with phytases E and F were equal to treatment 5 (0.40% NPP). These results indicate that the challenge of reducing the NPP was too high in relation to the amount of P released and used by the broiler to meet its requirements for growth. Han et al. (2009) have demonstrated that phytase supplementation of 500 FTU /kg of feed yields expected values of P release at a maximum of 0.081% for a maximum of 0.288% of available P, which is not sufficient to meet the requirements of the broilers, in accordance with the NRC (1994), explaining why the worst results were obtained for phytase-supplemented birds.

Many aspects could be related to these results such as the reduction in the P requirement with age (NRC 1994), an improvement in mineral retention (P, Ca, and Na) in the low levels of NPP (Han et al. 2009, Rutherfurd et al. 2012) and compensatory growth. Regarding FCR, phytase E showed a similar value in comparison to the use of phytase F and the highest level of NPP.

Increases in performance and bone quality were observed with the addition of 500 FTU/kg of phytase in the diets with low levels of NPP (Simons et al. 1990, Pillai et al. 2006, El-Sherbiny et al. 2010, Lalpanmawia et al. 2014, Shang et al. 2015, Dessimoni et al. 2019).

Regarding the uniformity, which was represented by the CV of the body weight, the broilers supplemented with enzyme D had the worst values for this parameter (P<0.05) followed by the enzymes A, B, and C. Broilers supplemented with enzymes E, F, and G had the best values and also similar values in comparison with broilers fed control diet (0.40% NPP). In this analysis, we observed that broilers receiving low levels of NPP had an increase in CV values; on the other hand, broilers fed diets supplemented with phytase, even without improvement in performance, showed an increase in CV values. This observation may be related to differences in phosphorus released by different phytase sources, even after using the same concentration (500 FTU/kg of feed). In addition, there is a wide range in the pH of the gastrointestinal tract of broilers fed or not with diets supplemented with phytase, mainly in the proventriculum and gizzards (Walk et al. 2012, Souza et al. 2015). This greater variation in the pH of the intestinal sections influences the digestibility of Ca and P, directly affecting the deposition of Ca, P and ash in the tibia (Walk et al. 2012). The association of the factors described above explains the variations in the uniformity of the broiler chickens.

It was possible to see differences between broilers receiving different phytases in tibia Ca percentage (P<0.05), where broilers fed diet with phytase F had higher values for Ca. Broilers receiving diets supplemented with phytase D had the lowest values and it was also the only treatment producing a statistically significant difference (P<0.05), in contrast to the ash and tP values found for broilers receiving diets with 0.40% NPP. Bone ash appears to be the most sensitive method to evaluate the effect of different levels of NPP from feed (Ravindran et al. 1995). However, Scholey et al. (2018) consider that bone strength is a better parameter to assess the effect of different levels of NPP in the diet associated with phytase supplementation. According to our results, the value of Ca percentage was the most sensitive to bone characteristics for different phytases at 21 days. In addition, broilers supplemented with phytases E, F, and G showed the highest percentage of Ca in tibia (P<0.05), while no difference was found compared with broilers fed control diet. This result shows a variation in Ca percentages when different phytases are used, suggesting a different approach to determining the inclusion of phytase, apart from the use of FTU in the current study. Despite the direct effect of phytase on tP, it was not possible to observe an effect (P>0.05) of phytase on this parameter. Similar results were observed by Han et al. (2009) using 500 FTU/kg of phytase in a diet with 0.13% NPP for broilers from 22 to 42 days.

Results of linear equation is in partial agreement with those presented by Han et al. (2009) where the authors observed a linear response between NPP supplementation and bone characteristics of birds on day 42. According to the linear equation obtained for each observed response variable, a performance trait or tibia ash was used to calculate the PP, example: FI of phytase A from 1 to 21 days = 0.97 kg (0.97 = 0.324 + 2.22X) where X = 0.293 and the PP release from phytase = 0.293 – 0.200 = 0.090%.

Because of the results of FCR, this parameter was not used to calculate the PP release in the period from 1 to 21 days. A PP release calculated for birds receiving phytase produced interesting results (Table V). In the period from 1 to 21 days of age, broilers showed a variation of 0.068% (0.149

- 0.081) in PP release between phytases when FI was used in the calculations. The differences were higher when the BWG was used to calculate PP, showing a variation of 0.078% (0.152 - 0.074) between phytases. The PP release calculated on the ash basis showed that the enzyme D did not increase ash, showing a difference of 0.111% in contrast with birds from treatment F.

The determination of efficacy in the release of PP due to phytase supplementation may vary according to the productive characteristic to be evaluated. It is possible to see that when FCR is used in the calculation, the releases have a greater variation among phytases. Hence, FI and BWG appear to be more accurate for the prediction of PP release for each phytase.

The values of PP released are in accordance with the results obtained from performance responses where, in general, the enzyme F had the best results and met the values recognized by the (NRC 1994). According to enzyme suppliers, commercial phytase had a PP release ranging from 0.100% to 0.150%. The supplementation of F enzyme released 0.149%, 0.158%, and 0.165% of PP for FI and 0.152%, 0.157%, and 0.169% of PP for BWG in the periods from 1 to 21, 1 to 35, and 1 to 42 days, respectively. The results suggests that there is a considerably range between commercial phytases, according to it PP release. Recently, Han et al. (2009) found 0.049% and 0.060% of PP released for FI and BWG, respectively at day 42 using a commercial phytase (*E. coli*-derived product, 500 FTU/g), demonstrating that this difference could be found even in other findings in literature.

CONCLUSIONS

The FTU system is not fit for comparing phytases, after all they are differing in in vivo performance, but not necessarily activity.

Feed intake and body weight gain seems to be the best way to evaluate the phytic phosphorus release by phytase. Of all enzymes tested, phytase F shows the best performance results and the greatest release of phytic phosphorus. Different commercial phytases should be supplemented at different levels to release the same amount of phytic phosphorus for birds. The results observed in this study suggests that it is necessary to have a better method to evaluate phytase activity in order to avoid subnormal levels of available P in feed when phytase is used, which may affect performance and bone characteristics as observed in this study.

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Antônio G. Bertechini designed and idealized the study, participated in the statistical analysis, and wrote a preliminary version of the manuscript. Matheus de P. Reis conducted the study, analysis of samples, statistical analysis, data interpretation and wrote a preliminary version of the manuscript. Felipe S. Dalólio was responsible for sample preparation and wrote the preliminary and final version of the manuscript. Julio Cesar C. de Carvalho contributed the donation of commercial phytases and worked on data interpretation.