Ileal digestibility of calcium and phosphorus in broilers fed diets with different phytases and Ca:available P ratios

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Abstract – The objective of this work was to determine the apparent ileal digestibility of calcium and phosphorus in broilers fed diets with different phytases and calcium:available phosphorus (Ca:aP) ratios. Two experiments were carried out: one with broilers with 22 to 33 days of age, and the other with broilers with 35 to 42 days. The Ca:aP ratios used were 4.5:1.0, 6.0:1.0, and 7.5:1.0 in the first period, and 3.5:1.0, 5.0:1.0, and 6.5:1.0 in the second. All diets were supplemented with 1,500 units of phytase activity per kilogram of six different sources of microbial phytase. At the end of each experiment, two broilers per replicate were slaughtered to collect the ileal content. In the samples of digested food, the Ca and P contents were determined for later calculation of the apparent ileal digestibility coefficients of these minerals. The Ca:aP ratio influenced the activity of phytases, and the highest digestibility was observed with lower ratios, in both experiments. The phytases that provided greater digestibility coefficients were A, D, and E. In both ages evaluated, the inclusion of phytases in the feed improved the digestibility of Ca and P, which was also affected by the content of calcium and by the phytase source used.

Index terms: microbial enzymes, phosphorus digestibility, phytase superdosing, stages of creation.

Digestibilidade ileal de cálcio e fósforo em frangos de corte alimentados com rações com diferentes fitases e relações cálcio:fósforo disponível

Resumo – O objetivo deste trabalho foi determinar a digestibilidade ileal aparente do cálcio e do fósforo em frangos de corte alimentados com rações com diferentes fitases e relações cálcio:fósforo disponível (Ca:Pd). Foram realizados dois experimentos: um com frangos de 22 a 33 dias de idade, e outro com frangos de 35 a 42 dias. As relações Ca:Pd usadas foram de 4,5:1,0, 6,0:1,0 e 7,5:1,0, no primeiro período, e de 3,5:1,0, 5,0:1,0 e 6,5:1,0, no segundo. Todas as dietas foram suplementadas com 1.500 unidades de atividade de fitase por quilograma de seis diferentes fontes de fitase microbiana. Ao final de cada experimento, duas aves por repetição foram abatidas para a coleta do conteúdo ileal. Nas amostras de digestas, foram determinados os teores de Ca e P para posterior cálculo do coeficiente de digestibilidade ileal aparente destes minerais. A relação Ca:Pd influenciou a atividade das fitases, e a maior digestibilidade foi observada nas menores relações, em ambos os experimentos. As fitases que proporcionaram os maiores coeficientes de digestibilidade foram A, D e E. Em ambas as idades avaliadas, a inclusão de fitases às dietas aumentou a digestibilidade de Ca e P, a qual também foi afetada pelo teor de cálcio e pela fonte de fitase utilizada.

Termos para indexação: enzimas microbianas, digestibilidade do fósforo, superdosagem de fitase, fases de criação.

Introduction

Phytases are enzymes commonly used in broiler feed, with the main purpose of disrupting phytic acid (Rama Rao et al., 2014; Wu et al., 2015). Cereals use the phytic acid molecule as a reserve source of phosphorous (~75%) in seeds for germination, whereas chicken have a low capacity of phytase synthesis; therefore, the addition of exogenous phytase (Beeson et al., 2017) is often needed to improve growth performance and nutrient utilization by broilers. However, the effectiveness of phytases in increasing P digestibility depends on the level of dietary calcium, P content, intestinal pH, phytate concentration, bird age and species, as well as on enzyme-related factors, such as phytase source and dosage, optimal pH range for action, and resistance to the action of proteases in the gastrointestinal tract (Adeola & Cowieson, 2011; Dersjant-li et al., 2015; Sousa et al., 2015).

Many studies have evaluated the inclusion of large doses of units of phytase activity (FTU) in feed, in order to enhance the availability of phytic phosphorus to broilers, highlighting the concept of "superdosing" (Manangi & Coon, 2008). The excess of calcium in diets also deserves attention in this context, since it can affect phytase activity (Selle et al., 2009). Rousseau et al. (2012) concluded that it is possible to reduce dietary P from feed, without hindering animal performance, if Ca concentration is concomitantly reduced.

Exogenous phytase allows the reduction of dietary P, but the conditions that optimize the efficiency of the enzyme still need to be determined, particularly in relation to Ca contents (Narcy et al., 2009). According to Kim et al. (2017), the contents of Ca and P recommended for broiler feeds still are to be debated. Moreover, little attention has been given to the possibility of reducing Ca concentrations in diets supplemented with phytases and with low available phosphorus (aP) levels. Therefore, studying the effect of dietary Ca concentrations on nutrient digestibility in broiler diets supplemented with different kinds of phytases is of great importance.

The objective of this work was to determine the apparent ileal digestibility of calcium and phosphorus in broilers fed diets with different phytases and Ca:aP ratios.

Materials and Methods

Two experiments were conducted in the poultry sector of the Department of Animal Science of Universidade Federal de Lavras (Ufla), located in the state of Minas Gerais, Brazil, using Cobb-500 male broilers. Each experiment corresponded to a breeding phase, comprehending the periods from 22 to 33 days of age and from 35 to 42 days. All procedures used in the experiments were approved by the ethics committee on animal use of Ufla (protocol number 004/11).

The experimental design was completely randomized, in a (6x3) + 1 factorial arrangement, corresponding to six sources of microbial phytases and three Ca:aP ratios, plus a positive control diet, without phytase addition. The feed was formulated according to the nutritional recommendations of Rostagno et al. (2011). For each treatment, four replicates of four and three chickens each were carried out, respectively, for the period from 22 to 33 days of age and from 35 to 42 days, totaling 304 and 228 broilers. In the first period, the Ca:aP ratios used were 4.5:1.0, 6.0:1.0, and 7.5:1.0, while in the second, the ratios were 3.5:1.0, 5.0:1.0, and 6.5:1.0 (Tables 1 and 2).

Different phytases were added to the diets at the concentration of 1,500 units of phytase activity (FTU) per kilogram of feed. The phytases used were: phytase A, a 6-phytase expressed by *Aspergillus oryzae*, genetically modified with two synthetic genes from the gene coding for phytase in *Citrobacter Braakii* ATCC 51113; phytase B, a 6-phytase produced by *A. oryzae*, genetically modified with the phytase gene of *Peniophora lycii*; phytase C, a 6-phytase produced by *Schizosaccharomyces pombe* ATCC 5233, genetically modified with the phytase gene of *Escherichia coli*; phytase D, the second generation of the genetic breeding of 6-phytase produced by *E. coli*; phytase E, the first generation of the genetic breeding of 6-phytase produced by *E. coli*; and phytase F, a "wild type" of 6-phytase, also produced by *E. coli*.

The Ca:aP ratios were defined according to P availability with the inclusion of 1,500 FTU kg^{-1}, as previously determined by Naves et al. (2015) and reinforced by Gautier et al. (2018). A total of 3.0 g kg^{-1} P were obtained: 2.0 g kg^{-1} P made totally available by 1,500 FTU kg^{1} plus 1.0 g kg^{-1} added P. The P contents in the diets supplemented with phytase were similar to that of the control.

One-day-old broilers were purchased from a hatchery and kept in a conventional shed until 21 or 34 days of age, receiving corn and soybean meal according to their nutritional requirements (Rostagno et al., 2011). Chickens were individually weighed at 22 and 35 days of age and sorted according to their weight. Then, they were transferred to cages (50x50x50 cm) in a metabolism room, so that the experimental units presented similar initial mean weight (1.046±0.010 and 2.204±0.080 kg, respectively, for the weighed ages).

During the experiment, chickens received feed and water ad libitum. For each evaluated period, 0.5% chromium oxide was added and mixed to the feed one day before slaughter. On the thirty-third and forty-second days, two chickens per replicate were slaughtered to collect ileal intestinal contents; for this, a 20-cm segment of the ileum was sampled, the digest was removed by finger pressure, and the ileal content was frozen at -80°C, until analysis.

Samples were lyophilized using the L101 lyophilizer (LioTop, São Carlos, SP, Brazil) and were kept in vacuum condition at -40ºC, until constant weight. Total Ca and P analyses were performed in the lyophilized feed and digests, using the methodology of Association of Official Analytical Chemists (Horwitz & Latimer, 2005). Chromium content in the feed and ileal digests was determined by atomic absorption spectrophotometry, according to Williams et al. (1962). Subsequently, the coefficients of apparent

Table 1. Composition and calculated and determined nutritional composition of the diet with six different microbial phytases and three calcium:available phosphorous ratios, supplied to broilers in the period of 22 to 33 days of age.

Ingredients	PCD(1)	Phytases A, B, and C			Phytases D, E, and F		
($g\ kg^{-1}$)		4.5:1.0	6.0:1.0	7.5:1.0	4.5:1.0	6.0:1.0	7.5:1.0
Corn	623.89		623.89			623.89	
Soybean meal	304.92		304.92			304.92	
Soybean oil	32.00		32.00			32.00	
Dicalcium phosphate	11.49		0.00			0.00	
Limestone	7.66	7.89	11.61	15.32	7.89	11.61	15.32
Salt	4.30		4.30			4.30	
DL-methionine	2.64		2.64			2.64	
L-lysine HCL	2.08		2.08			2.08	
L-threonine	0.47		0.47			0.47	
Mineral supplement(2)	0.50		0.50			0.50	
Vitamin supplement(3)	0.40		0.40			0.40	
Choline chloride	0.40		0.40			0.40	
Bacitracin zinc	0.25		0.25			0.25	
Phytase(4)	0.00		0.15			0.30	
Inert (kaolin)	4.02	15.27	11.56	7.85	15.12	10.41	7.70
Chromium oxide	5.00		5.00			5.00	
Nutritional composition ($g\ kg^{-1}$)							
Metabolizable energy ($kcal\ kg^{-1}$)	3,100		3,100			3,100	
Calculated crude protein	195.00		195.00			195.00	
Determined crude protein	196.20	197.80	196.10	195.30	195.84	197.20	196.80
Calculated calcium	7.50	4.50	6.00	7.50	4.50	6.00	7.50
Determined calcium	7.73	4.62	6.19	7.69	4.58	6.12	7.65
Available phosphorus	3.40		1.00			1.00	
Calculated total phosphorus	5.40		3.00			3.00	
Determined total phosphorus	5.75	3.39	3.42	3.45	3.37	3.48	3.41
Calculated lysine	10.78		10.78			10.78	
Calculated methionine + cystine	7.87		7.87			7.87	
Calculated threonine	7.01		7.01			7.01	
Phytase activity ($FTU\ kg^{-1}$)	0.00		1,500			1,500	

(1)Positive control diet. (2)Supplemented per kg of feed: 55 mg Zn, 0.18 mg Si, 0.70 mg I, 10 mg Cu, 78 mg Mn, and 48 mg Fe. (3)Supplemented per kg of feed: 0.64 mg folic acid, 11.60 mg pantothenic acid, 0.024 mg biotin, 2.0 mg butylhydroxytoluene, 14.8 mg niacin, 8,000 IU vitamin A, 1.2 mg vitamin B1, 16.20 IU vitamin E, 10.8 µg vitamin B12, 4.8 mg vitamin B2, 2.40 mg vitamin B6, 2,000 IU vitamin D3, and 1.92 mg vitamin K3. (4)Enzymatic supplementation in units of phytase activity (FTU) per kilogram: phytases A, B, and C, declared enzymatic activity of 10,000 FTU g^{-1}; and phytases D, E, and F, declared enzymatic activity of 5,000 FTU g^{-1}.

ileal digestibility of Ca and P were obtained with the following equations: indigestibility factor (IF) = percentage of chromium in the diet/percentage of chromium in the digest; and apparent ileal digestibility coefficient = [(percentage of nutrient in the diet - (percentage of nutrient in the digest x IF)) / percentage of nutrient in the diet] x 100.

The data were subjected to the analysis of variance, using the Sisvar, version 5.6, software (Ferreira, 2014). The different Ca:aP ratios and phytases evaluated were compared with each other using the Scott-Knott test, at 5% probability, in order to avoid overlapping of letters in the comparison of the means. In addition, P digestibility coefficients were compared by Dunnett's test, at 5% probability.

Table 2. Composition and calculated and determined nutritional composition of the diet with six different microbial phytases and three calcium:available phosphorous ratios, supplied to broilers in the period from 35 to 42 days of age.

Ingredients	PCD[(1)]	Phytases A, B, and C			Phytases D, E, and F		
(g kg^{-1})		3.5:1.0	5.0:1.0	6.5:1.0	3.5:1.0	5.0:1.0	6.5:1.0
Corn	669.17		669.17			669.17	
Soybean meal	263.73		263.73			263.73	
Soybean oil	30.73		30.73			30.73	
Dicalcium phosphate	9.78		0.00			0.00	
Limestone	6.60	5.69	9.41	13.12	5.69	9.41	13.12
Salt	4.19		4.19			4.19	
DL-methionine	2.47		2.47			2.47	
L-lysine HCL	2.47		2.47			2.47	
L-threonine	0.56		0.56			0.56	
Mineral supplement[(2)]	0.50		0.50			0.50	
Vitamin supplement[(3)]	0.30		0.30			0.30	
Choline chloride	0.20		0.20			0.20	
Phytase[(4)]	0.00		0.15			0.30	
Inert (kaolin)	4.30	14.85	11.13	7.41	14.70	10.98	7.26
Chromium oxide	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Nutritional composition (g kg^{-1})							
Metabolizable energy (kcal kg^{-1})	3,150		3,150			3,150	
Calculated crude protein	180.00		180.00			180.00	
Determined crude protein	179.80	181.20	179.95	181.50	180.75	181.06	181.49
Calculated calcium	6.50	3.50	5.00	6.50	3.50	5.00	6.50
Determined calcium	6.65	3.69	5.06	6.75	3.74	5.13	6.48
Available phosphorus	3.00		1.00			1.00	
Calculated total phosphorus	5.00		3.00			3.00	
Determined total phosphorus	5.25	3.15	3.25	3.17	3.07	3.14	3.21
Calculated lysine	10.10		10.10			10.10	
Calculated methionine + cystine	7.37		7.37			7.37	
Calculated threonine	6.56		6.56			6.56	
Phytase activity (FTU kg^{-1})	0.00		1,500			1,500	

[(1)]Positive control diet. [(2)]Supplemented per kg of feed: 55 mg Zn, 0.18 mg Si, 0.70 mg I, 10 mg Cu, 78 mg Mn, and 48 mg Fe. [(3)]Supplemented per kg of feed: 0.48 mg folic acid, 8.70 mg pantothenic acid, 0.018 mg biotin, 1.5 mg butylhydroxytoluene, 11.1 mg niacin, 6,000 IU vitamin A, 0.8 mg vitamin B1, 12.15 IU vitamin E, 8.10 μg vitamin B12, 3.6 mg vitamin B2, 1.80 mg vitamin B6, 1,500 IU vitamin D3, and 1.44 mg vitamin K3. [(4)]Enzymatic supplementation in units of phytase activity (FTU) per kilogram: phytases A, B, and C, declared enzymatic activity of 10,000 FTU g^{-1}; and phytases D, E, and F, declared enzymatic activity of 5,000 FTU g^{-1}.

Results and Discussion

There was no interaction between phytases and Ca:aP ratios for the apparent ileal digestibility of P, in the period from 22 to 33 days of age. Phytase D provided the highest digestibility of P, and the increase in the Ca:aP ratio from 4.5:1.0 to 7.5:1.0 reduced P exploitation up to 27.8% (Table 3). Chickens fed diets with the Ca:aP ratios of 4.5:1.0, supplemented with any of the evaluated phytases, and of 6.0:1.0, containing phytase B, showed ileal digestibility of P similar to that of chickens fed the control. It should be noted that ileal digestibility of P was lower in chickens fed all other experimental diets.

Phytase types and Ca:aP ratios interacted for the apparent ileal digestibility of Ca, determined in the period from 22 to 33 days (Table 3). The increase in the Ca:aP ratio from 4.5:1.0 to 7.5:1.0 decreased Ca utilization for all six microbial phytases. However, for the Ca:aP ratios of 4.5:1.0 and 7.5:1.0, phytases A and E provided the highest indices of ileal digestibility of Ca. Moreover, considering the ratio of 6.0:1.0, the highest Ca digestibility was observed using feed supplemented with phytase A. There was no difference between the control and supplemented diets containing phytase, according to Dunnett's test, for the three evaluated Ca:aP ratios.

In the period from 35 to 42 days of age, phytase types and Ca:aP ratios interacted as to the apparent ileal digestibility of P (Table 4). Regardless of the type of phytase, the highest utilization of P occurred for the Ca:aP ratio of 3.5:1.0, and P digestibility was impaired with increasing Ca:aP ratios. Only phytases A and F showed high catalytic efficiency with a wide range of Ca:aP ratios, with greater P utilization in the three assessed ratios. The following experimental diets had lower Ca use compared with the control: Ca:aP ratio of 3.5:1.0 and phytases B and C; Ca:aP of 5.0:1.0 and phytases A, B, C, E, and F; and Ca:aP of 6.5:1.0 and the six evaluated phytases.

Microbial phytases and the Ca:aP ratio also had a significant effect on apparent ileal digestibility of Ca, in the period from 35 to 42 days (Table 4). These results suggest that a lower Ca:aP ratio allows a better digestibility of Ca with less variability in the activity of phytases. In general, for the six studied phytases, the highest ileal digestibility of Ca was obtained with diets formulated with the Ca:aP ratio of 3.5:1.0, and the lowest, with the 6.5:1.0 ratio. However, chickens showed better Ca utilization when feed diets with the following combinations: Ca:aP ratio of 3.5:1.0 and phytases A, D, and E; Ca:aP of 5.0:1.0 and phytase D; and Ca:aP of 6.5:1.0 and phytases B, C, D, and F.

Table 3. Apparent ileal digestibility of calcium and phosphorous in 33-day-old broilers fed diets containing different microbial phytases and Ca:available P ratios(1).

Phytase(2)	Digestibility of Ca (%)			Average	Digestibility of P (%)			Average
	4.5:1.0	6.0:1.0	7.5:1.0		4.5:1.0	6.0:1.0	7.5:1.0	
A	73.6Aa	57.0Ba	43.4Ca	58.0	78.4	67.6*	54.0*	66.7b
B	65.7Ab	50.7Bc	35.6Cd	50.7	77.2	70.00	53.8*	67.0b
C	54.8Ad	43.1Bd	35.8Cd	44.6	74.6	66.4*	53.8*	64.9b
D	61.9Ac	45.3Bd	38.4Cc	48.5	80.7	68.7*	62.0*	70.8a
E	70.8Aa	52.4Bc	43.3Ca	55.5	74.3	69.8*	59.6*	67.9b
F	67.3Ab	54.3Bb	40.0Cb	53.8	77.8	67.0*	50.8*	65.2b
Average	65.7	50.5	39.4	-	77.2A	68.2B	55.7C	-
PCD(3)	-	-	-	54.5	-	-	-	74.0
CV (%)		6.3				5.6		
p-value								
Phytase x Ca:available P		0.05				ns		
Phytase		0.01				0.01		
Ca:available P		0.01				0.01		

(1)Means followed by equal letters, uppercase in the rows and lowercase in the columns, do not differ by the Scott-Knott test, at 5% probability. (2)Added to the diets at the concentration of 1,500 FTU kg^{-1}. (3)Positive control diet without phytase and formulated with 7.5 g calcium per kilogram of feed and 3.4 g available phosphorus per kilogram of feed. *Differs significantly from the control diet by Dunnett's test, at 5% probability. nsNonsignificant.

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For the two periods evaluated in the present study, the highest values of apparent ileal digestibility of Ca and P were observed with the diets containing the lowest Ca:aP ratios (4.5:1.0 in the period from 22 to 33 days, and 3.5:1.0 from 35 to 42 days), regardless of the phytase used. Phytase activity is impaired by high Ca contents because the excess of this mineral can bind to the phytate molecule and form complexes (calcium phytate) not efficiently degraded by phytases (Selle et al., 2009; Faridi et al., 2015).

The negative effect of high Ca concentrations on phytase activity was reported by Applegate et al. (2003), who found a reduction of 44.4% in phytate hydrolysis in the ileum due to increasing levels of Ca in the feed. Plumstead et al. (2008) observed a decrease in phytase efficiency when dietary Ca increased from 4.7 to 11.6 g kg^{-1}, reducing ileal digestibility of phytate P in 71%. Similarly, Walk et al. (2012) verified a 7% reduction in apparent ileal digestibility of P when Ca content increased from 6.4 to 10.3 g kg^{-1}. Amerah et al. (2014) evaluated the effect of different Ca:aP ratios (1.43, 2.14, 2.86, and 3.57:1.00) and phytase levels (0 and 1,000 FTU kg^{-1}) on nutrient digestibility of broilers with 5 to 21 days of age, and found a linear reduction in P digestibility with increasing Ca:aP ratios. According to Gautier et al. (2018), a balanced Ca:aP ratio is essential for the formulation of broiler diets.

Calcium in excess may also bind to inorganic P to form calcium phosphate, which not only is poorly assimilated by the intestine, but also has the capacity to adsorb other minerals and to prevent their absorption by the gastrointestinal tract (Rama Rao et al., 2014). Furthermore, the formation of calcium phytate and calcium phosphate complexes is favored by increased pH in the digestive system segments (Wilkinson et al., 2013; Sousa et al., 2015). Therefore, according to Lawlor et al. (2005), limestone – which is widely used as a source of Ca in poultry diets – may enhance the formation of these complexes in the gastrointestinal tract and diminish mineral digestibility due to its strong acid-binding capacity. The increase in pH not only decreases phytase action, by the formation of less catalytic substrates, but also reduces the efficiency of this enzyme since most of the commercialized microbial phytases have higher activity under acidic conditions (Selle et al., 2009). In broilers, the main sites of phytase activity are the crop and the upper digestive tract, which have the lowest pH (Dersjant-Li et al., 2015). Finally, it is worth mentioning than an increase in pH in the proventriculus can impair the action of pepsin, an important enzyme in the process of protein digestion, compromising the use of amino acids (Walk et al., 2012).

Table 4. Apparent ileal digestibility of calcium and phosphorous in 42-day-old broilers fed diets containing different microbial phytases and Ca:available P ratios.

Phytase[2]	Digestibility of Ca (%)			Average	Digestibility of P (%)			Average
	3.5:1.0	5.0:1.0	6.5:1.0		3.5:1.0	5.0:1.0	6.5:1.0	
A	84.4Aa	59.7Bc	49.5Cb	64.5	90.0Aa	82.9Ba*	74.2Ca*	82.4
B	75.4Ab	65.3Bb	56.3Ca	65.7	82.1Ab*	68.1Bc*	65.3Bb*	71.8
C	74.7Ab	66.2Bb	57.2Ca	66.0	77.0Ab*	68.6Bc*	68.6Bb*	71.4
D	85.2Aa	74.2Ba	57.0Ca	72.1	90.5Aa	84.0Ba	67.3Cb*	80.6
E	79.4Aa	49.9Bd	41.7Cc	60.0	85.5Aa	73.8Bb*	68.0Cb*	75.7
F	72.3Ab	60.5Bc	53.4Ca	62.0	88.4Aa	81.5Ba*	77.8Ba*	82.6
Average	78.5	62.6	52.5	-	85.6	76.5	70.2	-
PCD[3]	-	-	-	67.0	-	-	-	87.7
CV (%)		6.4				4.9		
p-value								
Phytase x Ca:available P		0.01				0.01		
Phytase		0.01				0.01		
Ca:available P		0.01				0.01		

[1]Means followed by equal letters, uppercase in the rows and lowercase in the columns, do not differ by the Scott-Knott test, at 5% probability. [2]Added to the diets at the concentration of 1,500 FTU kg^{-1}. [3]Positive control diet without phytase and formulated with 7.5 g calcium per kilogram of feed and 3.4 g available phosphorus per kilogram of feed. *Differs significantly from the control diet by Dunnett's test, at 5% probability.

As described in the literature, the addition of phytase in diets for broilers improves the digestibility of Ca and P. Ravindran et al. (2008) observed an increase of 27% in ileal digestibility of Ca, using a diet supplemented with 500 FTU kg^{-1}. Lalpanmawia et al. (2014) found improved ileal digestibility coefficients for P with diets supplemented with 500 FTU kg^{-1}. Oliveira et al. (2008) reported increased ileal digestibility coefficients for Ca and P due to the use of phytase, showing that the hydrolysis of the phytate-mineral complex does not only release P but also Ca, ready to be absorbed.

The microbial phytases evaluated in the present study provided different coefficients of ileal digestibility of Ca and P; the highest values were observed for phytases A, D, and E. These enzymes may differ in their activity along the digestive tract of broilers, due to their particularities regarding the optimum pH range for performance, thermal stability, catalytic properties, and resistance to the action of endogenous proteases (Konietzny & Greiner, 2002; Dersjant-Li et al., 2015). The obtained results are indicative that that variation in Ca digestibility is lower in diets with a lower Ca:aP ratio, regardless of the microbial phytase used.

The apparent ileal digestibility of Ca and P was higher, in absolute values, in older birds (35 to 42 days old). A possible explanation is that the digestive system of older animals is more efficient, with greater production of digestive secretions and intestinal absorption capacity of nutrients (Macari et al., 2002).

Conclusions

1. The inclusion of 1,500 units of phytase activity per kilogram improves the digestibility of calcium and phosphorous in Cobb-500 broiler diets, allowing to reduce the Ca:available P ratio to 4.5:1 for animals with 22 to 33 days of age and to 3.5:1 for animals with 35 to 42 days.

2. The digestibility of Ca and P is affected by the microbial phytase used.

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