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Effects of high phytase dosage on broiler performance and nutrient digestibility

Efeitos da alta dosagem de fitase no desempenho de frangos de corte e na digestibilidade de nutrientes

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Abstract: The study evaluated the effect of different doses of phytase and two diets (corn and soybean meal, and meat and bone meal) on the performance of broiler chickens and the digestibility of nutrients in feed. A total of 1320 Cobb 500 broilers were used for the performance experiment, 216 Cobb 500 broilers for the metabolism test, and 252 broiler chicks to determine the apparent and standardized digestibility coefficients of amino acids in the diets. The birds were individually weighed and distributed in a completely randomized design, comprising six treatments divided into a 2x3 factorial scheme (two diets and three levels of phytase composing the treatments): vegetable diets with 500, 1000, and 1500 FTU of phytase (T1, T2, T3); and meat and bone meal diets with 500, 1000, and 1500 FTU of phytase (T4, T5, T6). The average final weight gain and weight gain of animals that consumed diets with meat and bone meal and phytase at 500 FTU/kg were similar to those that consumed 1000 FTU/kg of phytase. The digestibility of amino acids for animals that consumed diets with corn and soybean meal and phytase at 500 FTU/kg was similar to those that consumed 1000 FTU/kg; statistically, the level of 1500 FTU/kg was equal to that of 500 FTU/kg. High doses of phytase did not promote improvements in performance and nutrient digestibility, as well as EMAn values; therefore, phytase supplementation at 500 FTU/kg is recommended.

Keywords: digestibility; enzyme; phytic acid

Resumo: O estudo avaliou o efeito de diferentes doses de fitase e duas dietas (milho e farelo de soja, e farinha de carne e ossos) no desempenho de frangos de corte e na digestibilidade de nutrientes das rações. Foram utilizados 1320 frangos de corte Cobb 500 para o experimento de desempenho, 216 frangos de corte Cobb 500 para o ensaio de metabolismo, e 252 pintos de corte para a determinação dos coeficientes de digestibilidade aparentes e estandardizados dos aminoácidos das dietas. As aves foram pesadas individualmente e distribuídos em delineamento inteiramente casualizado, totalizando seis tratamentos divididos em esquema fatorial 2x3 (duas dietas e três níveis de fitase compondo os tratamentos): dietas vegetais com 500, 1000 e 1500 FTU de fitase (T1, T2, T3); e dietas com farinha de carne e ossos com 500, 1000 e 1500 FTU de fitase (T4, T5, T6). O ganho de peso médio final e ganho

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de peso dos animais que consumiram dietas com farinha de carne e ossos e fitase a 500 FTU/kg foi similar com os que consumiram 1000 FTU/kg de fitase. E a digestibilidade dos aminoácidos para os animais que consumiram dietas com milho e farelo de soja e fitase com 500 FTU/kg foi similar com os que consumiram 1000 FTU/kg, e estatisticamente o nível de 1500 FTU/kg foi igual ao nível de 500 FTU/kg. Altas doses de fitase não promoveram melhora no desempenho e digestibilidade dos nutrientes, bem como nos valores de EMAn, com isso, recomenda-se a suplementação de fitase a 500 FTU/kg.

Palavras-chaves: ácido fítico; digestibilidade; enzima

1. Introduction

Broiler chicken feed in Brazil is formulated with ingredients of plant origin (corn and soybean meal), which represent 90% of the entire diet, contributing to the supply of energy, protein, vitamins, and minerals. These ingredients represent high costs in feed [1]. The use of enzymes contributes to improving the digestibility and availability of certain nutrients, such as phosphorus.

Ingredients of plant origin in bird diets contain some anti-nutritional compounds, such as phytate or phytic acid, a molecule that mainly contains phosphorus. Phytic acid forms insoluble salts with important amino acids and minerals, leading to decreased availability, as it is poorly digested by monogastric animals. Phytate hydrolysis is carried out by the enzyme phytase, and therefore its addition to the diet has been an alternative in current formulations since birds cannot produce it sufficiently [2,3].

Phytase supplementation aims to make the phosphorus contained in phytic acid molecules available, thereby reducing the need to include ingredients rich in this mineral. Besides phosphorus availability, phytic acid, due to its electronegative charge under intestinal conditions (pH and temperature), can reduce the solubility and digestibility of Ca, Fe, Zn, and protein, leading to detrimental effects [6].

An overdose of phytase can contribute to improving the digestibility coefficients of mineral matter and protein. This improvement in digestibility may be related to the degradation of phytate and the increased availability of myoinositol [4]. Therefore, the use of the phytase enzyme, both at levels recommended by manufacturers and in overdose, is of great interest to the sustainability of the poultry production chain. This enzyme can help maximize the digestion of natural feed components and reduce environmental excretions [5].

Walters et al. [7] observed improvements in bird performance and nutrient digestibility when evaluating overdoses of phytase (2,000 and 3,000 FTU/kg).

Furthermore, the use of phytase can reduce environmental pollution. Approximately 30% of P excretion is reduced by poultry and pigs with the inclusion of phytase in diets, and the digestibility of other nutrients linked to phytic acid is increased through the hydrolysis of the phytate molecule [8].

Another method to enhance phosphorus availability in animals has been the inclusion of meat and bone meal in feed, an ingredient of animal origin devoid of the phytic acid molecule. Supplementation with around 5% meat and bone meal can potentially reduce or eliminate the need for inorganic phosphorus supplementation, thereby lowering feed costs. The phosphorus in meat and bone meal, due to its lack of association with phytic acid, is considered more bioavailable than phosphorus in vegetable-based ingredients [9].

The present study was conducted under the hypothesis that phytase enhances bird performance and nutrient digestibility, thereby contributing to reduced environmental pollution. The objective was to evaluate the effects of different phytase doses and two diets—corn and soybean meal, and meat and bone meal—on the performance of broiler chickens and nutrient digestibility in their diets.

2. Material and Methods

2.1 Location, facilities and experimental management

This experiment was approved by the Ethics Committee on the Use of Production Animals of the Federal University of Viçosa (CEUAP/UFV) - protocol nº021/2019. It was conducted at the Poultry Production and Nutrition Teaching, Research, and Extension Unit of the Animal Husbandry Department of the Agricultural Sciences Center of the Federal University of Viçosa (UFV), from September to October 2019.

Food and water were provided ad libitum. The boxes were monitored throughout the day to assess the condition of the animals, availability of feed and water, temperature control, and any other adverse conditions. Animals that died during the experiment were promptly removed and weighed. The weights, date, and box were recorded regularly to adjust feed consumption and calculate feed conversion at the end of each experimental phase, following Sakamura & Rostagno [10].

2.2 Experimental treatments

The study comprised two experiments with six treatments organized into a 2x3 factorial scheme (two diets and three levels of phytase: 500, 1000, and 1500 FTU). Treatments were as follows: T1 - Dicalcium phosphate diet with 500 FTU; T2 - 1000 FTU; T3 - 1500 FTU; T4 - Meat and bone meal diet with 500 FTU; T5 - 1000 FTU; T6 - 1500 FTU. Each treatment had 10 replications with 22 birds per experimental unit (Table 1).

Table 1 Experimental treatments.

	Overdose Test
Treatments	Levels
1	Dicalcium phosphate diet (with 500 FTU)
2	T1 + 500 FTU (total 1000 FTU)
3	T1 + 1000 FTU (total 1500 FTU)
4	Meat and bone meal diet (with 500 FTU)
5	T4 + 500 FTU (total 1000 FTU)
6	T4 + 1000 FTU (total 1500 FTU)

The diets were formulated according to the recommendations of Rostagno et al. [11], and tailored for the average performance of regular broiler chickens. The diets included corn and soybean meal, as well as corn, soybean meal, and meat and bone meal, supplemented with 500 FTU of phytase (50 g/t), resulting in an increase of 0.15% available phosphorus, 0.15% calcium, and 50 kcal/kg ME from phytase. Tables 1 and 2 present the composition of the experimental rations.

Table 2 Composition of experimental rations for the phase from 1 to 21 days.

	Initial from 01 to 21 days of age					
Ingredients/Treatments	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**
Corn	54,97	54,97	54,97	56,04	56,04	56,04
Soybean meal	39,70	39,78	39,70	36,84	36,84	36,84
Soy oil	1,79	1,79	1,79	1,15	1,15	1,15
Limestone	1,00	1,00	1,00	0,46	0,46	0,46
Dicalcium phosphate	0,98	0,98	0,98	---	---	---
Common salt	0,51	0,51	0,51	0,54	0,54	0,54
DL-methionine, 99%	0,31	0,31	0,31	0,32	0,32	0,32
Lysine HCl, 76%	0,16	0,16	0,16	0,16	0,16	0,16
L-threonine, 98%	0,04	0,04	0,04	0,04	0,04	0,04
Vitamin supplement[2]	0,20	0,20	0,20	0,20	0,20	0,20
Mineral supplement[1]	0,13	0,13	0,13	0,13	0,13	0,13
Choline chloride, 60%	0,10	0,10	0,10	0,10	0,10	0,10
Salinomycin	0,05	0,05	0,05	0,05	0,05	0,05
Antioxidant (BHT)	0,01	0,01	0,01	0,01	0,01	0,01
Commercial Phytase	0,005	0,010	0,015	0,005	0,010	0,010
Meat and bone meal 43%	---	---	---	4,000	4,000	4,00
Total	**100**	**100**	**100**	**100**	**100**	**100**
Meta. Energy, kcal/kg	305	305	305	305	305	305
Crude protein %	23,12	23,12	23,12	23,62	23,62	23,62
Calcium%	0,93	0,93	0,93	0,93	0,93	0,93
Phosphorus available %	0,44	0,44	0,44	0,47	0,47	0,47
Sodium%	0,21	0,21	0,21	0,21	0,21	0,21
Digestible arginine %	1,43	1,43	1,43	1,46	1,46	1,46
Glycine + Digestible serine %	1,85	1,85	1,85	2,05	2,05	2,05
Digestible isoleucine %	0,90	0,90	0,90	0,88	0,88	0,88
Digestible methionine %	1,25	1,25	1,25	1,25	1,25	1,25
Met.+ Digestible Cys. %	0,92	0,92	0,92	0,92	0,92	0,92
Digestible methionine %	0,62	0,62	0,62	0,63	0,62	0,63
Digestible threonine %	0,82	0,82	0,82	0,82	0,82	0,82
Digestible tryptophan %	0,26	0,26	0,26	0,25	0,25	0,25
Digestible valine %	0,96	0,96	0,96	0,96	0,96	0,96

[1] Vitamin Supplement - Guarantee levels per kg of feed: Vit. A - 9375 IU; Vit. D3 - 2375 IU; Vit E - 35 IU; Vit B1 - 2.50 mg; Vit B2 - 6.25 mg; Vit B6 - 3.5 mg; Vit B12 - 0.015 mg; Nicotinic acid - 37.5 mg; B.C. Pantothenic - 12.5 mg; Vit. K3 - 1.88 mg; B.C. Folic - 0.875 mg; Biotin - 0.088 mg.
[2] Mineral supplement – Guarantee levels per kg of feed: Selenium - 0.375 mg; Manganese- 88 mg; Iron –62.5 mg; Zinc – 81.3 mg; Copper- 12.5 mg; Iodine- 1.25 mg. 3 Anticoccidial.

Table 3 Composition of experimental rations for the 22-to-42-day phase.

	Growth/termination 21 to 42 days of age					
Ingredients/Treatments	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**
Corn	59,53	59,53	59,53	60,38	60,38	60,38
Soybean meal	33,74	33,74	33,74	30,91	30,91	30,91
Soy oil	3,86	3,86	3,86	3,29	3,29	3,29
Limestone	0,80	0,80	0,80	0,62	0,62	0,62
Dicalcium phosphate	0,67	0,67	0,67	---	----	---
Common salt	0,48	0,48	0,48	0,42	0,42	0,42
DL-methionine, 99%	0,27	0,27	0,27	0,28	0,28	0,28
Lysine HCl, 76%	0,17	0,17	0,17	0,17	0,17	0,17
L-threonine, 98%	0,03	0,03	0,03	0,03	0,03	0,03
Vitamin supplement[2]	0,10	0,10	0,10	0,10	0,10	0,10
Mineral supplement[1]	0,10	0,10	0,10	0,10	0,10	0,10
Choline chloride, 60%	0,10	0,10	0,10	0,10	0,10	0,10
Salinomycin	0,05	0,05	0,05	0,05	0,05	0,05
Starch	0,05	0,05	0,05	0,05	0,05	0,05
Antioxidant (BHT)	0,01	0,01	0,01	0,01	0,01	0,01
Commercial Phytase	0,005	0,010	0,015	0,005	0,010	0,015
Meat and bone meal 43%	---	---	---	4,00	4,00	4,00
Total	**100**	**100**	**100**	**100**	**100**	**100**
Ener. Metabolizable, Kcal/kg	315	315	315	315	315	315
Crude Protein %	20,76	20,76	20,76	21,25	21,25	21,25
Calcium %	0,75	0,75	0,75	0,75	0,75	0,75
Available phosphorus %	0,37	0,37	0,37	0,46	0,46	0,46
Sodium %	0,20	0,20	0,20	0,20	0,20	0,20
Digestible arginine %	1,26	1,26	1,26	1,29	1,29	1,29
Digestible glycine + serine %	1,65	1,65	1,65	1,85	1,85	1,85
Digestible isoleucine %	0,79	0,79	0,79	0,78	0,78	0,78
Digestible lysine %	1,12	1,12	1,12	1,12	1,12	1,12
Met.+ cyst. Digestible %	0,83	0,83	0,83	0,83	0,83	0,83
Digestible methionine %	0,55	0,55	0,55	0,56	0,56	0,56
Digestible threonine %	0,74	0,74	0,74	0,74	0,74	0,74
Digestible tryptophan %	0,23	0,23	0,23	0,22	0,22	0,22
Digestible valine %	0,86	0,86	0,86	0,86	0,86	0,86

[1]*Vitamin Supplement - Guarantee levels per kg of feed: Vit. A - 9375 IU; Vit. D3 - 2375 IU; Vit E - 35 IU; Vit B1 - 2.50 mg; Vit B2 - 6.25 mg; Vit B6 - 3.5 mg; Vit B12 - 0.015 mg; Nicotinic acid - 37.5 mg; B.C. Pantothenic - 12.5mg; Vit. K3 - 1.88mg; B.C. Folic - 0.875mg; Biotin - 0.088 mg.*
[2]*Mineral supplement – Guarantee levels per kg of feed: Selenium - 0.375 mg; Manganese- 88 mg; Iron –62.5 mg; Zinc – 81.3 mg; Copper- 12.5 mg; Iodine- 1.25 mg. 3Anticoccidial.*

2.3 Performance experiment

In the performance experiment, 1320 one-day-old male Cobb 500 broilers were utilized. Birds were individually weighed and distributed across treatments to ensure similar average body weights. The animals were housed in 3-meter-high masonry sheds with fiber cement tiles. Performance parameters assessed included feed intake (FI, g/bird), weight gain (WG, g/bird), feed conversion ratio (FCR, kg/kg) at 21 and 42 days of age, and viability (VIAB, %). Mortality was recorded daily to adjust feed consumption as per Sakomura and Rostagno [10]. Food and water were provided ad libitum throughout the experimental period. The chicken bedding consisted of new sawdust.

2.4 Metabolism experiment

In the metabolism experiment, 216 male Cobb broilers were randomly assigned in a completely randomized design with six treatments and 6 replications per treatment unit, each containing 6 birds. The birds were housed in cages equipped with trays lined with plastic for total excreta collection, during the period from 14 to 23 days of age. Each cage was equipped with a nipple feeder and waterer, providing ad libitum access to food and water. Initially, the birds were fed a commercial diet based on corn and soybean meal for 13 days before being introduced to the experimental diets.

Total excreta were collected to determine apparent metabolizable energy (AME) corrected for nitrogen balance (AMEn). Following a 4-day adaptation period (days 14-18), excreta were collected twice daily (8 am and 6 pm) over four consecutive days (days 19-23). After the collection period, samples were thawed at room temperature, homogenized, and subsamples were stored in plastic containers at -18 ºC until analysis.

Excreta and feed samples were dried in a forced ventilation oven at 55°C for 72 hours. Samples were ground with a 1 mm screen mill. Dry matter content (DM) was determined by oven-drying samples overnight at 105°C [21]. Nitrogen content in excreta and feed samples was determined using the Kjeldahl method according to standard protocols [21]. Nitrogen excretion (NE) was calculated by multiplying the total excreted DM by the percentage of nitrogen in excreta, also in DM. Nitrogen intake (NI) was similarly calculated for feed samples.

Nitrogen retention (NR) was calculated as the difference between NI and NE. Gross energy content was determined using an adiabatic C5001 bomb calorimeter (IKA-Werke GmbH & Co.KG, Staufen, Germany). EMAn values were calculated based on diet and excreta analyses, following methods described by Sakomura and Rostagno [10].

2.5 Digestibility experiment

For digestibility, 252 male Cobb 500 broilers were housed in cages and distributed in a completely randomized design, with seven treatments, six replications, and six birds per experimental unit. To determine the standardized ileal digestibility coefficients (CSIDs) of amino acids, a protein-free diet was utilized to measure endogenous amino acid excretion (see Table 3). The birds received the experimental diet from 16 to 20 days of age. Celite® (Celite Corp., Lompoc, CA, USA), an acid-insoluble ash (AIA) source, was added to all diets at 10 g kg-1 as an indigestible marker, and IAA concentrations were determined using the method by Van Keulen and Young [12].

After a five-day adaptation period, all birds were euthanized by cervical dislocation, and their abdominal cavities were immediately opened to expose the digestive tract. The intestinal contents were removed from a 40 cm section just anterior to the ileo-cecal junction.

Ileal samples were frozen and stored at -20ºC until processing. Subsequently, they were lyophilized for 72 h at -40ºC using a lyophilizer (LH 0401, Terroni, São Carlos, Brazil).

Laboratory analysis for dry matter content of diets and ileal digesta was conducted for digestibility calculations. Amino acid content analysis of diets and excreta was performed by CBO - Análises Laboratories (Campinas, São Paulo, Brazil) using HPLC (high-performance liquid chromatography). Amino acid digestibility was calculated based on the analysis of diets and ileal digesta following methods described by Sakomura and Rostagno [(10)].

2.6 Statistic

All data were subjected to analysis of variance (ANOVA). To estimate the effect of the factors under study (levels and sources), as well as their interactions, the means were compared using the Tukey test at 5% significance. The analyses were performed using the statistical program R v. 3.5.1 [(19)].

3. Results

3.1 Performance experiment

The results for feed intake (FI), average final weight (AFW), weight gain (WG), and feed conversion (FCR) from 1 to 21 days and 1 to 42 days of age can be found in Tables 3 and 4, respectively.

There was no significant effect (P >0.05) on feed consumption and feed conversion during the period from 1 to 21 days of age among treatments. However, a significant phytase x diet interaction was observed for average final daily weight and weight gain (P <0.05). Birds fed diets containing meat and bone meal showed better average weight and weight gain when supplemented with 1000 FTU of phytase compared to the 1500 FTU level.

Table 3 Performance of broilers aged 1 to 21 days receiving the experimental diets.

		Feed			Probability			
Variables	**Enzyme**	**C.SB**	**M.BM**	**Average**	**Diets**	**Enzyme**	**E*D**	**CV %**
	500	1,23	1,25	1,24				
FI (kg/bird)	1000	1,25	1,26	1,25	0,34	0,27	0,87	6,52
	1500	1,27	1,30	1,28				
Average		**1,25**	**1,27**					
	500	0,98B	1,02Aab	1,00				
AFW (kg/bird)	1000	1,00	1,03^a	1,01	0,10	0,34	0,01	3,18
	1500	1,01	0,99b	1,00				

Average		**1,00**	**1,01**					
WG (kg/bird)	500	0,94B	0,98Bab	0,96				
	1000	0,95	0,98a	0,97	0,08	0,38	0,01	3,32
	1500	0,96	0,94b	0,95				
Average		**0,95**	**0,97**					
FCR (kg/kg)	500	1,31	1,28	1,30				
	1000	1,30	1,27	1,29	0,91	0,20	0,21	7,74
	1500	1,31	1,38	1,34				
Average		**1,31**	**1,31**					

CV= coefficient of variation. Average followed by different letters, uppercase in the rows and lowercase in the columns, differ from each other using the Tukey test.
C.SB = Corn and soybean meal.
M.BM = Meat and bone meal
*E*D = Effect of enzyme x diet interaction.*

No interaction (P >0.05) was observed for the variables evaluated—feed consumption, average weight gain, and total weight gain from 1 to 42 days of age. However, there was a significant effect of diet on feed conversion.

Table 4 Performance of broiler chickens aged 1 to 42 days receiving the experimental diets.

		Feed			Probability			
Variables	**Enzyme**	**M.SB**	**M.BM**	**Average**	**Diets**	**Enzyme**	**E*D**	**CV %**
FI (kg/bird)	500	5,02	5,11	5,07				
	1000	4,96	5,07	5,02	0,23	0,55	0,91	5,14
	1500	5,09	5,13	5,11				
Average		**5,02**	**5,10**					
AFW (kg/bird)	500	3,27	3,22	3,25				
	1000	3,32	3,26	3,29	0,07	0,23	0,64	2,91
	1500	3,30	3,29	3,29				
Average		**3,30**	**3,25**					
WG (kg/bird)	500	3,23	3,17	3,20				
	1000	3,27	3,21	3,24	0,08	0,25	0,65	2,96
	1500	3,25	3,24	3,24				
Average		**3,25**	**3,20**					
FCR (kg/kg)	500	1,55	1,61	1,54				
	1000	1,51	1,58	1,57	0,03	0,36	0,66	5,17
	1500	1,56	1,58	1,58				
Average		**1,54B**	**1,59B**					

CV= coefficient of variation. Average followed by different letters, uppercase in the rows and lowercase in the columns, differ from each other using the Tukey test.
C.SB = Corn and soybean meal.
M.BM = Meat and bone meal
*E*D = Effect of enzyme x diet interaction.*

3.2 Metabolism experiment

There was no significant effect (P>0.05) of ME inclusion levels on the values of apparent metabolizable energy and apparent metabolizable energy corrected for nitrogen balance, (Table 5).

Table 5 Average values of apparent metabolizable energy (AME) and apparent metabolizable energy corrected by nitrogen balance (AMEn).

		Feed			Probability			
Variables	**Enzyme**	**C.SB**	**M.BM**	**Average**	**Diets**	**Enzyme**	**E*D**	**CV %**
	500	3264,83	3253,37	3259,09				
AME	1000	3330,99	3301,62	3316,30	0,18	0,06	0,61	2,78
	1500	3393,17	3309,59	3351,38				
Average		**3329,66**	**3288,19**					
	500	3089,41	3089,45	3089,43				
AMEn	1000	3147,19	3132,00	3139,59	0,25	0,05	0,46	2,59
	1500	3212,91	3134,46	3173,68				
Average		**3149,83**	**3118,63**					

CV= coefficient of variation. Average followed by different letters, uppercase in the rows and lowercase in the columns, differ from each other using the Tukey test.
C.SB = Corn and soybean meal.
M.BM = Meat and bone meal
*E*D = Effect of enzyme x diet interaction.*

3.3 Digestibility experiment

Tables 6 and 7 present the digestibility results of essential and non-essential amino acids, methionine (Met), cystine (Cys), lysine (Lys), threonine (Thr), valine (Val), arginine (Arg), isoleucine (Ile), leucine (Leu), tyrosine (Tyr), serine (Ser), proline (Pro), phenylalanine (Phe), alanine (Ala), asparagine (Asp), glycine (Gly), histidine (His) and glutamine (Glu) the average apparent ileal digestibility coefficient of essential amino acids (CAID_EAA), non-essential amino acids (CAID_NEAA), total amino acids (CAID_TAA), methionine + cystine (Met+Cyst), phenylalanine + tyrosine (Phen+Tyr) and glycine + serine (Gly+Ser) from the experimental diets.

When evaluating the apparent and standardized ileal digestibility of essential and non-essential amino acids with different levels of phytase and two diets (corn and soybean meal, and meat and bone meal), higher amino acids digestibility was observed when the animals consumed feed with corn and soybean meal and phytase at 500 FTU and 1000 FTU/kg.

Supplementation with 500 and 1000 FTU/kg of phytase showed the best results when the animals consumed corn and soybean meal for CAID_EAA, CAID_TAA, and gly+ser, and for CAID_NEAA and Phen+Tyr the level of 1500 FTU was statistically similar to that of 500 FTU/kg (Tables 6 and 7).

Table 6 Apparent ileal digestibility coefficients of essential amino acids (CAID_EAA), non-essential amino acids (CAID_NEAA), total amino acids (CAID_TAA), methionine + cystine (Met+CYst), phenylalanine + tyrosine (Phen+Tyr) and glycine + serine (Gly+ Ser) of the experimental diets.

		Feed			Probability			
Variables	**Enzyme**	**C.SB**	**M. BM**	**Average**	**Diets**	**Enzyme**	**E*D**	**CV %**
	500	85,94Aa	81,56B	83,75				
CAID_EAA	1000	86,42Aa	83,03B	84,73	0,01	0,24	0,08	2,80
	1500	82,28b	83,92	83,10				
Average		**84,88**	**82,84**					
	500	83,99Aab	79,85B	82,42				
CAID_ NEAA	1000	84,51Aa	80,72B	82,61	0,02	0,26	0,41	3,49
	1500	80,00b	81,41	80,71				
Average		**83,17**	**80,66**					
	500	84,92Aa	80,64B	82,78				
CAID_TAA	1000	85,41Aa	81,79B	83,60	0,01	0,26	0,02	3,15
	1500	81,08b	82,57	81,83				
Average		**83,80**	**81,6**					
Met+cys	500	91,73	93,04	92,15A				
	1000	89,23	89,56	89,43AB	0,54	0,03	0,90	4,11
	1500	87,15	88,32	88,07B				
Average		**89,51**	**90,26**					
Phe+Tyr	500	84,21Aab	80,00B	82,10				
	1000	85,00Aa	81,47B	83,24	0,03	0,23	0,01	3,24
	1500	80,44b	82,29	81,36				
Average		**83,22**	**81,26**					
	500	78,59Aa	73,46B	76,03				
Gly+Ser	1000	79,57Aa	74,36B	76,96	0,01	0,16	0,02	4,31
	1500	73,54b	75,20	74,37				
Average		**77,23**	**74,34**					

CV= coefficient of variation. Average followed by different letters, uppercase in the rows and lowercase in the columns, differ from each other using the Tukey test.
C.SB = Corn and soybean meal.
M.BM = Meat and bone meal
*E*D = Effect of enzyme x diet interaction.*

Table 7 Standardized ileal digestibility coefficients of essential amino acids (CAID_EAA), non-essential amino acids (CAID_NEAA), total amino acids (CAID_TAA), methionine + cystine (Met+Cyst), phenylalanine + tyrosine (Phen+Tyr) and glycine + serine (Gly+ Ser) of the experimental diets.

		Feed			Probability			
Variables	**Enzyme**	**C.SB**	**M. BM**	**Average**	**Diets**	**Enzyme**	**E*D**	**CV %**
	500	86,11Aa	81,73B	83,92				
CAID_EAA	1000	86,58Aa	83,20B	84,89	0,01	0,24	0,008	2,79
	1500	82,45b	84,08	83,26				
Average		**85,04**	**83,00**					

CAID_ NEAA	500	84,14Aab	80,00B	82,07				
	1000	84,65Aa	80,86B	82,75	0,02	0,26	0,41	3,48
	1500	80,15b	81,55	80,85				
Average		**82,98**	**80,80**					
CAID_TAA	500	84,99Aa	80,72B	82,86				
	1000	85,495Aa	81,86B	83,68	0,01	0,26	0,02	3,14
	1500	81,166b	82,64	81,90				
Average		**83,886**	**81,74**					
Met+cys	500	94,263	95,20	94,50A				
	1000	91,765	91,72	91,78AB	0,75	0,03	0,90	4,00
	1500	89,68	90,49	90,42B				
Average		**92,04**	**92,43**					
Phe+Tyr	500	86,44Aab	82,26B	84,35				
	1000	87,24Aa	83,73B	85,48	0,03	0,23	0,01	3,15
	1500	82,67b	84,54	83,61				
Average		**85,45**	**83,51**					
Gly+Ser	500	82,37Aa	76,78B	79,57				
	1000	83,35Aa	77,67B	80,51	0,004	0,16	0,02	4,12
	1500	77,32b	78,51	77,92				
Average		**81,02**	**77,65**					

CV= coefficient of variation. Average followed by different letters, uppercase in the rows and lowercase in the columns, differ from each other using the Tukey test.
C.SB = Corn and soybean meal.
M.BM = Meat and bone meal
*E*D = Effect of enzyme x diet interaction.*

4. Discussion

4.1 Performance experiment

Phytase supplementation aims to make the phosphorus contained in phytic acid molecules available, making it possible to reduce the inclusion of ingredients. Phytic acid, due to its electronegative charge under intestinal conditions (pH and temperature), not only limits phosphorus availability but also reduces the solubility and digestibility of Ca, Fe, Zn, and protein, leading to adverse effects [6].

Contrary to our findings, Walters et al. [7] reported that high doses of phytase (≥1,500 FTU/kg) improved animal performance compared to standard inclusion rates. Studies have shown that overdosing phytase, such as at 2,000 FTU/kg, does not adversely affect growth performance, nutrient utilization, bone mineralization, or plasma indices in broiler chickens [13].

Despite the benefits observed in several studies of phytase overdose, it is necessary to be aware of some essential factors such as phytate concentration, energy and amino acid density, animal age, and dietary ion balance [6]. Phytate concentration determines the substrate available for phytase action; hence, high phytase doses may not be beneficial without sufficient substrate.

In their study, Liu et al. [14] noted worsened feed conversion (FCR) and weight gain in animals consuming diets with meat and bone meal initially. However, by the final phase, there was a 4.4% improvement in feed conversion.

In the phase from 1 to 21 days of age Meneghetti et al. [15], studied the supplementation of phytase overdose (1,500 to 10,000 FTU/kg), and found no difference in feed consumption and weight gain, However, they observed worsened feed conversion (FCR) for birds fed with levels of 1,500, 3,000 and 6,000 FTU/kg of phytase, differing from this study that found no difference between the variables when supplementing overdoses. Additionally, improvements in feed conversion were noted at 1,500 and 3,000 FTU/kg from 1 to 35 days of age, in contrast to our findings where diets supplemented with phytase did not influence any of the variables studied in the phase from 1 to 42 days of age.

In the phase from 1 to 42 days, supplementation of diets based on meat and bone meal containing phytase did not affect the variables studied. Strategies to enhance exogenous enzyme activity often involve reducing nutrients in the diet and removing sources of phosphorus from the diet [6].

However, in our study there was no absence of phosphorus sources in treatments, until treatment three, dicalcium phosphate was provided and, in the others, meat and bone meal, which is a source of phosphorus, was provided, which could explain the non-beneficial results of the phytase overdose. It is assumed that the interaction between dietary protein and phytate occurs in the intestine at a pH below the proteins' isoelectric point, depending on the concentration of phytate and protein in the medium [6].

The results of this study suggest that from 1 to 42 days of age, supplementation of diets containing meat and bone meal with phytase did not significantly affect performance variables. The presence of phosphorus sources such as dicalcium phosphate and meat and bone meal may have influenced the results, not favoring the benefits of phytase overdose. Therefore, adjusting the diet and considering the interaction between phytate and proteins, as well as other dietary factors, is crucial to optimizing phytase effectiveness and ensuring adequate animal performance.

4.2 Metabolism experiment

Meneghetti et al. [15] found no difference in AMEn values with the supplementation of overdoses of phytase in diets for broiler chickens, which is similar to the findings of the present study. Likewise, Litz et al. [16], when supplementing diets containing phytase (50 g/t) and meat and bone meal, found no difference between the treatments in the values of apparent metabolizable energy and apparent metabolizable energy corrected for nitrogen balance, results that are corroborated by this study.

Phytase supplementation, even at high doses, in diets containing meat and bone meal, corn, and soybean meal did not show an impact on these parameters, and apparent metabolizable energy was corrected for nitrogen balance. This suggests that the phytase effectiveness in improving dietary energy availability may be limited in certain dietary contexts, indicating the need for careful assessment of the specific conditions of each diet to optimize the use of this enzyme.

4.3 Digestibility experiment

In the absence of enzymes for phytate degradation, it can form complexes with proteins and form the phytate-protein complex. In addition to proteins, it can also bind to other substrates and enzymes, causing a decrease in their action, as well as a reduction in protein digestion and an increase in endogenous amino acid losses. Phytase supplementation can alleviate these harmful effects of phytate [17].

Phytase overdoses did not promote improved performance and nutrient digestibility. Effective overdoses reduce the need for amino acids in the diet, leading to an appreciation of the nutrients in corn and soy, as well as greater digestibility and a reduction in endogenous losses [18]. Phytase supplementation provides greater digestibility of amino acids, which may result from phytate breakdown and subsequent release of nutrients, or also due to lower losses of endogenous amino acids [7]. Similar results were found by Cowieson et al. [20] in which, increasing the inclusion concentration above 1000 FYT/kg did not result in additional changes in the digestibility of amino acids, both essential and non-essential.

In a study by Liu et al. [14] evaluating diets containing meat and bone meal and phytase, it was observed that the ileal digestibility of most amino acids was not affected by the addition of phytase. However, glycine showed greater digestibility in diets with phytase and 60 g/kg of meat and bone meal. However, in this study, despite phytase supplementation, the digestibility of essential and non-essential amino acids was lower with the use of meat and bone meal, when compared to conventional diets.

The absence of specific enzymes for phytate degradation results in the formation of phytate-protein complexes, in addition to binding to other substrates and enzymes, impairing protein digestion and increasing endogenous losses of amino acids. The use of phytase is effective in mitigating these negative effects, improving the digestibility of amino acids due to the breakdown of phytate and the release of nutrients. However, excessive doses of phytase do not present additional improvements in performance and nutrient digestibility, although they can reduce the need for amino acids in the diet and enhance the nutrients present in corn and soy. Research suggests that including of phytase in diets containing meat and bone meal may not enhance the digestibility of all amino acids, highlighting the variability of phytase effects depending on diet composition. Therefore, phytase supplementation must be carefully adjusted to maximize its benefits in animal nutrition.

5. Conclusion

High doses of phytase did not promote improvements in performance, nutrient digestibility, as well as EMAn values; hence, phytase supplementation at 500 FTU/kg is suggested. Diets based on corn and soybean meal show higher digestibility of amino acids, both essential and non-essential, especially when supplemented with phytase.

Conflict of interests

The authors declare no conflicts of interest.

Author contributions

Data curation: Soares NS. Formal analysis: Soares NS. Investigation: Soares NS, Salgado HR. Project administration: Soares NS, Salgado HR, Albino LFT. Visualization: Soares NS, Salgado HR, Pinheiro SG, Ferreira TS. Writing – original draft: Soares NS. Writing – review & editing: Soares NS, Kaneko IN, Pinheiro SG, Ferreira TS. Supervision: Salgado HR, Kaneko IN, Jacob RF, Albino LFT. Validation: Kaneko IN. Software: Jacob RF. Resources: Albino LFT. Funding acquisition: Albino LFT.

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References

1. Costa FGP, Brandão PA, Brandão JS, Silva, JHV. Efeito da enzima fitase nas rações de frangos de corte, durante as fases pré-inicial e inicial. Ciênc. Agrotec. 2007; 31 (3): 865-870. doi: https://doi.org/10.1590/S1413-70542007000300037.

2. Woyengo TA, Nyachoti CM. Review: Supplementation of phytase and carbohydrases to diets for poultry. Can. J. Anim. Sci. 2011; 91 (2): 177-192. doi: https://doi.org/10.4141/cjas10081.

3. Abreu MT, Fassani EJ, Silveira MMBM, Viveiros MP. Complexo enzimático à base de xilanase, β-glucanase e fitase em rações para poedeiras comerciais leves em pico de produção. B. Indústr. Anim. 2018; 75:17-24. doi:https://doi.org/10.17523/bia.v75n1p17.

4. Fernandes JIM, Horn D, Ronconi EJ, Buzim R, Lima FK, Pazdiora DA. Effects of phytase superdosing on digestibility and bone integrity of broilers. J. Appl. Poult. Res. 2019; 28:390-398. doi: https://doi.org/10.3382/japr/pfz001.

5. Franceschina CS, Pires PGS, Franceschi CH, Mendes JV. A utilização de fitase na dieta de poedeiras. Nutritime Revista eletrônica. 2016; 13 (1): 1983-9006.

6. Cowieson AJ, Nji FF. Phytate-free nutrition: A new paradigm in monogastric animal production. Animal Feed Science and Technology. 2016; 222: 180-189. doi: https://doi.org/10.1016/j.anifeedsci.2016.10.016.

7. Walters HG, Coelho M, Coufal CD, Lee JT. Effects of Increasing Phytase Inclusion Levels on Broiler Performance, Nutrient Digestibility, and Bone Mineralization in Low-Phosphorus Diets. Journal of Applied Poultry Research. 2019; 28 (4): 1210-1225. doi: https://doi.org/10.3382/japr/pfz087.

8. Kies AK, Hemert KHFV, Sauer WC. Effect of phytase on protein and amino acid digestibility and energy utilisation. World's Poultry Science Journal. 2007; 57: 109-116. doi: https://doi.org/10.1079/WPS20010009.

9. Selle PH, Cowieson AJ, Ravindran V. Consequences of calcium interactions with phytate and phytase for poultry and pigs. Livestock Science, 2009; 124 (1-3), 126-141. doi: https://doi.org/10.1016/j.livsci.2009.01.006.

10. Sakamura NK, Rostagno HS. Métodos de pesquisa em nutrição de monogástricos. 2ª ed., Jaboticabal:Funep; 2016. 262p.

11. Rostagno HS, Albino L FT, Hannas MI, Donzele JL, Sakomura NK, Perazzo FG, Saraiva A, Teixeira MV, Rodrigues PB, Oliveira RF, Barreto SLT, Brito CO. Tabelas Brazileiras para aves e suínos: composição de alimentos e exigências nutricionais. 4a ed. Minas Gerais: Viçosa; 2017. p.488.

12. Van Keulen J, Young BA. Evaluation of acid-insoluble ash as natural marker in ruminant digestibility studies. Journal of Animal Science. 1977; (4): 282-287.

13. Babatunde OO, Cowieson AJ, Wilson JW, Adeola O. Influence of age and duration of feeding low-phosphorus diet on phytase efficacy in broiler chickens during the starter phase. Poult. Sci. 2019; 0:1-10. doi: https://doi.org/10.3382/ps/pez390.

14. Liu SY, Cowieson AJ, Selle PH. The influence of meat-and-bone meal and exogenous phytase on growth performance, bone mineralisation and digestibility coefficients of protein (N), amino acids and starch in broiler chickens. Anim. Nutr. 2016; 2(2): 86-92. doi: https://doi.org/10.1016/j.aninu.2016.03.003.

15. Meneghetti C, Bertechini AG, Rodrigues PB, Fassani EJ, Brito JAG, Reis MP, Garcia Jr AAP. Altos níveis de fitase em rações para frangos de corte. Arq. Bras. Med. Vet. Zootec. 2011; 63 (3): 624-632.

16. Litz FH, Fernaandes EA, Bueno JPR, Silva MCA, Carolino ACXG, MARTINS JMS, Fagundes NS, Nascimento JR, DV. Digestibility, Determination of Metabolizable Energy and Bone Mineralization of Broilers Fed with Nutritionally Valued Phytase. Brazilian Journal of Poultry Science. 2017; 19. doi: https://doi.org/10.1590/1806-9061-2016-0325.

17. Pereira AA, Junqueira OM, Alva JCR, Sgavioli S, Praes MFFM, Griep Júnior DN. Utilização de rações de poedeiras comerciais formuladas com fitase e níveis de proteína bruta sobre a excreção de fósforo, nitrogênio e cálcio. Ars Veterinaria. 2010; 26 (3): 178-183.

18. Siegert W, Zuber T, Sommerfeld V, Krieg J, Feuerstein D, Kurrle U, Rodehutscord M. Prececal amino acid digestibility and phytate degradation in broiler chickens when using different oilseed meals, phytase and protease supplements in the feed. Poultry Science. 2019; 1 (98): 5700-5713. https://doi.org/10.3382/ps/pez355

19. R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

20. Cowieson, AJ, Ruckebush JP, Sorbara JOB, Wilson JW, Guggenbuhl P, Ross FF. A systematic view on the effect of phytase on ileal amino acid digestibility in broilers. Animal Feed Science and Technology. 2017; 225: 182-194. doi: https://doi.org/10.1016/j.anifeedsci.2017.01.008

21. Detmann E, Souza MA,Valadares Filho, SC. et al. Métodos para análise de alimentos. Suprema: Visconde do Rio Branco. 2012. 214p.