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Standardized ileal amino acid digestibility of plant-based feedstuffs and phytase supplementation in broiler chicken diets

[*Digestibilidade ileal estandardizada de aminoácidos de alimentos de origem vegetal e suplementação de fitase em dietas para frangos de corte*]

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ABSTRACT

The objective was to determine the standardized ileal amino acid digestibility (SIAAD) in soybean meal (SBM), corn + SBM, wheat + SBM and a protein free diet (PFD) associated with phytase. A total of 672 Cobb 500 (493±10g) male chicks were distributed in a completely randomized design with twelve treatments (PFD, PFD + SBM, PFD + corn + SBM and PFD + wheat + SBM, supplemented with 0, 500 and 1000 FTU), eight replicates and seven birds per cage from 14 to 23 days. Phytase supplementation at 500 FTU increased the SIAAD of methionine in SBM ($P<0.05$) and threonine in corn + SBM ($P<0.05$). There was no significant effect ($P>0.05$) for the SIAAD of methionine, arginine and histidine in wheat + SBM as the phytase supplementation. However, the SIAAD of lysine, threonine, isoleucine, phenylalanine, valine, cystine, alanine, aspartic acid, glutamic acid, glycine, serine and tyrosine differed ($P<0.05$). In general, the SIAAD for SBM, corn + SBM and wheat + SBM are 90.32, 88.65 and 83.97% (0 FTU); 91.31, 88.81 and 88.36% (500 FTU); and 91.36, 87.09 and 87.87% (1000 FTU). In conclusion, the efficacy of phytase for improve the SIAAD vary according to the feedstuff and level of supplementation.

Keywords: amino acids, broilers, digestibility, phytase, phosphorus

RESUMO

Objetivou-se determinar a digestibilidade ileal estandardizada dos aminoácidos (SIAAD) do farelo de soja (FS), milho + FS, trigo + FS e uma dieta livre de proteína (PFD) com fitase. Um total de 672 pintos machos (493±10g), Cobb 500, foi distribuído em um delineamento inteiramente ao acaso, com 12 tratamentos (PFD, PFD + FS, PFD + milho + FS e PFD + trigo + FS, suplementados com 0, 500 e 1000 FTU), oito repetições e sete aves por gaiola, no período de 14 a 23 dias. A suplementação de fitase em 500 FTU aumentou o SIAAD da metionina do FS (P<0,05) e da treonina do milho + FS (P<0,05). Não houve efeito significativo (P>0,05) para o SIAAD de metionina, arginina e histidina no trigo + farelo de soja à medida que os níveis de fitase aumentaram. No entanto, o SIAAD de lisina, treonina, isoleucina, fenilalanina, valina, cistina, alanina, ácido aspártico, ácido glutâmico, glicina, serina e tirosina diferiu (P<0,05). Em geral, a SIAAD do FS, milho + FS e trigo + FS foi de 90,32, 88,65 e 83,97% sem a suplementação de fitase (0 FTU); 91,31, 88,81 e 88,36% (500 FTU); e 91,36, 87,09 e 87,87% (1000 FTU). Em conclusão, a eficácia da suplementação com fitase para aumentar a SIAAD varia de acordo com o alimento e o nível de suplementação.

Palavras-chave: aminoácidos, frangos de corte, digestibilidade, fitase, fósforo

INTRODUCTION

Plant-based feedstuffs such as corn, wheat, and soybean meal (SBM) are the main components of poultry diets. However, their composition includes phytic acid, a powerful chelator of proteins, amino acids, starch, cations and enzymes (pepsin, trypsin, and α-amylase) that reduce the solubility and digestibility of those nutrients due

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to the formation of insoluble complexes (Cowieson *et al*., 2017). Thus, alternatives to mitigate the undesirable effects of phytic acid should be sought.

Phytase supplementation in cereal-based diets can be used as a strategy to overcome endogenous enzyme deficiencies and improve the utilization of dietary nutrients, especially in early life stages of poultry. This practice is adopted in broiler chicken farming to increase the digestibility of raw materials, improve production performance, and increase nutrient utilization efficiency, ultimately reducing production costs (Meng *et al*., 2005; Dessimoni *et al*., 2019). Phytase inclusion improves the digestibility of amino acids in ingredients individually and in compound diets (Rutherfurd *et al*., 2002; Cowieson *et al*., 2017; Walters *et al*., 2019). Rutherfurd *et al*. (2012) found that the amino acid digestibility of wheat, corn and SBM can increase by 11.7%, 4.0% and 6.5%, on average, when phytase is added to the diet. The use of the exogenous phytase enzyme in diets can not only allow for a reduction in inorganic phosphorus supplementation, but also promote the release of amino acids and other nutrients. This process is known as the extra-phosphoric effect of phytase.

The objective of the present study was to determine the standardized ileal amino acid digestibility in soybean meal, corn + soybean meal, wheat + soybean meal and a protein free diet associated with different levels of the phytase supplementation in diets for broiler chickens.

MATERIAL AND METHODS

The experiment was conducted at the Unit for Teaching, Research and Extension in Poultry Production and Nutrition at the Department of Animal Science at the Federal University of Viçosa, located in Viçosa - MG, Brazil. All experimental procedures complied with the norms established by the Ethics Committee in the Use of Production Animals - CEUAP-UFV (approval no. 056/2013).

A total of 672 male broiler chicks of the Cobb 500 line with an average weight of 493 ± 10 g were used in the study. The birds were evaluated in a completely randomized experimental design with twelve treatments (PFD, PFD + SBM, PFD + corn + SBM and PFD + wheat + SBM, supplemented with 0, 500 and 1000 FTU of phytase), eight replicates and seven birds per cage during the experimental period of 14 to 23 days of age, to determine the standardized ileal amino acid digestibility (SIAAD) in SBM, corn + SBM and wheat + SBM, by the ileal content collection method. From the 1st to the 13th day of age, the birds were reared as recommended in the manual for the management of the line, with water and feed available *ad libitum*. At 14 days of age, the birds were weighed and transferred to cages in metal structures. The cages were equipped with nipple drinkers and trough feeders. Water and feed were available *ad libitum* throughout the experimental period.

The total amino acid composition of SBM, corn and wheat were analyzed by high-performance liquid chromatography (HPLC) (AOAC, 1995) (Table 1). The experimental treatments were a protein-free diet (PFD) with supplementation of 0, 500 and 1000 FTU of phytase; 70% of PFD + 30% SBM with supplementation of 0, 500 and 1000 FTU of phytase; 20% PFD + 50% corn + 30% SBM with supplementation of 0, 500 and 1000 FTU of phytase; 20% PFD + 50% wheat + 30% SBM with supplementation of 0, 500 and 1000 FTU of phytase, totaling 12 treatments.

A PFD with supplementation of 0, 500 and 1000 FTU of phytase are described in Table 2. The ingredients in the proper proportions (SBM, corn and wheat) were added to replace starch in protein-free diets with supplementation of 0, 500 and 1000 FTU of phytase. Celite was used as acid-insoluble ash (AIA) as a marker to determine the digestibility of amino acids and protein as well as quantify endogenous amino acid excretion. The premix of minerals and vitamins that were used in the diets did not contain rice hulls as an excipient and diluent. Thus, there was no interference from silica content in the AIA analysis.

Table 1. Total amino acid composition of soybean meal, corn, and wheat, on an as-is basis (%)

Amino acid	Soybean meal	Corn	Wheat
Lysine	2.82	0.23	0.29
Threonine	1.83	0.30	0.31
Methionine	0.64	0.17	0.17
Cystine	0.52	0.14	0.20
Alanine	2.00	0.67	0.38
Arginine	3.32	0.36	0.49
Aspartic acid	5.11	0.54	0.52
Glutamic acid	8.47	1.66	3.45
Glycine	1.91	0.31	0.46
Histidine	1.20	0.25	0.25
Isoleucine	2.04	0.29	0.37
Leucine	3.49	1.08	0.73
Phenylalanine	2.41	0.45	0.51
Serine	2.34	0.41	0.50
Tyrosine	1.79	0.29	0.30
Valine	2.14	0.41	0.46
Protein	44.97	8.22	11.07
Total amino acids	43.19	7.86	9.77

Table 2. Composition of experimental protein free diet (PFD) used for the determination of standardized ileal amino acid digestibility for plant-based feedstuffs supplemented with different levels of phytase for broilers from 14 to 23 days of age

Protein free diet (PFD)	PFD + 0 FTU	PFD + 500 FTU	PFD + 1000 FTU
Sugar	5.000	5.000	5.000
Soybean oil	5.000	5.000	5.000
Dicalcium phosphate	2.100	1.397	1.186
Limestone	1.000	1.077	1.100
Salt	0.450	0.450	0.450
Corn cob	4.000	4.000	4.000
Mineral supplement[1]	0.110	0.110	0.110
Vitamin supplement[2]	0.110	0.110	0.110
Choline chloride (60%)	0.200	0.200	0.200
BHT[3]	0.010	0.010	0.010
Acid-insoluble ash (Celite™)	1.000	1.000	1.000
Phytase[4]	0.000	0.009	0.018
Starch	81.020	81.637	81.825
Total	100.000	100.000	100.000

[1]Provides per kg of diet: manganese - 70.0mg; iron - 50.0mg, zinc - 65.0mg; copper - 10.0mg; iodine - 1.0mg, selenium - 0.30mg.

[2]Provides per kg of diet: vitamin A - 7500 IU, vitamin D3 - 1900 IU, vitamin E - 28 IU, K3 - 1.5mg, vitamin B1 - 2.0mg, vitamin B2 - 5.0mg, vitamin B6 - 2.8mg, nicotinic acid - 30mg, pantothenic acid - 10.0mg, vitamin B12 - 0.012mg, biotin - 0.07mg; folic acid - 0.07mg.

[3]Butylated hydroxytoluene 99%.

[4]Phytase – AB Vista Quantum.

*Soybean meal replaced 30% of the starch in the diet, whereas wheat and corn replaced 50% of it in the diets.

*The diets were enriched with 0.13% available P and 0.143% Ca, respectively.

On the 23rd day of age, all birds were sacrificed by cervical dislocation. The ileal digesta was harvested, stored in plastic bottles, and subsequently lyophilized.

Samples of feeds and digesta were analyzed for dry matter (Official…, 2006, method 934.01); nitrogen (Official…, 2006, method 968.06), to calculate the crude protein content (N × 6.25); and acid-insoluble ash (Joslyn, 1970). The amino acid content was also analyzed by HPLC (Official…, 1995). Amino acid digestibility was calculated using acid-insoluble ash (AIA) to estimate the indigestibility factor, using equations proposed by Sakomura and Rostagno (2016).

Endogenous correction to estimate the standardized ileal digestibility of crude protein and amino acids was calculated using the PFD without phytase addition. Endogenous excretion for each enzymatic treatment (i.e., PFD with 0, 500 and 1000 FTU) was also analyzed, with the SIAAD adjusted for the enzyme-free diets and the diets with 500 and 1000 FTU, respectively.

The data were subjected to analysis of variance and means were compared by the Student-Newman-Keuls test at the 5% probability level (SAS, 2014).

RESULTS AND DISCUSSION

The absolute values of endogenous amino acid losses from the digesta of broilers fed PFD diets vary according phytase supplementation levels (Table 3). However, the differences observed in the present study were not statistically significant (P>0.05). According to Moughan *et al*. (2014) endogenous nitrogen losses are influenced by the type of feed, body weight, content and quality of diet ingredients, amount of fiber, dry matter intake and presence of antinutritional factors (trypsin inhibitor, tannins, and lectins).

Table 3. Mean values of ileal endogenous amino acids (mg/g of dry matter intake) determined using protein-free-diet (PFD) supplemented with 0, 500 and 1000 FTU of phytase for broilers

Amino acids	Protein-free-diet			P Value	[2]CV (%)
Phytase[1]	0 FTU	500 FTU	1000 FTU		
Lysine	0.018	0.025	0.024	0.237	18.60
Threonine	0.045	0.057	0.053	0.304	15.50
Methionine	0.010	0.018	0.016	0.137	25.28
Cystine	0.013	0.017	0.015	1.000	27.00
Alanine	0.024	0.042	0.041	0.224	33.55
Arginine	0.026	0.044	0.048	0.167	29.94
Aspartic acid	0.050	0.063	0.068	0.097	13.02
Glutamic acid	0.055	0.071	0.071	0.255	16.94
Glycine	0.027	0.034	0.035	0.194	14.99
Histidine	0.010	0.013	0.015	0.122	15.96
Isoleucine	0.021	0.027	0.026	0.216	15.67
Leucine	0.033	0.043	0.041	0.221	15.11
Phenylalanine	0.021	0.026	0.024	0.261	14.11
Serine	0.036	0.045	0.041	0.365	15.71
Tyrosine	0.018	0.023	0.020	0.344	16.70
Valine	0.028	0.035	0.035	0.271	16.17
Protein	0.804	0.869	0.812	0.999	17.71
Total amino acids	0.459	0.618	0.603	0.184	16.96

[1]Phytase – AB Vista Quantum; [2]Coefficient of variation.

In diet with SBM the SIAAD of methionine increased (P<0.05) when 500 and 1000 FTU of phytase were supplemented (Table 4). In general, the average SIAAD of SBM was 90.32, 91.31 and 91.36%. However, the SIAAD of the other amino acids of SBM were not influenced by phytase supplementation (P>0.05). Other authors also observed a significant effect of phytase supplementation on the amino acids' digestibility from plant-based feedstuffs (Ravindran *et al*., 2001; Rutherfurd *et al*., 2012; Kiarie *et al*., 2015).

In the diet with corn + SBM, phytase supplementation at the dose of 500 FTU improved the SIAAD of threonine (P<0.05) (Table 5). In general, the average SIAAD of corn + SBM was 88.65, 88.81 and 87.09%. However, the SIAAD of aspartic acid, glutamic acid, glycine, isoleucine, leucine, phenylalanine, serine, tyrosine, valine, and protein decreased when the phytase supplementation level was raised to 1000 FTU. The increase in P release by 1000 FTU supplementation may have caused an imbalance in the Ca:P ratio. In addition, the Ca:P ratio of the

PFD with 1000 FTU supplementation was higher compared to the other diets (0 and 500 FTU). Thus, the higher Ca content may have increased the complexation with phytic acid residues forming calcium phytate, reducing the digestibility of some amino acids. Another important fact is that high-dose phytase supplementation can alter electrolyte balance, as phytase influences sodium secretion in the intestinal lumen, altering amino acid digestibility (Ravindran 2013).

Table 4. Standardized ileal amino acid digestibility coefficients in diets based on soybean meal supplemented with 0, 500 and 1000 FTU of phytase for broiler chickens

Amino acids	Soybean meal			P value	CV%[2]
Phytase[1]	0 FTU	500 FTU	1000 FTU		
Lysine	92.09	92.98	92.75	0.157	1.22
Threonine	88.07	88.24	88.16	0.108	1.61
Methionine	92.68 B	97.34 A	97.99 A	0.032	2.39
Cystine	83.25	85.19	85.50	0.114	3.31
Alanine	89.87	91.08	91.46	0.500	2.16
Arginine	92.70	93.82	94.15	0.618	2.49
Aspartic acid	89.42	89.49	89.58	0.204	2.27
Glutamic acid	93.09	93.46	93.44	0.103	1.98
Glycine	86.73	86.99	86.84	0.104	1.99
Histidine	90.90	93.13	92.97	0.114	1.92
Isoleucine	90.65	91.27	91.55	0.130	1.26
Leucine	91.36	91.45	91.47	0.191	1.18
Phenylalanine	92.06	92.44	92.74	0.181	1.16
Serine	89.82	90.35	89.87	0.211	1.48
Tyrosine	91.71	93.00	92.47	0.081	1.31
Valine	90.70	90.80	90.86	0.141	1.46
Mean amino acids	90.32	91.31	91.36	0.073	3.01
Protein	90.27	90.29	90.43	0.151	1.37

A,B - Means followed by different letters in the same row are significantly different according to the SNK test (P<0.05); [1]Phytase – AB Vista Quantum; [2]Coefficient of variation.

Contrary to the data observed in the present study, Walters *et al*. (2019) identified that phytase supplementation above 1000 FTU increased the digestibility of amino acids in diets based on corn and SBM in broilers with 14 and 28 days of age. Kiarie *et al*. (2015) also observed an increase in the ileal digestibility of amino acids from diets based on corn and SBM supplemented with different levels of phytase for broilers with 21 days of age.

The SIAAD of methionine, arginine, and histidine in the diets with wheat + SBM did not differ significantly (*P*>0.05) as the phytase levels were increased (Table 6). However, a difference was detected (*P*<0.05) for the SIAAD of essential amino acids (lysine, threonine, isoleucine, phenylalanine, and valine). The SIAAD of non-essential amino acids increased (*P*<0.05) (cystine, alanine, aspartic acid, glutamic acid, glycine, serine, and tyrosine) between the treatments without and with phytase supplementation. The inclusion of phytase increase the mean SIAAD in the diets with wheat + SBM (*P*<0.05). In general, the average SIAAD of wheat + SBM was 83.97, 88.36 and 87.87%. Nevertheless, the treatments supplemented with 500 FTU and 1000 FTU of phytase were similar in this aspect. Gallardo *et al*. (2018) observed an increase in ileal amino acids digestibility in broilers fed diets containing 30% of wheat bran in the diets supplemented with 500 FTU of phytase, except for the amino acids isoleucine, methionine, tryptophan, alanine, glycine and tyrosine.

Table 5. Standardized ileal amino acid digestibility coefficients in diets based on corn + soybean meal supplemented with 0, 500 and 1000 FTU of phytase for broiler chickens

Amino acids	Corn + Soybean meal			P value	CV%[2]
Phytase[1]	0 FTU	500 FTU	1000 FTU		
Lysine	89.91	89.80	88.39	0.580	1.22
Threonine	84.83B	87.58A	84.96B	0.019	1.61
Methionine	92.66	91.50	90.73	0.318	2.39
Cystine	82.75	82.49	80.32	0.114	3.31
Alanine	89.10	88.28	87.66	0.500	2.16
Arginine	89.92	91.20	90.23	0.618	2.49
Aspartic acid	86.73A	87.31A	84.01B	0.020	2.27
Glutamic acid	91.28A	91.69A	90.01B	0.041	1.98
Glycine	84.55A	84.99A	82.24B	0.036	1.99
Histidine	90.22	90.39	88.80	0.114	1.92
Isoleucine	89.05AB	88.57AB	87.28B	0.012	1.26
Leucine	90.37A	89.96A	88.67B	0.023	1.18
Phenylalanine	90.52A	90.90A	89.01B	0.017	1.16
Serine	87.39AB	88.15AB	85.92B	0.008	1.48
Tyrosine	90.57A	90.22A	88.83B	0.019	1.31
Valine	88.50A	87.96A	86.40B	0.006	1.46
Mean amino acids	88.65	88.81	87.09	0.124	2.98
Protein	88.49A	89.25A	86.44B	0.005	1.37

A,B - Means followed by different letters in the same row are significantly different according to the SNK test (P<0.05); [1]Phytase – AB Vista Quantum; [2]Coefficient of variation.

Table 6. Standardized ileal digestibility coefficients of amino acids in diets based on wheat + soybean meal supplemented with 0, 500 and 1000 FTU of phytase for broiler chickens

Amino acids	Wheat + soybean meal			P value	CV (%)[2]
Phytase[1]	0 FTU	500 FTU	1000 FTU		
Lysine	84.78B	88.54A	88.24A	0.006	1.22
Threonine	80.51B	85.74A	86.12A	0.001	1.61
Methionine	87.40	88.69	88.07	0.318	2.39
Cystine	79.32B	85.50A	87.59A	0.011	3.31
Alanine	82.03B	85.45A	84.96A	0.049	2.16
Arginine	83.46	89.41	87.23	0.161	2.49
Aspartic acid	80.31B	85.04A	86.90A	0.020	2.27
Glutamic acid	90.23B	93.66A	93.92A	0.001	1.98
Glycine	79.17B	85.03A	85.01A	0.036	1.99
Histidine	84.76	88.69	86.20	0.114	1.92
Isoleucine	85.13B	89.30A	88.71A	0.013	1.26
Leucine	85.59B	89.66A	88.41A	0.001	1.18
Phenylalanine	86.81B	91.21A	89.43A	0.009	1.16
Serine	83.11B	88.45A	86.74A	0.008	1.48
Tyrosine	86.83B	91.10A	89.30A	0.012	1.31
Valine	84.14B	88.36A	89.12A	0.006	1.46
Mean amino acids	83.97B	88.36A	87.87A	0.009	2.90
Protein	85.57B	88.17A	87.16A	0.041	1.37

A,B - Means followed by different letters in the same row are significantly different according to the SNK test (P<0.05); [1]Phytase – AB Vista Quantum; [2]Coefficient of variation.

Table 7 shows, only descriptively, the percentage contribution of phytase doses (500 and 1000 FTU) on the SIAAD of SBM. In general, for the amino acids lysine, threonine, glutamic acid, glycine, histidine, serine and tyrosine it was possible to observe a numerical reduction in the contribution to SIAAD with 1000 FTU supplementation.

In the present study, after the dose of 500 FTU, release efficiency decreases due to the reduced amount of substrate for enzymatic action. According to Ravindran (2013), in the presence of adequate concentrations of enzyme, the reaction rate increases as the substrate concentration is elevated up to a maximum value. This is because there is more substrate than the enzyme can cleave. In this way, as the amount of substrate is reduced, no linear increase in cleavage occurs with the growing phytase levels. On this basis, higher phytase levels are not significantly effective, thus obeying the "law of diminishing returns". Some important points must be clarified to understand the effects of the action of supplemented phytase in diets for broilers with different plant-based feedstuffs. First, in the present study, FTU analysis of phytase added to diets was not performed. The phytase nutritional matrix information described in the methodology at the bottom of table 2 was used. Second, recovery analysis of phytase supplemented in different concentrations (500 and 1000 FTU) in SBM, corn + SBM and wheat + SBM. And third, the levels of phytic acid in feedstuffs have not been analyzed. Data from the Brazilian Tables for Poultry and Swine were used (Rostagno *et al*., 2011). Thus, these factors are important to increase the accuracy of the real impact of supplementing different doses of phytase on the SIADD in plant-based feedstuffs used as a basis for broiler diets.

Table 7. Phytase contribution (FTU/kg) to the standardized ileal amino acid digestibility in soybean meal for broiler chickens (%)

					Soybean meal	
Amino acids	Total	0	500	1000	Phytase contribution	
					500	1000
Lysine	2.820	2.597	2.622	2.616	0.025	0.019
Threonine	1.830	1.612	1.615	1.613	0.003	0.002
Methionine	0.640	0.593	0.623	0.627	0.030	0.034
Cystine	0.520	0.433	0.443	0.445	0.010	0.012
Alanine	2.000	1.797	1.822	1.829	0.024	0.032
Arginine	3.320	3.078	3.115	3.126	0.037	0.048
Aspartic acid	5.110	4.569	4.573	4.578	0.004	0.008
Glutamic acid	8.470	7.885	7.916	7.914	0.031	0.030
Glycine	1.910	1.657	1.662	1.659	0.005	0.002
Histidine	1.200	1.091	1.118	1.116	0.027	0.025
Isoleucine	2.040	1.849	1.862	1.868	0.013	0.018
Leucine	3.490	3.188	3.192	3.192	0.003	0.004
Phenylalanine	2.410	2.219	2.228	2.235	0.009	0.016
Serine	2.340	2.102	2.114	2.103	0.012	0.001
Tyrosine	1.790	1.642	1.665	1.655	0.023	0.014
Valine	2.140	1.941	1.943	1.944	0.002	0.003
Protein	44.970	40.594	40.603	40.666	0.009	0.072

Table 8 shows, only descriptively, the percentage contribution of phytase doses (500 and 1000 FTU) on the SIAAD of corn + SBM. In corn + SBM, SIAAD improved with phytase supplementation up to the maximum level of 1000 FTU, especially for lysine, threonine, aspartic acid, glutamic acid, and glycine (Table 8). Walters *et al*. (2019) evaluated different levels of phytase supplementation (250, 500, 750, 1000, 2000 and 3000 FTU/kg) in diets based on corn and SBM for broilers. These authors observed improve in digestibility of amino acids with the supplementation of increasing levels with phytase, with a more pronounced effect in increasing digestibility of tryptophan, threonine, valine, arginine, methionine, and histidine in broilers at 28 days of age.

Table 8. Phytase contribution (FTU/kg) to the standardized ileal digestibility of amino acids in corn + soybean meal for broiler chickens (%)

Corn + soybean meal						
Amino acids	Total	0	500	1000	Phytase contribution	
					500	1000
Lysine	0.769	0.692	0.691	0.680	0.078	0.089
Threonine	0.558	0.474	0.489	0.474	0.069	0.084
Methionine	0.220	0.204	0.201	0.200	0.019	0.020
Cystine	0.183	0.151	0.151	0.147	0.032	0.036
Alanine	0.746	0.665	0.659	0.654	0.087	0.092
Arginine	0.942	0.847	0.859	0.850	0.083	0.092
Aspartic acid	1.445	1.253	1.262	1.214	0.183	0.231
Glutamic acid	2.694	2.459	2.470	2.425	0.224	0.269
Glycine	0.583	0.493	0.496	0.479	0.087	0.104
Histidine	0.389	0.351	0.352	0.345	0.037	0.044
Isoleucine	0.606	0.539	0.536	0.529	0.070	0.077
Leucine	1.270	1.147	1.142	1.126	0.128	0.144
Phenylalanine	0.756	0.684	0.687	0.673	0.069	0.083
Serine	0.726	0.635	0.640	0.624	0.086	0.102
Tyrosine	0.544	0.492	0.490	0.483	0.054	0.061
Valine	0.678	0.600	0.596	0.586	0.082	0.092
Protein	14.081	12.460	12.567	12.171	1.514	1.910

For wheat + SBM, the better SIADD were achieved when 500 FTU of phytase were added, for both essential and non-essential amino acids, except for threonine, cystine, alanine, aspartic acid and glutamic acid (Table 9).

Table 9. Phytase contribution (FTU/kg) to the standardized ileal digestibility of amino acids in wheat + soybean meal for broiler chickens (%)

Wheat + soybean meal						
Amino acids	Total	0	500	1000	Phytase contribution	
					500	1000
Lysine	1.805	1.530	1.598	1.593	0.068	0.062
Threonine	1.171	0.943	1.004	1.008	0.061	0.066
Methionine	0.410	0.358	0.364	0.361	0.005	0.003
Cystine	0.334	0.265	0.286	0.293	0.021	0.028
Alanine	1.281	1.051	1.095	1.088	0.044	0.038
Arginine	2.124	1.773	1.899	1.853	0.126	0.080
Aspartic acid	3.273	2.629	2.783	2.844	0.155	0.216
Glutamic acid	5.419	4.890	5.075	5.090	0.186	0.200
Glycine	1.223	0.968	1.040	1.040	0.072	0.071
Histidine	0.767	0.650	0.680	0.661	0.030	0.011
Isoleucine	1.307	1.113	1.167	1.159	0.055	0.047
Leucine	2.230	1.909	1.999	1.972	0.091	0.063
Phenylalanine	1.540	1.337	1.405	1.377	0.068	0.040
Serine	1.499	1.246	1.326	1.300	0.080	0.054
Tyrosine	1.144	0.993	1.042	1.022	0.049	0.028
Valine	1.370	1.153	1.211	1.221	0.058	0.068
Protein	15.221	13.025	13.420	13.267	0.396	0.242

According to Cowieson *et al*. (2017), phytase supplementation improves the ileal digestibility of amino acids regardless of the supplementation dose. The authors went on to state that the most pronounced effects are seen in lysine, methionine, and threonine from various protein sources, in the diet of broiler chickens. In the present study, the effects of phytase supplementation were greatest for arginine, glutamic acid, and methionine, in SBM; leucine, aspartic acid and glutamic acid, in the diets based on corn + SBM; and arginine, aspartic acid and glutamic acid, in the diets based on wheat + SBM. These findings indicate the existence of differences regarding the diet ingredients and the phytase supplementation doses used. The phytate concentration of SBM, corn and wheat are 0.36, 0.18 and 0.22%, respectively ((Rostagno *et al*. 2017). However, it is important to point out that these values can vary from 0.17 to 0.22% for corn, 0.35 to 0.45% for SBM and 0.18 to 0.29% for wheat (Selle and Ravindran 2007). In addition, the crude protein content of SBM, corn and wheat in the present study was 44.95, 8.22 and 11.07%, respectively. This explains the different effects found for the different levels of phytase supplementation in diets. After all, the phytate content of feedstuffs and the interactions between amino groups and phytate and starch-protein in the diet can influence endogenous loss and mucus production (Cowieson *et al*., 2004). Qaisrani *et al*. (2018) report that mucus is made up of about 5% mucin that is mainly formed by threonine, serine, and proline. Therefore, the increase or depletion in the production of mucus alters the ileal digestibility of amino acids in the diet.

Therefore, the lack of substrate impedes the progressive release of amino acids; however, the extra-phosphoric effects are not limited to phosphorus release or amino acid digestibility, but also to other nutrients (Ca, Mg and Na) and enzymes (pepsin) (Liu *et al*., 2009; Kiarie *et al*., 2015). Considering that endogenous losses change as the amount of phytase addition is increased, the strategy of using different PFD should be better investigated to prevent under- or overestimates of the phytase contribution values. In addition, for new studies evaluating endogenous losses and SIADD associated with supplementation with phytase for broiler chickens, it is necessary to carry out FTU analyzes of the enzyme, enzymatic recovery in the diets and levels of phytic acid in the feedstuffs used in formulation of diets.

CONCLUSIONS

The standardized ileal amino acid digestibility of plant-based feedstuffs varies according to the phytase supplementation level. The respective standardized ileal amino acid digestibility for soybean meal, corn + soybean meal and wheat + soybean meal is 90.32, 88.65 and 83.97% (0 FTU); 91.31, 88.81 and 88.36% (500 FTU); and 91.36, 87.09 and 87.87% (1000 FTU).

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