Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X 2022 / v.24 / n.4 / 001-010

http://dx.doi.org/10.1590/1806-9061-2021-1452

Original Article

Impact of the Supplementation of Exogenous Protease and Carbohydrase on the Metabolizable Energy and Standardized Ileal Amino Acid Digestibility of Soybean Meals in Two Brazilian Regions

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■Keywords

Amino acids, broilers, enzymes, metabolizable energy, soybean meal.

Submitted: 31/January/2021
Approved: 13/September/2022

ABSTRACT

This study aimed to evaluate the effects of different exogenous protease and carbohydrase in broiler diets on the nitrogen-corrected apparent metabolizable energy (AMEn) and standardized ileal amino acid digestibility (SIAAD) of soybean meals (SBM) in two Brazilian regions (Minas Gerais-MG and Rio Grande do Sul-RS). The total excreta collection of 528 14-d-old chicks was used to determine AMEn in a completely randomized design in a 2 (SBM MG and RS) x 5 (enzyme A, B, C, D and basal diet) + 1 (reference diet, RD) factorial arrangement, totaling 11 treatments, 8 repetitions, and 6 birds per experimental unit. Two experimental treatments (T1 and T6) without enzyme supplementation formulated with SBM MG and RS were used as negative control (NC). The RD without the inclusion of SBM MG and RS was used to correct the nitrogen balance. To determine the SIAAD, ileal content was collected from of broilers and the same experimental design and treatments of the previous trial were used except for the RD, which was replaced with a nitrogen-free diet (NFD) to quantify the excretion of endogenous amino acids. Soybean meal from MG showed the highest levels (p<0.05) of AME and AMEn (3,188 kcal/kg and 2,700 kcal/kg, respectively) in comparison to SBM RS (3,121 kcal/kg and 2,549 kcal/kg, respectively) and, when supplemented with the exogenous enzyme C, also improved the SIAAD (p<0.05), as compared to other enzymes.

INTRODUCTION

Soybean meal (SBM) is the main protein ingredient in Brazilian broiler diets. However, the nutritional composition of SBM is inconsistent for different reasons: the genetics of cultivars, the region where it is grown, as well as the amount and type of fertilization, storage, and processing. The nutritional value of SBM is limited by the presence of several anti-nutritional compounds such as trypsin inhibitors, saponins, and oligosaccharides, which inhibit feed intake and nutrient utilization by broilers (Frikha *et al.*, 2012). The thermal processing of SBM reduces most of these effects; however, excess heat increases the incidence of Maillard reactions, which occur between amino acid (AA) amines and reduce sugars in the diet (Qin *et al.*, 1998).

Exogenous enzymes can reduce the nutritional diversity of feed ingredients by improving its nutritional consistency. Moreover, enzymes improve nutrient utilization of ingredients that are nutritionally deficient, enhancing the precision of diet formulations (Ravindran, 2013). As SBM availability and its use increases, so does the number of proteases available in the market. Several digestibility studies have shown that exogenous microbial protease supplementation increases protein hydrolysis and solubility, improving the ileal AA digestibility of broilers (Caine *et al.*, 1998; Romero *et al.*, 2014; Dalólio *et al.*, 2016; Bertechini *et al.*, 2020).

The beneficial effects of enzyme supplementation on poultry diets rich in non-starch polysaccharides (NSP) cereals are well-established (Annison, 1992; Bedford & Classen, 1992). On the other hand, the general belief used to be that ingredients with low NSP (such as corn and SBM) would not benefit from enzyme supplementation, as nutrients found in corn and SBM are generally believed to be highly digestible (Zanella & Sakomura 1999). However, corn contains approximately 0.9% to 6% soluble NSP and 28% insoluble NSP, while SBM contains approximately 6% soluble NSP and 18 to 21% insoluble NSP (Knudsen, 1997; Choct, 2006). Cowieson (2010) states that although diets based on corn and SBM contain a low NSP content, supplementation with exogenous carbohydrases is being widely used in diets for broiler chickens. Dalólio *et al.* (2017) observed a difference in the AMEn and SIAAD values of full-fat-soybean meal samples from five different Brazilian regions. According to these authors, the standardization of industrial thermal processes and the use of exogenous enzymes may be relevant factors increasing AMEn and SIAAD values in broilers. Thus, it is necessary to conduct studies to assess the variation between different SBMs and the use of exogenous enzymes in diets for broilers.

Therefore, the objective of this study was to evaluate the effects of different exogenous proteases and carbohydrases on the standardized ileal amino acids digestibility coefficients and nitrogen-corrected apparent metabolizable energy of two SBMs from two Brazilian regions fed to broilers.

MATERIALS AND METHODS

All experimental procedures were approved by the Ethics Committee on the Use of Production Animals (CEUAP) at the Federal University of Viçosa (UFV) (approval no. 101/2014).

A total of 520 14-d-old male Cobb 528 chicks, from day 1 to 13, were reared in a poultry house with a concrete floor covered with wood shavings. The birds received feed and water *ad libitum*, and the pre-starter diet was formulated according to Rostagno *et al.* (2011). At 14 days of age, the birds were transferred to a metabolic room and placed into battery cages. The experimental design was made of a 2 (SBM MG and RS) x 5 (enzyme A, B, C, D and basal diet) + 1 (reference diet, RD) factorial arrangement, totaling 11 treatments, 8 repetitions, and 6 birds per experimental unit. Two experimental treatments (T1 and T6) without enzyme supplementation formulated with SBM MG and RS were used as negative control (NC) (Table 1). The RD (Table 2) is based on corn and SBM with 20.8% crude protein (CP) and 3,000 kcal of ME/kg of feed. The treatments were formulated by replacing 40% of the RD with soybean meal (SBM) from the MG and RS states. Enzymes A (500ppm), B (125ppm), C (200ppm) and D (200ppm) were added according to the manufacturer's recommendation. The enzyme phytase (100ppm) was added to all diets.

Table 1 – Experimental treatments to determine the levels of ME.

Treatments	Diets
T1	RD+SBM MG (NC)
T2	T1 + Enzyme A
T3	T1 + Enzyme B
T4	T1 + Enzyme C
T5	T1 + Enzyme D
T6	RD+SBM RS (NC)
T7	T6 + Enzyme A
T8	T6 + Enzyme B
T9	T6 + Enzyme C
T10	T6 + Enzyme D
T11	RD

NC = Negative control; RD = Reference diets

All diets contained 1,000 phytase units (FYT) of RONOZYME® Hiphos GT per kg of feed.

Enzyme A, Cibenza DP100, is a protease produced by *Bacillus licheniformis* and contains 600 units/gram; Enzyme B, Poultry Grow, is a protease produced by *Streptomyces fradie* and contains 25,000 units/g; Enzyme C, RONOZYME® ProAct, is the result of the fermentation of *Bacillus licheniformis* containing *Nocardiopsis prasina* genes, and the level of activity of this enzyme is defined by the amount of product needed to degrade 1 µM of the substrate-Suc-Ala-Ala-Pro-Phe-N-succinyl Ala-Ala-Pro-Phe-p-nitroanilide-per minute at pH 9 and 37° C. The product used contains 75,000 PROT units /gram of enzyme. Enzyme D, RONOZYME® VP is produced by *Aspergillus aculeatus* and contains 50 FGB/g of endo-1,3(4)-β-glucanase as well as pentosanase, hemicelulase, and pectinase activity. RONOZYME® HiPhos GT is a 6-microbial phytase expressed by synthetic genes of *Aspergillus oryzae*, which has a phytase activity (FYT) of 10,000 FYT units /g. One phytase unit is the amount of enzyme needed to release 1 µmol of inorganic phosphate under regular conditions (acetate buffer 0.25 M, pH 5.5, temperature of 37° C, and five µmol of sodium phytate). The diets were fed *ad libitum*. The experimental period lasted 10 days, the first five days as adaptation period for the diets, and the last five days for total excreta collection (twice a day) to prevent fermentation and nutrient loss.

Table 2 – Composition of the RD used as a percentage of the natural matter.

Ingredients	%
Corn	59.070
Soybean meal	34.760
Soybean oil	2.170
Dicalcium phosphate	1.527
Limestone	0.912
Common Salt	0.482
L-Lysine HCL (98%)	0.213
DL-Methionine (99%)	0.285
Vitamin supplement[1]	0.110
Mineral supplement[2]	0.110
Choline chloride 60%	0.100
Salinomycin 12%[3]	0.050
Avilamycin 10%[4]	0.010
Antioxidant[5]	0.010
Phytase[6]	0.010
Total	100.00
Calculated composition	
Metabolizable energy (kcal/kg)	3000
Crude Protein (%)	20.80
Digestible lysine (%)	1.174
Digestible methionine (%)	0.562
Digestible methionine + cystine (%)	0.846
Digestible threonine (%)	0.763
Digestible tryptophan (%)	0.231
Calcium (%)	0.819
Available phosphorus (%)	0.394
Sodium (%)	0.210

[1]Composition/kg of the feed: vit. A, 8.250 UI; vit. D3, 2.090 UI; vit. E 31 UI; vit. B1, 2,20 mg; vit B2, 5,50 mg; vit. B6, 3,08 mg; pantothenic acid, 11,0 mg; biotin, 0,077 mg; vit. K3, 1,65 mg; folic acid; 0,770 mg; nicotinic acid 33,0 mg; vit. B12, 0,013 mg; selenium, 0,330 mg.

[2]Composition per kg of feed: manganese, 77.0 mg; iron, 55.0 mg; zinc, 71.5 mg; copper, 11.0 mg; iodine 1.10 mg.

[3]Anticoccidial (Coxistac).

[4]Growth promoter (Surmax).

[5]Butyl Hydroxytoluene (BHT).

[6] RONOZYME Hiphos GT with 10,000 FYT /g.

Total excreta collection was carried out from day 19 to 24. At the end, samples were homogenized and sub-samples were pre-dried in a forced-ventilation oven at 55° C. Dry matter (DM), nitrogen (N), and gross energy (GE) of excreta and feed were determined according to the methodology described by Silva and Queiroz (2002). Apparent metabolizable energy (AME) and nitrogen-corrected apparent metabolizable energy (AMEn) were calculated using the equations proposed by Sakomura & Rostagno (2016):

$$AME\ (kcal/kg) = (GE_{ingested} - GE_{excreted}) / DM_{ingested}$$

$$AMEn\ (kcal/kg) = GE_{ingested} - (GE_{excreted} + 8.22 * [N_{ingested} - N_{excreted}]) / DM_{ingested}$$

In the AA digestibility trial, a total of 528 Cobb 500 male chickens with 25 days of age were allocated in the same experimental design and treatments as the previous trial. The experimental treatments are described in Table 3.

Table 3 – Experimental treatments used to determine the standardized ileal amino acid digestibility coefficients.

Treatments	Diets
T1	NFD+SBM MG (NC)
T2	T1 + Enzyme A
T3	T1 + Enzyme B
T4	T1 + Enzyme C
T5	T1 + Enzyme D
T6	NFD+SBM RS (NC)
T7	T6 + Enzyme A
T8	T6 + Enzyme B
T9	T6 + Enzyme C
T10	T6 + Enzyme D
T11	NFD

NC = Negative control; NFD = Nitrogen free diet.

A NFD was formulated to determine AA endogenous losses. The treatments were based on replacing 40% of the NFD-starch with SBM from the states of MG and RS. Celite™ 1% was added to all diets as an indigestible marker. The enzyme phytase was added to all diets (Table 4).

Table 4 – Composition of the experimental diets (as a percentage of natural matter).

Ingredients / Diets	NFD	NFD + SBM MG	NFD + SBM RS
Corn Starch	82.705	42.705	42.705
Sugar	5.000	5.000	5.000
Soybean meal	-	40.000	40.000
Soybean oil	5.000	5.000	5.000
Dicalcium phosphate	1.622	1.622	1.622
Limestone	0.803	0.803	0.803
Common Salt	0.450	0.450	0.450
Corn cob	3.000	3.000	3.000
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
Celite™	1.000	1.000	1.000
Phytase[3]	0.010	0.010	0.010
Total	100.000	100.000	100.000

[1]Mineral Supplement - amount per kg of feed: manganese, 77.0 mg; iron, 55.0 mg; zinc, 71.5 mg; copper, 11.0 mg; iodine 1.10 mg.

[2]Vitamin Supplement - amount per kg of feed: vit. A, 8.250 UI; vit. D3, 2.090 UI; vit. E 31 UI; vit. B1, 2,20 mg; vit B2, 5,50 mg; vit. B6, 3,08 mg; pantothenic acid, 11,0 mg; biotin, 0,077 mg; vit. K3, 1,65 mg; folic acid; 0,770 mg; nicotinic acid 33,0 mg; vit. B12, 0,013 mg; selenium, 0,330 mg.

[3] RONOZYMEHiPhos®GT with 10,000 FYT/g.

At the end of the fifth day of diet adaptation, all the birds were euthanized by CO_2. Contents of ileum from the Meckel's diverticulum to 40cm proximal to the ileo-caecal junction were collected by gentle pressure between the thumb and finger. The ileal content of all broilers in a replicate was pooled, immediately stored at -20°C, and subsequently freeze-dried. The amino acid content was determined by high-pressure liquid chromatography. The DM and the indigestibility factor (IF) were calculated according to Joslyn (1970). Both apparent ileal amino acid digestibility (AIAAD) and standardized ileal amino acid digestibility (SIAAD) were calculated according to the methodology proposed by Sakomura & Rostagno (2016).

AIAAD coefficient = 1 2 [(AA/AIA)i/(AA/AIA)d],

where (AA/AIA)d = ratio of amino acid to acid-insoluble ash in diet and (AA/Ti)i = ratio of amino acid to acid-insoluble ash in ileal digesta.

SIAAD = AID 1 {[BEAA (g/kg DMI)]/ [Ingredient amino acid (g/kg DM)]},

where BEAA = basal endogenous loss of the amino acid; and ingredient amino acid = concentration of the amino acid in the ingredient.

The data were submitted to ANOVA and means were compared by the Student-Newman Keuls (SNK) test (p<0.05) using Statistical Analysis System (SAS, 2002).

Table 5 – Composition analysis of the MG and RS soybean meals (natural matter).

Nutrient	SBM MG	SBM RS
Dry Matter %	89.03	88.48
Gross energy ME, kcal/kg	4,169	4,101
Crude Protein %	45.20	43.82
Total amino acids %	44.56	44.04
Total essential amino acids %	24.10	24.53
Total nonessential amino acids %	20.47	19.52
Lysine %	2.82	2.77
Methionine %	0.43	0.50
Met+Cys %	0.93	1.06
Threonine %	1.81	1.89
Arginine %	3.59	3.84
Gly+Ser %	4.58	4.81
Valine %	2.10	2.05
Isoleucine %	1.86	1.79
Leucine %	3.39	3.30
Histidine %	1.25	1.28
Phenylalanine %	2.27	2.30
Tyrosine %	1.60	1.75
Alanine %	2.17	2.11
Aspartic acid %	5.09	4.17
Glutamic acid %	8.57	8.23
Cystine %	0.50	0.56
Proline %	2.54	2.70

RESULTS AND DISCUSSION

Apparent metabolizable energy (AME) and AMEn did not interact (p>0.05) between SBM and enzyme supplementation; therefore, analyses were independent. Enzyme supplementation did not present a significant AME and AMEn difference (p>0.05), but SBM showed statistical difference (p<0.05).

Soybean meal (MG) diets had higher AME values than SBM (RS) diets (p<0.05), ranging from 3,188 kcal/kg and 3,121 kcal/kg, respectively, a difference of 67 kcal/kg in DM basis. This difference may be explained by the fact that the SBM had different levels of gross energy (GE) (SBM MG and RS presented 4,683 kcal/kg and 4,635 kcal/kg in DM basis, respectively, a difference of 48 kcal/kg). The AMEn values were 2,700 kcal/kg and 2,549 kcal/kg for MG and RS respectively, a total of 151 kcal/kg difference. This may be explained by the fact that the GE level from SBM MG is higher than that of RS, and the level of N retention provided by the SBM RS supplemented with Enzyme C is higher than that SBM MG supplemented with Enzyme C (p<0.05) by 62.52% and 57.69%, as shown in Table 6.

These levels of N retention may have influenced the increase in AMEn difference when corrected for N balance. According to Sakomura & Rostagno (2016), birds with different degrees of N retention display different amounts of excreted energy for feeds of equal digestibility. That is because, during growth, the protein retained in the bird's body is not catabolized into N products for excretion, therefore not contributing to the energy in the excreta. The average AMEn amount of the SBM was 84 kcal/kg, higher than the energy found by Rostagno *et al.* (2011). This difference can be due to the phytase in the basal diet, and also the quality of the SBMs tested. In terms of N retention, an interaction was observed between SBMs and enzymes; therefore, it was evaluated independently (Table 7).

There was no significant effect (p>0.05) of the enzymes (A, B, C, D) on SBM MG on N retention. However, enzyme C led to higher N retention when

Table 6 – Apparent metabolizable energy (AME), nitrogen-corrected apparent metabolizable energy (AMEn), and nitrogen retention values, in DM basis, of two soybean meals from different Brazilian regions with and without supplementation of enzymes A, B, C, and D.

Soybean meal	Enzyme	AME, kcal/kg DM	AMEn, kcal/kg DM	Nitrogen retention (%)
Minas Gerais (MG)	NC	3163	2679	56.78
	Enzyme A	3171	2682	57.39
	Enzyme B	3181	2693	57.37
	Enzyme C	3191	2700	57.69
	Enzyme D	3233	2748	56.95
SEM		27.37	27.91	0.36
Rio Grande do Sul (RS)	NC	3076	2522	55.93
	Enzyme A	3107	2533	58.34
	Enzyme B	3117	2546	57.98
	Enzyme C	3149	2543	62.52
	Enzyme D	3158	2603	55.97
SEM		33.12	31.40	2.68
Main averages				
Enzyme	NC	3119	2600	56.35
	Enzyme A	3139	2608	57.86
	Enzyme B	3149	2620	57.67
	Enzyme C	3170	2621	60.10
	Enzyme D	3195	2675	56.46
Soybean meal	MG	3188 A	2700 A	57.23
	RS	3121 B	2549 B	58.15
ANOVA *p*-value	Soybean meal	0.0039	0.0001	0.1164
	Enzyme	0.2473	0.1451	0.0006
	SBM * Enz	0.9799	0.9994	0.0142
	CV (%)	3.14	3.36	4.44

NC = Negative control.

Different letters in the same column indicate statistical difference. ANOVA (<0.05)

CV (%) = Coefficient of variation.

Table 7 – Nitrogen retention (%) and effect of the interaction between the soybean meals and supplementation of exogenous enzymes.

Soybean meal	Enzymes				
	NC	Enzyme A	Enzyme B	Enzyme C	Enzyme D
Minas Gerais	56.78aA	57.39aA	57.37aA	57.69 aB	56.95aA
Rio Grande do Sul	55.93bA	58.34abA	57.98 bA	62.52 aA	55.97 bA

NC – Negative control.

"A" and "B" letters in the same column indicate statistical difference, SNK (p<0.05); "a" and "b" letters in the same line indicate statistical difference, SNK (p<0.05).

used with the SBM RS (p<0.05) as compared to the treatments without enzyme, with enzyme B and D providing the same level of N retention as A. Birds submitted to the treatments without enzyme and with enzymes A, B, and D had the same percentage of N retention (p<0.05) for both SBMs. Enzyme C provided the best result (p<0.05) when added to the SBM RS. Tables 8, 9, and 10 present the AADC results of the SBMs with and without enzymes. It was observed that the DC of CP, Phe, and Tyr were not affected by SBMs or enzymes (p>0.05). By assessing the enzymes separately, it was observed that enzymes C and D provided the higher amounts of DC (p<0.05) as compared to the other enzymes. The DC of CP of the SBM MG was higher (p<0.05) than that of the SBM RS.

Table 8 – Standardized ileal amino acid digestibility coefficients (SIAADC) of two soybean meals from different Brazilian regions with and without supplementation of enzymes A, B, C, and D.

Soybean meal	Enzyme	SIAADC (%)						
		Protein	TAA	EAA	NEAA	Lys	Met	Met+Cys
Minas Gerais (MG)	NC	89.09	92.78	91.64	94.04	91.74	95.51	93.77
	Enzyme A	89.80	93.14	92.33	94.37	92.51	97.00	94.32
	Enzyme B	89.77	92.28	91.74	93.41	92.04	93.26	90.92
	Enzyme C	90.99	94.54	93.67	95.95	94.06	97.58	97.92
	Enzyme D	91.50	93.78	93.46	94.96	93.73	97.73	95.81
SEM		0.98	0.88	0.95	0.96	0.92	0.87	0.81
Rio Grande do Sul (RS)	NC	88.73	94.05	93.00	94.42	92.16	96.70	96.76
	Enzyme A	88.41	94.03	92.80	94.99	92.70	96.55	97.70
	Enzyme B	89.85	94.53	93.25	95.40	92.96	97.30	98.03
	Enzyme C	90.38	94.90	94.12	95.49	93.00	98.39	98.01
	Enzyme D	90.50	94.26	93.52	94.93	92.60	98.37	98.66
SEM		0.95	0.36	0.51	0.43	0.34	0.88	0.69
Main averages								
Enzyme	NC	88.91	93.41	92.32	94.23	91.95	96.11	95.26
	Enzyme A	89.10 BC	93.58	92.57	94.68	92.61	96.77	96.01
	Enzyme B	89.81 B	93.41	92.50	94.41	92.50	95.28	94.48
	Enzyme C	90.68 A	94.72	93.90	95.72	93.53	97.99	97.96
	Enzyme D	91.00 A	94.02	93.49	94.95	93.16	98.05	97.23
Soybean meal	MG	90.23 A	93.30	92.57	94.55	92.81	96.22	94.55
	RS	89.57 B	94.35	93.34	95.05	92.68	97.46	97.83
ANOVA *p*-value	SBM	0.005	0.001	0.001	0.001	>1.000	0.001	0.001
	Enzyme	0.001	0.001	0.001	0.001	0.001	0.001	0.001
	Enz x SBM	0.295	0.004	0.023	0.001	0.006	0.001	0.001
	CV (%)	1.121	0.796	0.778	0.559	0.998	1.532	1.073

NC = Negative control. TAA = Total Amino Acids. EAA = Essential Amino Acids. NEAA = Non-essential Amino Acids. Different letters in the same column indicate statistical difference (SNK, $p<0.05$). CV (%) = Coefficient of variation.

Table 9 – Continuation.

Soybean meal	Enzyme	SIAADC (%)						
		Tre	Arg	Gly+Ser	Val	Ileu	Leu	His
Minas Gerais (MG)	NC	90.19	98.60	90.38	90.54	88.60	88.52	90.82
	Enzyme A	89.91	98.83	90.54	91.94	90.18	90.08	92.10
	Enzyme B	89.52	98.91	90.44	90.58	89.40	89.10	91.19
	Enzyme C	93.95	99.82	93.01	89.57	90.81	91.46	95.66
	Enzyme D	93.47	99.46	90.63	92.77	91.43	91.29	95.24
SEM		2.12	0.50	1.12	1.26	1.02	1.30	2.28
Rio Grande do Sul (RS)	NC	95.18	99.52	92.81	87.70	90.02	90.45	93.20
	Enzyme A	95.64	99.53	92.24	87.46	89.62	89.99	93.02
	Enzyme B	95.36	99.57	92.48	88.89	90.61	90.71	93.57
	Enzyme C	97.44	99.58	93.74	90.04	92.35	91.12	94.16
	Enzyme D	96.12	99.58	92.50	89.32	91.13	90.76	94.11
SEM		0.90	0.03	0.59	1.09	1.06	0.42	0.52
Main averages								
Enzyme	NC	92.69	99.06	91.59	89.12	89.31	89.49	92.01
	Enzyme A	92.78	99.18	91.39	89.70	89.90	90.03	92.56
	Enzyme B	92.44	99.24	91.46	89.74	90.00	89.90	92.38
	Enzyme C	95.69	99.70	93.38	89.81	91.58	91.29	94.91
	Enzyme D	94.80	99.52	91.57	91.05	91.28	91.02	94.68
Soybean meal	MG	91.41	99.12	91.00	91.08	90.08	90.09	93.00
	RS	95.95	99.56	92.75	88.68	90.75	90.61	93.61
ANOVA *p*-value	SBM	0.001	0.001	0.001	0.001	0.018	0.032	0.003
	Enzyme	0.001	0.001	0.001	0.001	0.001	0.001	0.001
	Enz x SBM	0.002	0.001	0.036	0.001	0.037	0.002	0.001
	CV (%)	1.378	0.192	0.829	1.360	1.354	1.164	0.962

Different letters in the same column indicate statistical difference (SNK, $p<0.05$). CV (%) = coefficient of variation.

Table 10 – Continuation.

Soybean meal	Enzyme	SIAADC (%)						
		Phe	Tyr	Ala	Asp	Glu	Cys	Pro
Minas Gerais (MG)	NC	91.52	95.47	89.14	95.23	96.07	92.27	88.43
	Enzyme A	92.66	95.76	89.32	95.47	96.84	92.01	87.74
	Enzyme B	91.74	96.10	88.76	94.61	95.54	88.92	87.01
	Enzyme C	92.22	95.61	92.22	96.13	97.21	98.21	94.27
	Enzyme D	93.03	96.38	91.46	95.68	96.19	94.16	91.64
SEM		0.62	0.37	1.55	0.56	0.66	2.41	2.05
Rio Grande do Sul (RS)	NC	91.60	95.33	89.70	94.29	95.61	96.80	93.62
	Enzyme A	91.37	95.27	91.26	95.10	96.27	98.73	92.91
	Enzyme B	91.89	95.21	91.35	95.63	96.86	98.68	93.18
	Enzyme C	92.53	95.48	90.45	96.69	96.72	97.67	93.42
	Enzyme D	92.63	95.57	91.65	94.05	96.26	98.91	93.57
SEM		0,56	0.15	0.79	1.06	0.49	0.90	0.29
Main averages								
Enzyme	NC	91.56 B	95.40 A	89.42	94.76	95.84	94.53	91.03
	Enzyme A	92.01 B	95.51 A	90.29	95.28	96.55	95.37	90.33
	Enzyme B	91.81 B	95.65 A	90.06	95.12	96.20	93.80	90.10
	Enzyme C	92.37 AB	95.54 A	91.33	96.41	96.97	97.94	93.84
	Enzyme D	92.83 A	95.98 A	91.56	94.87	96.22	96.54	92.61
Soybean meal	MG	92.23 A	95.86 A	90.18	95.42	96.37	93.11	89.82
	RS	92.00 A	95.37 B	90.88	95.15	96.34	98.16	93.34
ANOVA *p*-value	SBM	0.262	0.003	0.005	0.090	<1.000	0.001	0.001
	Enzyme	0.002	0.213	0.001	0.001	0.001	0.001	0.001
	Enz x SBM	0.099	<1.000	0.001	0.001	0.001	0.001	0.001
	CV (%)	0.985	0.755	1.194	0.743	0.450	1.214	1.370

NC = Negative control. SIAADC = Standardized Ileal Amino Acid Digestibility Coefficient. Different letters in the same column indicate statistical difference (SNK, $p<0.05$). CV (%) = Coefficient of Variation.

The amounts of TAA, EAA, NEAA, Lys, Met, Met+Cys, Thr, Arg, Gly+Ser, Val, Ile, Leu, His, Ala, Asp, Glu, Cys, and Pro interacted with SBMs and enzyme supplementation and have been studied separately, as shown in tables 11 and 12.

Enzyme C with SBM MG resulted in the highest DC for TAA, NEAA, Met+Cys, Arg, Gly+Ser, Cys, and Pro when compared to the others. SBM MG supplemented with enzyme D improved the DC; however, this improvement was lower than the one obtained with the supplementation of enzyme C, providing results that were similar to the sum of EAA and to the content of Lys, Met, Thr, Leu, His, and Ala ($p<0.05$).

Protease can improve AA digestibility by increasing protein hydrolysis and solubility (Caine *et al.*, 1998). This explains the improvement observed in the SIAAD when enzyme C was used. Stefanello *et al.* (2016) found similar results, except for TAA, which had a higher digestibility. Angel *et al.*, (2011) observed that the digestibility of the CP was higher when they used corn and SBM-based diets supplemented with increasing levels of the same enzyme C used in this study, in concentrations varying from 7,500 to 60,000 PROT/kg in 22 days-old broilers. This result is very similar to the present study: 1.9% improvement in the digestibility of the CP.

When Angel *et al.* (2011) studied the supplementation of the same enzyme C to broilers, they reported an increase in the apparent digestibility of Arg, Ile, Lys, Thr, His, Asp, Cys, and Ser. Cowieson & Ravindran (2008) also detected improvements in the ileal digestibility of Lys, Cys, Thr, Ile, Asp, and Glu in turkeys fed diets supplemented with 15,000 PROT/kg. Romero *et al.* (2013) evaluated a combination of protease, xylanase, and amylase, noticing an increase in the digestibility of Cys (5.4%), Thr (4.4%), Gly (3.6%) and Val (3.3%); their results for the digestibility of Thr were similar to the ones found in this study.

The addition of enzymes A and B to the SBM MG did not increase SIAAD ($p>0.05$), except for Met and Glu. For the remaining AA, the supplementation of the enzyme A provided the same DC as enzyme C ($p<0.05$). For Arg, the supplementation of enzymes A and B improved DC as compared to non-supplementation ($p<0.05$); enzymes D and C did not lead to such improvement ($p<0.05$). In the case of Val, the supplementation with enzyme A provided a similar DC to enzyme D, which had the highest DC

Table 11 – Standardized ileal amino acid digestibility coefficients (SIAADC) of two soybean meals (SBM) from different Brazilian regions with and without supplementation of enzymes A, B, C, and D. Effect of the interaction between the soybean meals and the enzymes.

Amino acids	SBM	Enzyme				
		NC	Enzyme A	Enzyme B	Enzyme C	Enzyme D
TAA	MG	92.78 Bc	93.14 Bbc	92.28 Bc	94.54 Aa	93.78 Abc
	RS	94.05 Aa	94.03 Aa	94.53 Aa	94.90 Aa	94.26 Aa
EAA	MG	91.64 Bb	92.33 Ab	91.74 Bb	93.67 Aa	93.46 Aa
	RS	93.00 Ab	92.80 Ab	93.25 Ab	94.12 Aab	93.52 Aab
NEAA	MG	94.04 Ac	94.37 Bc	93.41 Bd	95.95 Aa	94.96 Ab
	RS	94.42 Ab	94.99 Aab	95.40 Aab	95.49 Aab	94.93 Aab
Lysine	MG	91.74 Ab	92.51 Ab	92.04 Ab	94.06 Aa	93.73 Aa
	RS	92.16 Aa	92.70 Aa	92.96 Aa	93.00 Ba	92.60 Ba
Methionine	MG	95.51 Ab	97.00 Aa	93.26 Bc	97.58 Aa	97.73 Aa
	RS	96.70 Aa	96.55 Aa	97.30 Aa	98.39 Aa	98.37 Aa
Met+Cys	MG	93.77 Bc	94.32 Bc	90.92 Bd	97.92 Aa	95.81 Bb
	RS	96.76 Ab	97.70 Aab	98.03 Aab	98.01 Aab	98.66 Aab
Threonine	MG	90.19 Bb	89.91 Bb	89.52 Bb	93.95 Ba	93.47 Ba
	RS	95.18 Ab	95.64 Ab	95.36 Ab	97.44 Aa	96.12 Ab
Arginine	MG	98.60 Bd	98.83 Bc	98.91 Bc	99.82 Aa	99.46 Ab
	RS	99.52 Aa	99.53 Aa	99.57 Aa	99.58 Ba	99.58 Aa
Gly+Ser	MG	90.38 Bb	90.54 Bb	90.44 Bb	93.01 Aa	90.63 Bb
	RS	92.81 Ab	92.24 Ab	92.48 Ab	93.74 Aa	92.50 Ab

NC = Negative control.

Different capital letters in the same column ("A" and "B") and small letters in the same line (a, b, c and d) indicate statistical difference (SNK, $p<0.05$).

Table 12 – Continuation.

Amino acids	SBM	Enzyme				
		NC	Enzyme A	Enzyme B	Enzyme C	Enzyme D
Valine	MG	90.54 Ab	91.94 Aa	90.58 Ab	89.57 Ab	92.77 Aa
	RS	87.70 Bb	87.46 Bb	88.89 Bab	90.04 Aab	89.32 Bab
Isoleucine	MG	88.60 Bc	90.18 Aabc	89.40 Abc	90.81 Babc	91.43 Aab
	RS	90.02 Ab	89.62 Ab	90.61 Ab	92.35 Aab	91.13 Aab
Leucine	MG	88.52 Bc	90.08 Abc	89.10 Bbc	91.46 Aa	91.29 Aa
	RS	90.45 Aa	89.99 Aa	90.71 Aa	91.12 Aa	90.76 Aa
Histidine	MG	90.82 Bc	92.10 Bb	91.19 Bc	95.66 Aa	95.24 Aa
	RS	93.20 Aa	93.02 Aa	93.57 Aa	94.16 Ba	94.11 Ba
Alanine	MG	89.14 Ab	89.32 Bb	88.76 Bb	92.22 Aa	91.46 Aa
	RS	89.70 Ab	91.26 Aab	91.35 Aab	90.45 Bab	91.65 Aab
Aspartic acid	MG	95.23 Aab	95.47 Aab	94.61 Bb	96.13 Aab	95.68 Aab
	RS	94.29 Bc	95.10 Ab	95.63 Ab	96.69 Aa	94.05 Bc
Glutamic acid	MG	96.07 Ab	96.84 Aa	95.54 Bc	97.21 Aa	96.19 Ab
	RS	95.61 Bc	96.27 Bb	96.86 Aa	96.72 Ba	96.26 Ab
Cystine	MG	92.27 Bc	92.01 Bc	88.92 Bd	98.21 Aa	94.16 Bb
	RS	96.80 Ab	98.73 Aab	98.68 Aab	97.67 Aab	98.91 Aab
Proline	MG	88.43 Bc	87.74 Bc	87.01 Bc	94.27 Aa	91.64 Bb
	RS	93.62 Aa	92.91 Aa	93.18 Aa	93.42 Aa	93.57 Aa

NC = Negative control.

Different capital letters in the same column A, B) and small letters in the same line (a.b and c) indicate statistical difference (SNK, $p<0.05$).

($p<0.05$). Soybean meal supplemented with enzyme C had the best DC values ($p<0.05$) for Tre, Gly+Ser, Asp, and Glu as compared to the diet without enzyme supplementation, as well as the treatments with enzymes A, B, and D; except for Glu, which performed better ($p<0.05$) with enzyme B.

The DC values of the SBM RS were higher or similar ($p<0.05$) to that of the SBM MG, except for Val and Lys. For these two AA, the SBM MG supplemented with enzymes C and D showed the best results ($p<0.05$). Bertechini *et al.* (2020) reported the highest AA digestibility when a monocomponent protease, the

same used in the present study, was added to SBM at 200ppm, providing a dose of 15,000 units/g.

The overall effects of enzyme C on AADC, EAA, and NEAA were 3.07%, 2.21% and 2.03%, respectively. Similar values were found by Cowieson *et al.*, (2018), who report an approximation of the effect of 3.7% reported by Cowieson & Ross (2013) based on a meta-analysis of 25 independent studies. The effect of enzyme C on Thr and Cys had higher increases of 4.16% and 6.43%, showing similar responses to Cowieson *et al.* (2018), which is related to previous results reported by Angel *et al.* (2011) and Cowieson & Ross (2013). These protease effects on absolute digestibility can be explained by the fact that AA are present in large amount in the intestinal mucosa. Cowieson & Ross (2013) noted that the effect of exogenous protease on the digestibility of AA in various diets fed to both poultry and swine had a relatively consistent pattern that favored AA that are found in high concentrations in endogenous proteins (especially mucin).

Enzyme C showed the best SIAAD results among the enzymes evaluated, followed by enzyme D ($p<0.05$). In a meta-analysis study by Lee *et al.* (2018), different effects were observed between the enzymes analyzed in both poultry and pigs, which could be explained by the difference between the enzymes derived from bacteria or fungi, and also by the different characteristics of protein sources. This could explain the fact that enzymes A and B do not provide an improvement in the SIAAD in the same way as enzyme C. There are different possible modes of action of the enzyme carbohydrase in poultry diets: improving access by endogenous enzymes to the cellular content due to hydrolysis of the arabinoxylans of the cell wall (Cowieson, 2005); increasing concentration of digestive enzymes in young animals, particularly amylase (Ritz *et al.*, 1995; Gracia *et al.*, 2003); modulating the intestinal microbiota (Fernandez *et al.*, 2000), and lowering losses of endogenous AA, particularly due to changes to pancreatic amylase (Jiang *et al.*, 2008) and secretion of mucin (Cowieson & Bedford 2009). These factors can explain why carbohydrases could improve the DC of some AA, leading to better results than those of enzymes A and B.

This evidence shows that the quality of SBMs can vary for different reasons. By adding enzymes to the SBMs, we were able to improve the DC of the AA, particularly when enzymes C and D were used, provided statistical equality in the DC for several AA among SBM; this means that when the SBM was not supplemented with enzymes, the DC was lower and so was the nutritional value of the diet. In contrast, in the diets without enzyme supplementation, the DC of NEAA, Lys, Met, and Ala remained unchanged ($p<0.05$), regardless of the SBM used. However, the coefficient of digestibility of TAA, EAA, NEAA, Met, Met+Cys, Gly+Ser, Val, Leu, Asp, Cys, and Pro improved when enzyme C was added, making them similar among SBM. This observation is in line with Ravindran (2013), who said that the supplementation of proteases reduces inconsistencies among batches of ingredients, improving the nutritional value of low-quality feed ingredients and reducing discrepancies between good- and bad-quality feed ingredients.

In summary, enzymes A, B, C, and D did not increase the level of AME and AMEn in the SBM. The values of AME and AMEn are higher in the MG than in the SBM RS. The addition of enzyme C to the SBM RS provided the highest level of N retention in broilers. Enzyme C showed the best SIAAD for the SBM MG, increasing total AA by 1.9%, EAA by 2.2%, Lys by 2.5%, Met by 2.2%, Met+Cys by 4.4%, and Thr by 4.2%. Enzymes A and B did not significantly improve the SIAAD. In general, the AA of the SBM RS is more digestible than those of the SBM MG.

ACKNOWLEDGEMENTS

We would like to thank DSM Nutritional Products, UFV, CAPES, CNPq, and FAPEMIG for the financial support for the development of this research.

DECLARATION OF CONFLICTING INTERESTS

The authors declared no potential conflicts of interests with respect to the research, authorship, and/or publication of this article.

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