https://doi.org/10.17221/134/2020-CJAS

Effect of protease supplementation on the digestibility of amino acids in animal-origin meals for broiler diets

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Citation: da Silva J.M.S., de Oliveira N.R., Gouveia A.B.V.S., Vieira R.A., dos Santos R.O.F., Minafra C.S., dos Santos F.R. (2021): Effect of protease supplementation on the digestibility of amino acids in animal-origin meals for broiler diets. Czech J. Anim. Sci., 66: 29–37.

Abstract: Enzymes benefit digestion and absorption of the ingredients and their addition to an animal-origin meal (AOM) can improve its nutritional quality. This research aimed to evaluate the effect of protease on nutrient digestibility, amino acids, and metabolism of AOM energy for broilers. Four hundred and eighty broiler chickens were distributed in a completely randomized design (4 × 2 factorial scheme), eight treatments, six replicates containing 10 birds/replicate. Treatments consisted of poultry viscera meal, swine viscera meal (SVM), bovine meat and bone meal and basal diet; with and without protease addition. Two tests were performed. In the first test, the total excreta collection method was used with birds at 13 to 20 days of age and 25% of the reference feed was replaced by AOM. In the second test, a protein-free diet was administered to birds at 21 to 24 days of age and AOM replaced 25% of the starch. The inclusion of protease increased the apparent metabolizable energy corrected for nitrogen balance of SVM by 15.99% and the apparent metabolizable crude energy by 5.7%, and it also raised the coefficient of true ileal digestibility of the amino acids in the AOMs by 5.67% on average. The inclusion of protease improved the apparent metabolizable crude energy of AOMs, apparent metabolizable dry matter of bovine meat and bone meal, coefficient of true ileal digestibility of essential amino acids and apparent metabolizable energy corrected for nitrogen balance of SVM. Dietary supplementation of protease may be a potential strategy to improve the digestibility of amino acids for broilers, a possibility of using animal-origin meals as a protein source of diets.

Keywords: additive; by-product; digestion; exogenous enzymes; nutrition; poultry

One of the main challenges to poultry production is the high cost of feedstuffs, which can represent up to 70% of total expenses. A strategy to overcome this problem is to use alternative feeds that provide good animal performance at a reasonable cost without harming the environment. One of these alternatives is to use various types of meal made from animal by-products, or animal-origin meal (AOM), which is an important source of calcium, phosphorus, amino acids and energy, to partially replace conventional feeds (Zhang and Adeola 2017). According Mahmoudnia et al. (2011) it is possible to use up to 6% of poultry by-product meal in feeding broiler chickens, without negatively influencing protein digestibility, apparent metabolizable energy (AME) and apparent metabolizable energy corrected for nitrogen balance (AMEn) values.

https://doi.org/10.17221/134/2020-CJAS

It is a consensus among animal nutritionists that knowledge of the energy content and coefficients of amino acid digestibility is necessary to formulate diets that meet the nutritional needs of poultry and reduce the feed costs (Troni et al. 2016).

The beneficial effects of using commercial exogenous enzymes for birds are already known, for example, pentosanase, protease, cellulase, beta-glucanase, phytase, pectinase and amylase are able to degrade pentosans, proteins, cellulose, starch and phytate, improving later the digestibility of nutrients and their absorption in the intestines of birds. Higher values of apparent digestibility of dry matter (DM), organic matter (OM), crude protein (CP) and ether extract (EE) were recorded for broilers fed diets supplemented with enzymes. Exogenous enzymes are provided in diets to dispense with endogenous production, improve digestibility and reduce production costs (Yadav and Jha 2019).

In their study Mahmood et al. (2017) concluded that the level of crude protein could be reduced to 19% with the inclusion of 3% of the meal made from a bird by-product supplemented with exogenous protease. Exogenous enzymes are provided in diets to forgo the need for endogenous production, improve digestibility and reduce production costs (Yadav and Jha 2019). In a study performed by Mahmood et al. (2017) the authors concluded that the crude protein level could be reduced to 19% with the inclusion of 3% of poultry by-product meal supplemented with exogenous protease.

Therefore, quantification of the nutrient extraction from AOM with the addition of proteolytic enzymes can provide relevant information to the poultry industry, enabling the inclusion of larger amounts of AOM in the diet and making the productive chain more economically and environmentally sustainable.

The objective of this study was to determine the energy values and digestibility of amino acids from three types of AOM (poultry viscera meal, swine viscera meal and bovine meat and bone meal), with and without protease addition of broiler diets.

MATERIAL AND METHODS

The experimental protocol was approved on June 2, 2018 by the Ethics Committee on the Use of Animals (CEUA) of Federal Institute of Goiás, under No. 7089010616.

The experiment was conducted at the Poultry Facilities of the Federal Institute of Goiás, Rio Verde, Goiás, Brazil. Four hundred eighty chicks of the Cobb 500 line were distributed in a 4 × 2 factorial arrangement by a completely randomized design, providing eight treatments with six replicates and 10 birds per replicate. Treatments consisted of (poultry viscera meal, swine viscera meal and bovine meat and bone meal and basal diet) × (with and without proteolytic enzyme addition). The enzyme applied was RONOZYME® ProAct (Royal DSM, Heerlen, The Netherlands); this enzyme is a protease obtained from the fermentation of *Bacillus lincheniformis*, containing genes transcribed from *Nocardiopsis prasina*. The enzymatic activity for this enzyme is defined by the amount of enzyme needed to degrade 1 µmol of *p*-nitroaniline from 1 µM of the substrate (Suc-Ala-Ala-Pro-Phe-*N*-succinyl Ala-Ala-Pro-Phe-*p*-nitroanilide) per minute, in a pH of 9.0 and 37 °C. The product used has 75 000 units of protease/g of enzyme.

In test 1, the total excreta collection method with birds at 13 to 20 days of age was used and 25% of the reference feed was replaced by animal-origin meal (Table 1).

The chicks were initially kept on the floor on sawdust bedding, and 13 days after hatching they were transferred to metabolic cages. The birds received water *ad libitum* during the entire experimental period. The coefficients of apparent metabolizable dry matter (AMDM), apparent metabolizable crude energy (AMCE), apparent metabolizable energy and apparent metabolizable energy corrected for nitrogen balance equal to zero were determined by the total excreta collection method (Sakomura and Rostagno 2007).

The experimental period lasted eight days, four for adaptation to the installations and experimental diets and four for excreta collection. The samples were weighed to measure the total excreta, homogenized, and divided into aliquots, which were dried in a ventilated oven at 55 °C for 72 h (ICNT-CA G-001/1 method) and milled. Then the samples were subjected to a laboratory analysis to determine dry matter content (ICNT-CA G-003/1 method) and nitrogen content (ICNT-CA N-001/1 method) according to the techniques described by Detmann et al. (2012). In turn, the crude energy was deter-

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Table 1. Centesimal composition and nutritional levels of the reference feed

Ingredient	Quantity (g/kg)
Corn	739.11
Soy meal	223.68
Dicalcium phosphate	16.09
Lime	9.26
Salt	4.81
DL-methionine	1.46
L-lysine	1.68
L-threonine	0.01
Mineral premix[1]	1.10
Vitamin premix[2]	1.10
60% choline chloride	1.00
Coccidiostat	0.55
Butylated hydroxytoluene	0.10
Antibiotic	0.05
Sum	1 000.00
Values calculated	
Metabolizable energy (kcal/kg)	3 000.00
Crude protein (%)	24.45
Calcium (%)	0.819
Available phosphorus (%)	0.391
Sodium (%)	0.210
Arginine (%)	1.365
Dig. glutamic acid + serine (%)	1.940
Dig. isoleucine (%)	0.835
Dig. lysine (%)	1.266
Dig. methionine + cysteine (%)	0.962
Dig. threonine (%)	0.822
Dig. tryptophan (%)	0.229
Dig. valine (%)	1.006

[1]Supplied per kg of complete diet: 7 000 IU of vitamin A, 2 000 IU of vitamin D_3, 25 IU of vitamin E, 2.0 mg of menadione, 4.0 mg of riboflavin, 25.0 mg of niacin, 12.0 mg of D-pantothenic acid, 4.0 mg of vitamin pyridoxine, 0.01 mg of vitamin B_{12}, 1.0 mg of folic acid and 0.08 mg of biotin; [2]supplied per kg of complete diet: 10 mg of copper as copper sulphate, 1 mg of iodine as calcium iodate, 60 mg of iron as ferrous sulphate, 70 mg of manganese as manganese sulphate, 0.3 mg of selenium as sodium selenite, and 70 mg of zinc as zinc sulphate

mined with an IKA model C200 calorimeter (IKA®, Staufen, Germany). The laboratory results for the three types of AOM, the reference feed and excreta samples were used to calculate the values of AME and AMEn by means of the equations proposed by Matterson et al. (1965).

Table 2. Centesimal composition of the protein-free diet

Ingredient/diet	PFD (g/kg)	PFD + AOM (g/kg)
Starch	826.80	616.30
Sugar	50.00	37.50
AOM	–	250.00
Soy oil	50.00	37.50
Dicalcium phosphate	16.20	12.20
Lime	8.00	6.00
Salt	4.40	3.40
Corncob	30.00	22.50
Mineral premix[1]	1.30	1.30
Vitamin premix[2]	1.30	1.30
Choline chloride	2.00	2.00
Celite™	10.00	10.00
Total	1 000.00	1 000.00

AOM = animal-origin meal; PFD = protein-free diet
[1]Supplied per kg of complete diet: 7 000 IU of vitamin A, 2 000 IU of vitamin D_3, 25 IU of vitamin E, 2.0 mg of menadione, 4.0 mg of riboflavin, 25.0 mg of niacin, 12.0 mg of D-pantothenic acid, 4.0 mg of vitamin pyridoxine, 0.01 mg of vitamin B_{12}, 1.0 mg of folic acid and 0.08 mg of biotin; [2]supplied per kg of complete diet: 10 mg of copper as copper sulphate, 1 mg of iodine as calcium iodate, 60 mg of iron as ferrous sulphate, 70 mg of manganese as manganese sulphate, 0.3 mg of selenium as sodium selenite, and 70 mg of zinc as zinc sulphate

In test 2, birds with ages from 21 to 25 days were provided with a protein-free diet (PFD) and AOM replaced 25% of the starch (Table 2). The PFD contained 1% Celite™ (Supleco Inc., Bellefonte, PA, USA) to increase the levels of acid-insoluble ash (AIA) used as a marker of indigestible matter (Sakomura and Rostagno 2007). After the experimental period, all the birds were killed by cervical dislocation, and the abdominal cavity was opened for the collection of the entire content of the ileal segment, starting 5 cm from the ileocaecocolic junction and traversing 30 cm toward the jejunum. The samples were frozen and then lyophilized, milled, and prepared for laboratory analyses. The crude protein content was ascertained by the ICNT-CA N-001/1 method according to Detmann et al. (2012). The AIA present in the diets and digesta was measured according to the method described by Pereira et al. (2017). The amino acids present in the feed, AOM additives and ileal digesta

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were determined by high-performance liquid chromatography performed by the Brazilian subsidiary of Adisseo company (São Paulo, Brazil).

The true digestibility of amino acids was calculated by the equations proposed by Sakomura and Rostagno (2007).

The data were evaluated by an analysis of variance (ANOVA). When effects were deemed significant (α = 0.05), Tukey's test was used to compare the means. All statistical analyses were performed using Sisvar software v5.6 (Ferreira 2008). The statistical model used was:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

where:

Y_{ijk} – observed value for the variable under study referring to k-th repetition of the combination of the i-th level of factor "α" with the j-th level of factor "β";

μ – the constant common to all observations;

α_i – effect of the i-th level of inclusion of animal-origin meals on the value observed in Y_{ijk};

β_j – effect of the j-th level of inclusion or not of exogenous protease on the value observed in Y_{ijk};

$(\alpha\beta)_{ij}$ – effect of the interaction of the i-th level of factor "α" with the j-th level of factor "β";

ε_{ijk} – experimental sampling error that receives level "i" of factor "α" and level "j" of factor "β" in repetition "k".

RESULTS

The poultry viscera meal (PVM) had the highest concentrations of protein, fat and total amino acids. As expected, the bovine meat and bone meal (BMBM) showed the highest ash composition (Table 3).

There was a significant interaction regarding AME and AMEn (kcal/kg) between the AOMs and the addition of enzyme ($P < 0.05$) (Table 4).

The diets with and without enzyme had a higher energy value for SVM ($P < 0.05$).

Table 3. Proximal chemical and amino acid composition of the three meal types: bovine meat and bone (BMBM), poultry viscera (PVM) and swine viscera (SVM)

Composition (%)	BMBM	SD	CV	PVM	SD	CV	SVM	SD	CV
Moisture	6.43	0.10	1.55	7.53	0.27	3.64	3.40	0.22	6.42
Mineral matter	26.59	0.02	0.06	18.46	0.22	1.22	18.61	0.29	1.56
Ether extract	11.54	0.14	1.23	17.48	0.24	1.35	16.51	0.25	1.50
Crude protein	54.48	0.22	0.40	55.42	0.21	0.38	49.45	0.22	0.44
Amino acids (%)									
Methionine	0.77	0.08	10.44	0.88	0.01	1.43	0.77	0.01	0.94
Methionine + cystine	1.32	0.21	15.64	1.52	0.14	8.99	1.11	0.01	0.92
Lysine	2.92	0.07	2.30	2.61	0.01	0.32	2.38	0.02	0.70
Threonine	1.80	0.09	4.84	1.75	0.27	15.62	1.54	0.20	12.86
Arginine	3.80	0.36	9.34	3.33	0.19	5.50	3.00	0.36	12.00
Isoleucine	1.60	0.36	22.37	1.77	0.17	9.77	1.41	0.08	5.47
Leucine	3.16	0.36	11.46	3.15	0.02	0.54	2.80	0.01	0.44
Valine	2.46	0.01	0.49	2.13	0.37	17.14	1.99	0.38	19.00
Histidine	0.99	0.01	0.88	1.00	0.01	0.82	1.01	0.01	0.88
Phenylalanine	1.70	0.01	0.48	1.78	0.25	13.79	1.62	0.02	1.11
Cystine	0.40	0.01	1.39	0.60	0.10	17.27	0.46	0.01	1.21
Serine	2.11	0.32	15.12	2.95	0.34	11.47	2.42	0.29	12.04
Alanine	4.05	0.34	8.33	3.74	0.23	6.14	3.54	0.36	10.26
Aspartic acid	2.14	0.03	1.32	2.63	0.35	13.34	2.39	0.36	15.10
Glutamic acid	3.60	0.32	8.89	3.78	0.33	8.74	3.55	0.33	9.34

CV = coefficient of variation (%); SD = standard deviation

The levels of amino acids were determined by high-performance liquid chromatography by the Adisseo company

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Table 4. Apparent metabolizable energy (AME) and apparent metabolizable energy corrected for nitrogen balance (AMEn) of animal-origin meals for broiler chickens in the initial phase, based on dry matter

Feed	AME[1] (kcal/kg)			AMEn[1] (kcal/kg)		
	no enzyme	enzyme	mean	no enzyme	enzyme	mean
SVM	3 176.19^{Ab}	3 684.14^{Aa}	3 430.16	3 058.32^{Ab}	3 547.41^{Aa}	3 302.87
PVM	2 742.63^{Ba}	2 778.83^{Ba}	2 760.73	2 422.84^{Ba}	2 454.81^{Ba}	2 438.83
BMBM	2 633.47^{Ba}	2 824.53^{Ba}	2 729.00	2 520.88^{Ba}	2 703.78^{Ba}	2 612.33
Mean	2 850.76	3 095.83		2 667.35	2 902.00	
Probability						
SEM		65.28			62.01	
Feed (A)		0.000 0			0.000 0	
Enzyme (E)		0.002 8			0.002 7	
A × E		0.047 0			0.042 1	
CV		7.610 0			7.710 0	

BMBM = bovine meat and bone; CV = coefficient of variation (%); PVM = poultry viscera; SEM = standard error of the mean; SVM = swine viscera

[1]Mean of six repetitions of 10 birds each; ^{A,B,a,b}means followed by the same uppercase letters in the row and lowercase letters in the column differ significantly by Tukey's test ($P < 0.05$)

Table 5. Coefficients of apparent metabolizable crude energy (AMCE) and apparent metabolizable dry matter (AMDM) of animal-origin meals for broiler chickens, based in cry matter

Feed	AMCE[1] (%)			AMDM[1] (%)		
	no enzyme	enzyme	mean	no enzyme	enzyme	mean
SVM	74.03	85.87	79.95^{A}	64.10^{Aa}	54.81^{Aa}	59.46
PVM	52.33	53.02	52.67^{B}	54.06^{Aa}	58.06^{Aa}	56.06
BMBM	76.63	82.19	79.41^{A}	24.38^{Ba}	55.27^{Ab}	39.82
Mean	67.66^{b}	73.69^{a}		47.52	56.04	
Probability						
SEM		1.54			3.17	
Feed (A)		0.000 0			0.000 3	
Enzyme (E)		0.002 0			0.026 7	
A × E		0.515			0.000 4	
CV		7.560			21.220 0	

BMBM = bovine meat and bone; CV = coefficient of variation (%); PVM = poultry viscera; SEM = standard error of the mean; SVM = swine viscera

[1]Mean of six repetitions of 10 birds each; ^{A,B,a,b}means followed by the same uppercase letters in the row and lowercase letters in the column differ significantly by Tukey's test ($P<0.05$)

The inclusion of protease did not affect the energy levels of BMBM ($P > 0.05$) and PVM ($P > 0.05$). However, the addition of protease increased the energy values of the SVM by 15.99% ($P < 0.05$).

There was no influence between the evaluated ingredients and the addition of enzymes for the apparent metabolizable crude energy (AMCE) of the three animal-origin meals studied ($P > 0.05$) (Table 5).

The highest values of AMCE were for SVM ($P < 0.05$) and BMBM ($P < 0.05$). The inclusion of protease in the AOMs increased the AMCE by 5.70% ($P < 0.05$). There was a significant interaction between ingredients and enzyme for AMDM ($P < 0.05$).

For the AOMs without enzyme inclusion, BMBM had the lowest AMDM value ($P < 0.05$). The inclusion of protease had a positive effect on the AMDM value only in BMBM ($P < 0.05$).

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Table 6. Coefficient of true ileal digestibility of animal-origin meals with and without addition of protease for broiler chickens

Amino acids	Animal-origin meals			Without enzyme[1]	With enzyme[1]	Probabilities				
	SVM[1]	PVM[1]	BMBM[1]			feed	enzyme	A × E	CV	SEM
Methionine	90.23	92.98	90.22	88.56[b]	94.77[a]	0.668	0.027	0.066	5.71	2.138
Methionine + cystine	92.53	93.27	91.94	89.95[b]	95.21[a]	0.871	0.025	0.197	4.71	1.781
Lysine	94.79	94.83	95.01	93.03[b]	96.73[a]	0.988	0.012	0.072	2.80	0.886
Threonine	84.89	90.35	87.35	83.90[b]	91.15[a]	0.381	0.037	0.319	7.48	2.673
Arginine	94.73	94.41	91.74	90.98[b]	96.27[a]	0.255	0.005	0.370	3.47	1.325
Isoleucine	89.25	91.48	90.08	86.13[b]	94.41[a]	0.781	0.008	0.110	6.09	2.243
Leucine	92.18	93.02	91.57	89.00[b]	95.52[a]	0.827	0.005	0.167	4.31	1.622
Valine	91.43	92.17	90.90	88.11[b]	94.89[a]	0.889	0.008	0.175	4.97	1.855
Histidine	87.10	89.96	88.76	86.00[a]	91.21[a]	0.706	0.084	0.071	6.61	2.392
Phenylalanine	86.96	87.78	87.10	86.20[a]	88.36[a]	0.978	0.536	0.789	8.24	2.934
Cystine	95.40	92.05	91.47	91.41[a]	94.54[a]	0.274	0.150	0.065	4.65	1.765
Serine	84.07	90.80	86.63	84.13[a]	90.21[a]	0.231	0.069	0.594	7.41	2.638
Alanine	94.51	93.31	90.56	90.61[a]	94.97[a]	0.211	0.029	0.158	4.01	1.519
Aspartic acid	86.03[B]	94.16[A]	94.46[A]	89.27[a]	93.82[a]	0.220	0.081	0.008	5.53	2.068
Glutamic acid	92.89	95.54	95.64	92.77[a]	96.61[a]	0.265	0.023	0.022	3.31	1.280
Crude protein	91.81	91.55	90.36	90.06[a]	92.45[a]	0.819	0.241	0.809	4.51	1.577

BMBM = bovine meat and bone; CV = coefficient of variation (%); PVM = poultry viscera; SEM = standard error of the mean; SVM = swine viscera

[1]Mean of six repetitions of 10 birds each; [A,B,a,b]means followed by the same uppercase letters in the row and lowercase letters in the column differ significantly by Tukey's test ($P < 0.05$)

There were higher coefficient of true ileal digestibility (CTID) values for aspartic acid of PVM and BMBM ($P < 0.05$) (Table 6).

With the exception of glutamic acid, serine, phenylalanine, histidine, aspartic acid and cystine, there was no significant correlation between the AOMs × enzyme for the CTID of the amino acids ($P > 0.05$). The same effect was observed for crude protein ($P > 0.05$). The addition of protease caused an average increase of 5.67% in the CTID of the essential amino acids, except for histidine and phenylalanine.

DISCUSSION

Rostagno et al. (2017) reported lower values for moisture and CP (6.10% and 51.70%) for BMBM, but higher values for mineral material (MM) and EE (12.20% and 29.90%).

Troni et al. (2016), evaluating the chemical and energy composition of feeds for broiler poultry, among them BMBM, found the following results: 5.50% DM, 39.55% MM, 9.60% EE, and 48.06% CP. Also, Rostagno et al. (2017), with respect to SVM, gave values similar to ours, with the largest discrepancies being for the levels of DM (3.31 × 6.00%) and MM (16.19 × 27.90%). The variation of the nutritional quality of BMBM is directly related to the type of used raw material, such as the inclusion of varied portions of entire carcasses, parts of carcasses, hooves and heads of chickens (Matias et al. 2015).

For lysine and methionine levels, Rostagno et al. (2017) reported the respective values of 2.73% and 0.70% for BMBM; 3.09% and 1.06% for PVM, and 2.60% and 0.74% for SVM. Vieira et al. (2014), analysing BMBM, found values of 1.70% and 0.45% for lysine and methionine. Carvalho et al. (2012) investigated PVM and found values of 2.13% for lysine and 0.58% for methionine. The variation in the results observed for these amino acids can be attributed to different nutritional composition as well as to the methods employed in producing the AOMs, mainly processing time and temperature (Tschirner and Simon 2015).

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In this study, the results obtained for AMEn of the AOMs without enzyme were equal to 2 520.88 kcal/kg for BMBM, 2 422.84 kcal/kg for PVM and 3 058.32 kcal/kg for SVM.

However, Rostagno et al. (2017) gave AMEn values of 2 373.0 kcal/kg for BMBM, 3 682.0 kcal/kg for PVM and 2 240.0 kcal/kg for SVM. In turn, Cowieson and Roos (2016) reported AMEn values equal to 2 778.0 kcal/kg and 4 293.0 kcal/kg for BMBM and PVM, respectively, while Troni et al. (2016), also studying broilers, obtained AMEn values of 1 574.0 kcal/kg for BMBM and 3 705.0 kcal/kg for PVM. The differences in the composition of raw materials of the AOMs, the processing method and age when determining the energy values are factors that explain the variations in the results for AMEn, as reported by Amorin et al. (2015). The higher energy values for SVM found in this study can be attributed to the lower mineral matter value of this ingredient. This occurs because when calcium and sodium ions are present in lower concentrations, saponification of the fats present in the particular AOM is less pronounced, increasing their use by the birds, with greater release of AME (Troni et al. 2016).

The energy present in food is a product resulting from the transformation of nutrients during metabolism, being one of the most important factors to be considered in animal nutrition. The energy available in poultry feed is usually expressed in the form of apparent metabolizable energy (AME) or true metabolizable energy. The latter differs from AME because the former involves correcting of DM values due to endogenous and metabolic losses. These losses are estimated by the faecal energy metabolic rate and endogenous urinary energy determined with fasting roosters.

The presented values of AME and AMEn interacted between the AOMs with and without enzyme supplementation. There was a significant difference due to the use of protease only in the values of AME and AMEn of SVM. Likewise, the addition of protease also improved the AMCE value of all three AOMs and the AMDM value of BMBM. This fact indicates that the addition of protease allowed greater availability of nutrients to the birds.

Studies evaluating the effect of the inclusion of enzymes, especially protease, on the energy values of the ingredients of animal feeds are scarce. On the other hand, investigations attesting to a positive effect of proteases on the energy values of diets are more common (Olukosi et al. 2015; Mahmood et al. 2017). Gallardo et al. (2017) evaluated the addition of a complex composed of carbohydrase and protease to diets with maize distillers' dried grain with solubles and observed an increase of AMEn, suggesting that the presence of protease improves the digestion of nutrients in the birds' gut. On the other hand, Scotta et al. (2016) found that the addition of a meal containing α-amylase from maize residues had a significant effect on the values of AME and AMEn, and there was a positive effect on the coefficient of metabolizable energy.

The improvement in AMEs of the enzyme-included diet may have been due to better digestion of fats (Perera et al. 2019). In addition, the increase in energy values may suggest greater availability of energy, through greater availability of amino acids, as found with the inclusion of protease in this study.

The coefficients of true ileal digestibility of amino acids of the PVM, SVM and BMBM were higher than those found in the literature consulted (Scotta et al. 2016; Rostagno et al. 2017). These results demonstrate that the AOMs used in this experiment were adequately processed, with nearly complete hydrolysis, resulting in higher digestibility values of the amino acids (Tuncil et al. 2018). Another factor was the use of the proteolytic enzyme, which improved the CTID of the majority of the amino acids. This occurred due to enzymatic hydrolysis, which breaks down proteins into smaller peptides and free amino acids, in turn increasing their solubility and consequently digestibility (Paiva et al. 2015; Cowieson and Roos 2016; Cheong et al. 2018).

Similar results were observed by Carvalho et al. (2020), who concluded that the use of animal by-product meal as a source of protein in the starter feed of broiler chickens improved digestibility and performance of broiler chicks. The use of protease during the starter rearing period is recommended mainly for vegetable-based diets.

CONCLUSION

In closing, we found that the three animal-origin meals (SVM, PVM and BMBM) presented AMEn values of 3 058.32 kcal/kg, 2 422.84 kcal/kg and 2 520.88 kcal/kg, respectively. The addition of the proteolytic enzyme was efficient in improving the AMEn value of SVM, the AMCE of all the AOMs, the AMDM value of BMBM and

https://doi.org/10.17221/134/2020-CJAS

the CTID of the majority of the amino acids studied. Dietary supplementation of protease may be a potential strategy to improve the digestibility of amino acids for broilers, a possibility of using animal-origin meals as a protein source of diets.

Acknowledgment

The authors express thanks to the Federal Institute of Goiano, Rio Verde Campus, and the companies Adisseo, Patense and BRF for financial support.

Conflict of interest

The authors declare no conflict of interest.

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Received: May 27, 2020
Accepted: November 12, 2020