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Metabolizable energy and digestible amino acids of full-fat soybean without or with protease supplementation in diets for broilers

Energia metabolizável e aminoácidos digestíveis da soja integral sem ou com a suplementação de protease em rações para frangos de corte

Felipe Santos Dalólio[1*], Luiz Fernando Teixeira Albino[2], Horácio Santiago Rostagno[2], Diego Ladeira da Silva[2], Maurílio de Lucas Xavier Júnior[2], Vinícius Duarte de Oliveira[2]

[1]Universidade Federal de Viçosa/UFV, Departamento de Engenharia Agrícola, Viçosa, MG, Brazil
[2]Universidade Federal de Viçosa/UFV, Departamento de Zootecnia, Viçosa, MG, Brazil
[*]Corresponding author: felipesantos181@hotmail.com
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ABSTRACT

Two experiments were carried out to determine the energy values and the true ileal digestibility of amino acids of nine toasted full-fat soybeans (FFSB), without or with the addition of monocomponent protease. In the first experiment, to determine the energy values, the method of excreta collection was used with 1120 broiler chicks at 14 days old, distributed in randomized blocks in a 10x2 factorial arrangement with nine full-fat soybeans (30% included) plus a control diet (70%) without or with the addition of protease, totaling 20 treatments with 8 replicates and 7 birds per cage. In the second experiment, 1120 broiler chickens from 24 to 28 days of age were distributed in randomized blocks in a 10x2 factor arrangement, with nine full-fat soybeans (40% included) + a protein free diet (60%), without or with the addition of protease, totaling 20 treatments with 8 replicates and 7 birds per cage. Ileal digesta collection was used to determine the true digestibility coefficients and the digestible amino acid content of full-fat soybean. The average values of nitrogen-corrected apparent metabolizable energy (AMEn) and their respective metabolization coefficients (CAMEn) were 3207 kcal/kg and 62.57%, respectively. The average values of digestibility coefficients of crude protein and essential and nonessential amino acids were 86.79, 87.90 and 84.34%, respectively. The inclusion of protease improved (P<0.05) all evaluated parameters. Therefore, its use is recommended in diets containing full-fat soybeans for broiler chickens.

Index terms: Digestibility coefficients; metabolizability; monocomponent enzyme.

RESUMO

Foram realizados dois experimentos para determinar os valores energéticos e os coeficientes de digestibilidade ileal verdadeiros de aminoácidos de nove sojas integrais tostadas, sem ou com a adição de protease monocomponente. No primeiro experimento, para a determinação dos valores energéticos, foi utilizado o método de coleta total de excretas, com 1120 pintos de corte com 14 dias de idade, distribuídos em blocos casualizados, em arranjo fatorial 10x2, com nove sojas integrais (30% de inclusão) + uma ração referência (70%), sem ou com adição de protease, totalizando 20 tratamentos, com 8 repetições e 7 aves por gaiola. No segundo experimento foram utilizados 1120 frangos dos 24 aos 28 dias de idade, distribuídos em blocos casualizados, em arranjo fatorial 10x2, com nove sojas integrais (40% de inclusão) + uma ração isenta de proteína (60%), sem ou com adição de protease, totalizando 20 tratamentos, com 8 repetições e 7 aves por gaiola. A coleta da digesta ileal foi utilizada para determinar os coeficientes de digestibilidade verdadeiros e o teor de aminoácidos digestíveis da soja integral. Os valores médios de energia metabolizável aparente corrigida para balanço de nitrogênio (EMAn) e o seu respectivo coeficiente de metabolizabilidade (CEMAn) foram de 3207 kcal/kg e 62,57%, respectivamente. Os valores médios dos coeficientes de digestibilidade da proteína bruta e dos aminoácidos, essenciais e não essenciais foram de 86,79, 87,90 e 84,34%, respectivamente. A inclusão de protease melhorou (P<0,05) todos os parâmetros avaliados. Portanto, recomenda-se sua utilização em rações formuladas com soja integral para frangos de corte.

Termos para indexação: Coeficientes de digestibilidade; metabolizabilidade; enzima monocomponente.

INTRODUCTION

For a better utilization of the diets by the animals, besides the chemical and energy composition, the knowledge of the concentration of digestible amino acids (AA) is important, since the amino acids that make up the protein of the feed are not completely available for the animal (Gomes et al., 2010). Therefore, it is necessary to determine the digestibility coefficients of the AA in certain feeds, aiming at obtaining the values of digestible AA. This enables the formulation of more effective and accurate diets and reduces negative impact of environmental pollution. It also leads a cost reduction since protein is one of the most expensive ingredients.

Soybean meal is the main protein source added to the diets. This is more evident in broilers farming, which requires more dense diets with adequate balance of digestible AA. However, the intense use of soybean meal causes the poultry sector to depend on it, raising the production costs (Ncobela; Chimonyo, 2015). Therefore, the search for new protein feeds that can replace partially or completely soybean meal in diet formulation is needed.

Full-fat soybean (FFSB) presents about 36% of crude protein (CP) and 18% of ether extract (EE) (Rostagno et al., 2011), wich is an excellent alternative to soybean meal and to the inclusion of vegetable oil (Costa et al., 2015). Moreover, the digestibility of AA, EE and metabolizable energy of the FFSB is similar to the conventional soybean meal and vegetable oil mixture (Café et al., 2000), commonly used to enhance energy levels and dietary AA contents.

However, the presence of some antinutritional factors in FFSB, such as protease inhibitors (particularly trypsin and chymotrypsin), lectins, allergenic proteins, saponins, protein-protein interaction as well as the Mailard reaction at the thermal processing of fresh grain, change the use of nutrients by broilers and limit their use in diet formulation (Valencia et al., 2009; Rocha et al., 2014).

According to Carvalho et al. (2008) the high temperatures inactivates the antinutritional factors of soybean, allowing its use in the whole form. However, toasting may not be sufficient to inactivate all the antinutritional factors and may damage pancreatic enzymes, such as trypsin, in broiler chickens (Clarke; Wiseman et al., 2005).

The use of exogenous enzymes, especially proteases, has been successfully evaluated (Yu et al., 2007; Angel et al., 2011) to improve the availability of nutrients and the supplementation endogenous enzymes. It reduces the problem of incomplete standardization in thermal process. Also, the addition of protease in diets for broilers promotes increase in the digestibility of CP and AA (Freitas et al., 2011). Moreover, the supplementation with proteases provides larger degradation of conglycinin and β-conglycinin present in raw soybeans (Graham et al., 2002); both considered allergenic proteins (Zilic et al., 2011).

Thus, the objective of this study was to evaluate the values of nitrogen-correted apparent metabolizable energy, digestible amino acids, and their respective coefficients of digestibility of full-fat soybean samples without or with the addition of protease in diets for broilers.

MATERIAL AND METHODS

All procedures using animals for research were approved by the ethics committee for experimentation and use of animals in research at UFV, under protocol 043/2015.

Two biological trials were carried out in the Poultry Sector in the Animal Science Department of the Universidade Federal de Viçosa. In the first trial, 1120 male Cobb 500 broiler chicks at 14 days of age with average weight of 440.0 ± 5.0g were used. From birth to 13 days of age, the broilers were raised in brick shed with floor covered with wood shavings, where they received proper diet for the initial period, following the recommendations of Rostagno et al. (2011).

At 14 days of age, the broilers were transferred to metal batteries (cages) provided with platform type feeder, nipple drinker and a plastic covered tray for excreta collection. The experimental design used was a complete randomized block**s** in a 10 x 2 factor arrangement (a control basal diet and nine samples of toasted FFSB, without and with the addition of protease), totaling 20 treatments and eight replicates with seven birds per experimental unit. The product used has 75.000 protease units/ g of enzyme, and diets were provided as "on top" without their respective nutritional value at the proportion of 200 ppm (15.000) according to the recommendations of the manufacturer. This protease is manufactured from the fermentation of *Bacillus lincheniformis* containing gene transcripted Nocardiopsis prasina, being considered as monocomponent protease. The enzymatic activity for this enzyme is defined as the amount of enzyme needed to degrade 1 μmol of the substrate (Suc-Ala-Ala-Pro-Phe-N-succinyl Ala-Ala-Pro-Phe-p-nitroanilide) per minute in pH of 9.0 at 37 ºC.

The basal diet was formulated according to the recommendations of Rostagno et al. (2011) (Table 1). The experimental diets were composed of 70% of the reference basal diet and 30% of inclusion of each sample of toasted FFSB to be evaluated for each treatment, without or with the addition of protease. Broilers were fed diets *ad libitum*. Samples of toasted FFSB were from nine feed mills (Samples 1 to 9), belonging to the same grain producer company. The thermal process used been for toasting rotating drum. In this process the whole soybean enters a tube which is subjected to a dry air stream. Reaching a temperature of 120-125 ºC in tosting machine output.

The experiment lasted 10 days with 5 days for adaptation and 5 days for total excreta collection. The total excreta collection were carried out daily and then, the samples were placed in a freezer at a temperature of -20 °C to prevent bacterial fermentation. Samples were weighed, homogenized and representative sub-samples were taken for pre-drying at 55 °C in a forced ventilation oven for 72 hours for subsequent determination of values of dry matter (DM), CP and nitrogen (N), according to the methodology proposed by Silva and Queiroz (2002).

For determining the content of DM, N, CP, crude fiber (CF) and EE of samples of FFSB, the methodology proposed by Silva and Queiroz (2002) was used. Analyzes of urease index, protein solubility, and trypsin inhibitor were carried out in soybean samples according to Associação Nacional dos Fabricantes de Rações - ANFAR (2005). Based on the results achieved for N and gross energy (GE) of the diets and excreta, determined in the Parr-1261 bomb calorimeter, values

Table 1: Composition of experimental diets used for the determination of metabolizable energy values and content of digestible amino acids of full-fat soybean samples.

Diet reference basal[1]	(%)	PFD[2]	(%)
Ingredients		Ingredients	
Corn	72.466	Starch	80.310
Soybean meal	22.368	Sugar	5.000
Soy oil	1.000	Soy oil	5.000
Dicalcium phosphate	1.609	Dicalcium phosphate	2.100
Limestone	0.926	Limestone	0.700
Salt	0.481	Salt	0.450
DL-methionine (99%)	0.146	Potassium carbonate	1.000
L-lysine HCl (78%)	0.168	Corn cob	4.000
L-threonine (98%)	0.001	Vitamin Supplement[3]	0.080
Vitamin Supplement[3]	0.110	Mineral Supplement[4]	0.150
Mineral Supplement[4]	0.010	Choline chloride (60%)	0.200
Choline chloride (60%)	0.100	Antioxidant[6]	0.010
Anticoccidial[5]	0.055	Acid insoluble ash (CeliteTM)	1.000
Antioxidant[6]	0.010	Total	100.000
Total	100.000	Composition analyzed basal diet reference	
Calculated composition		Gross energy	3902
Metabolizble Energy (Kcal/kg)	3050	Metabolizable Energy (Kcal/kg)	3035
Crude protein (%)	16.132	Crude protein (%)	16.35
Digestible lysine (%)	0.847	-	-
Digestible methionine (%)	0.395	-	-
Digestible Met + Cist (%)	0.610	-	-
Digestible threonine (%)	0.550	-	-
Digestible tryptophan (%)	0.166	-	-
Calcium (%)	0.819	-	-
Non-phytate phosphorus (%)	0.391	-	-
Sodium (%)	0.210	-	-

[1]Diet reference used to evaluate the metabolizable energy values of nine samples of FFSB. [2]FPD: Protein free diet, used to evaluate the true digestibility of amino acids in nine samples of FFSB. [3]Composition for kg of product: manganese, 75.000 mg; iron, 20000 mg; zinc, 50.000 mg; copper, 4.000 mg; cobalt, 200 mg; iodine 1.500 mg and qsp, 1.000 g. [4]Composition for kg of product: vit. A, 12.000.000 UI; vit. D3, 2.200.000 UI; vit. E 30.000 UI; vit. B1, 2.200 mg; vit B2, 6.000 mg; vit. B6, 3.300mg; pantothenic acid, 13.000mg; biotin, 110mg; vit. K3, 2.500 mg; folic acid, 1.000mg; nicotinic acid 53.0000 mg; niacin, 25.000 mg; vit. B12, 16.000 µg; selenium, 0.25 g and qsp., 1.000g. [5]Salinomycin 12%. [6]Butyl Hydroxy Toluene.

of nitrogen-corrected apparent metabolizable energy (AMEn) were estimated using the equations proposed by Matterson et al. (1965). After that, the metabolization coefficients of the nitrogen-corrected apparent energy (CAMEn) were set.

In the second trial, 1120 male Cobb 500 broilers were 24 days old and an average weight of 890.00 ± 10.0g were used. Broilers were distributed in randomized blocks in a 10 x 2 factor arrangement (nine FFSB - 40% included + a protein free diet (PFD) - 60%, without or with the addition of monocomponent protease) (Table 1), totaling 20 treatments and eight replicates with seven birds each. Celite™ acid-insoluble ash was added at 1% into all experimental diets. The enzyme used in the experiment, its respective characteristics and proportion of inclusion were above cited in the first trial. Until the 23rd day, the animals were raised fed basal diet following the recommendations of Rostagno et al. (2011). Subsequently, the broilers were sheltered in batteries from the 24th day to the 28th day of age, fed the experimental diets.

After a 4th day adaptation period, the broilers were slaughtered by electronarcosis for collection of ileal digesta. Then, broilers were opened in the abdominal cavity, removing the entire intestinal content, which was 40 cm from the terminal ileum portion prior to the ileocecal junction. Before slaughter, the broilers were encouraged to consume feed to prevent the emptying of the digestive tract, which affects the ileal digesta collection procedure.

Samples of ileal digesta were freeze-dried under vacuum, at a temperature of -40 °C for 72 hours, and laboratory analyses were carried out to verify the amino acidic content by HPLC (High Performance Liquid Chromatography), according to the methodology described by Association of Official Agricultural Chemists - AOAC (1995). Dry matter and CP of digesta were also determined according to Silva and Queiroz (2002). The acid-insoluble ash and indigestible factor were carried out according to Joslyn (1970). The calculations of the true ileal digestibility of amino acids were performed using the methodology proposed by Sakomura and Rostagno (2007).

Dataset for the first and second experiments were submitted to analysis of variance (ANOVA), and means were analyzed by Student-Newman-Keulls (SNK) test at 5%. All analyzes were carried out using the Statistical and Genetics Analysis System software - UFV (SAEG, 2007).

RESULTS AND DISCUSSION

From the chemical composition of toasted FFSB samples, it can be inferred that this feed presents a good profile of CP and EE in addition to reduced CF content (Table 2). These results corroborate the findings by Rostagno et al. (2011) and Ravindran et al. (2014). The GE sample values of FFSB of the present study were higher than those found by Rostagno et al. (2011), of 5032kcal/kg, except for the sample 4. This occurred due to the lower EE content in this sample.

The concentration of trypsin inhibitor factor in samples 4 and 5 were over the acceptable limit of 4.1 TIF mg/g, for the FFSB (Ravindran et al., 2014). The urease index on samples 4 and 5 were over than the range (0.03 to 0.05) recommended by Araba and Dale (1990). However, the protein solubility values of all samples ranged between 70 and 85%, as recommended by Parsons et al. (1991). According to Nunes et al. (2015) the evaluation the effectiveness of the termal treatment used to deactivate anitinutritional factors in FFSB, correlates urease index

Table 2: Chemical composition (% natural matter), protein solubility (%), urease index and trypsin inhibitor factor of samples of toasted full-fat soybeans.

Analysis	Toasted full-fat soybean samples								
	1	2	3	4	5	6	7	8	9
GE (kcal/kg)	5173	5099	5237	4646	5307	5216	5216	5108	5190
DM (%)	90.86	91.05	90.97	90.78	90.83	90.91	90.98	90.90	90.88
CP (%)	34.96	36.87	37.81	39.77	35.58	34.59	38.93	36.23	36.84
EE (%)	21.71	20.71	20.39	10.25	22.09	20.86	19.98	20.94	21.82
CF (%)	4.73	3.57	2.45	4.93	4.90	3.75	2.90	3.72	4.76
PS (%)	74.99	73.52	74.53	84.10	72.68	74.19	83.10	73.75	76.90
UI (pH)	0.05	0.05	0.05	0.11	0.36	0.05	0.05	0.05	0.05
TIF (mg/g)	1.63	1.15	1.40	8.96	4.38	1.54	1.12	1.30	1.90

GE = gross energy; DM = dry matter; CP = crude protein; EE = ether extract; CF = crude fiber; PS = protein solubility KOH (0.2%); UI = urease index in units of pH; TIF = Trypsin inhibitor factor (TIFmg/g of protein).

and protein solubility. Since, high values of urease index indicate sub-haeting within the same type of processing.

In this study it was observed that there is variation among the FFSB samples. The discrepancy between the chemical composition data and the FFSB can occur due to type of processing, type of cultivar used, type of planting, and the place of origin (Valencia et al., 2009). According to Freitas et al. (2005) for the same type of processing there is no complete standardization of conditions of temperature, humidity, time and pressure. Therefore, we can infer that the toasted FFSB samples had no standardization among the various production units.

The nitrogen-corrected apparent metabolizable energy (AMEn) of FFSB samples, without addition of protease, were similar to those reported by Ravindran et al. (2014), by 3228 kcal/kg and lower than those reported by Valencia et al. (2009) and Rostagno et al. (2011), by 3439 and 3463 kcal/kg, respectively (Table 3).

Among the analyzed samples, the CP and EE of FFSB sample 4 showed greater variability. Acoording to

Table 3: Nitrogen-corrected apparent metabolizable energy (AMEn) values and their respective metabolization coefficient (CEMAn) of toasted full-fat soybean samples without and with addition of protease expressed in natural matter (kcal/kg), for the first experiment.

FFSB	Enzyme	AME_n	$CAME_n$
Sample 1	without	3063	59.22
	with	3243	62.68
Sample 2	without	3199	62.75
	with	3438	67.43
Sample 3	without	3232	61.71
	with	3343	63.84
Sample 4	without	3220	69.52
	with	3519	75.75
Sample 5	without	3269	61.59
	with	3343	62.99
Sample 6	without	3289	63.06
	with	3510	67.30
Sample 7	without	3060	58.66
	with	3154	60.47
Sample 8	without	3254	63.70
	with	3388	66.32
Sample 9	without	3267	62.94
	with	3275	63.11
Enzyme	without	3207 b	62.57 b
	with	3357 a	65.54 a
Diference		150	2.97
CV(%)		6.94	7.08
		P – value	
FFSB[1]		0.004	0.004
E		0.002	0.002
FFSB x E		0.852	0.952

FFSB = full-fat soybeans samples; E = enzyme; FFSB x E = interaction of factors FFSB and E; a-b Different letters in the same column differ by SNK test (P<0.05); CV = Coefficient of variation. [1]The marginal mean values of FFSB samples are presented in Table 4.

Nunes et al. (2005) the factors that most influences the use of energy feedtuffs by broilers are the levels of CP and EE. This is due to the difference in caloric increment of protein and fat, changing the energy balance for nitrogen excretion and deposition of body tissue. Overall, the protease enhanced values of AMEn and CAMEn compared to those without addition (Table 3). Similar results was observed by Yu et al. (2007), Freitas et al. (2011) and Matias et al. (2015), when they evaluated soybean meal based diets as a protein source, supplemented or not with protease.

The enzymatic activity of protease provided an average of 150 kcal/kg, which is 4.5% more energy in the FFSB samples evaluated in this study. This indicates better utilization of CP from the FFSB, providing greater amount of AA available for protein metabolism and other nutrients. By hydrolyzing the reserve proteins of whole soybean grains, protease releases some nutrients, such as starch, promoting the use of energy from the diet (Fru-Nji et al., 2011). Accordingly, protease releases the potential energy contained in the starch-protein bonds, and this effect is more evident in varieties with lower EE, as CP is increased with the dilution effect, as observed in sample 4 (Table 3).

When analyzing the average marginal effect on the type of FFSB in the use of energy, it is clear that the sample 7 was higher than sample 4 (P<0.05), however, did not differ from the others.

According to Lemme et al. (2004) a substantial amount of AA and protein passes through the gastrointestinal tract of broilers and are not utilized neither completely digested. Protease supplementation also increases hydrolysis of antinutritional protein-origin factors such as trypsin and lectin inhibitors, enhancing the use efficiency of AA (Ghazi et al., 2002), even as supplied as "on top" (Fru-Nji et al., 2011; Kamel et al., 2015).

The content of total AA in different samples of toasted FFSB was similar to those reported by Rostagno et al. (2011) (34%). However, they were lower than those found by Valencia et al. (2009) and Ravindran et al. (2014), by 43 and 52%, respectively, especially related to the concentration of methionine (Table 5).

The true ileal digestibility coefficient of essential AA of toasted FFSB samples was on average 87.90%. These values were similar to those reported by Rostagno et al. (2011), which were 87.10%. The inclusion of protease enhanced true ileal digestibility coefficient of essential AA (P<0.05) (Table 6).

On average, the inclusion of protease enhanced the digestibility of essential AA by 2.5%. The higher variation was observed on digestibility coefficients of threonine, methionine and valine. Threonine is associated to the production of mucin, which in turn, stimulates the production of mucus by the gastrointestinal tract (Star et al., 2012). Full-fat soybeans contain some antinutritional factors, such as allergenic proteins. Also, it was observed low standardization on some FFSB, as samples 4 and 5. Probably, these factor influenced on results observed for threonine digestibility coefficient (up to 7.34%). Valine is located in the inner part of the soybeans (Tavernari et al., 2013). It is digestibility increased, on average, by 4.10% due to inclusion of protease,

Table 4: Marginal means values of the full-fat soybean samples for nitrogen-corrected apparent metabolizable energy (AMEn) and the respective metabolization coefficient (CEMAn), for the first experiment.

FFSB	AMEn	CAMEn
Sample 1	2963 ab	57.29 ab
Sample 2	3018 ab	59.18 ab
Sample 3	3006 ab	57.40 ab
Sample 4	2952 b	63.54 ab
Sample 5	3009 ab	56.70 ab
Sample 6	3039 ab	58.25 ab
Sample 7	3028 a	58.05 a
Sample 8	3015 ab	59.02 ab
Sample 9	3000 ab	57.78 ab
P-value	<0.001	<0.001
CV%	2.43	2.37

FFSB = full-fat soybean; a-b Different letters in the same column differ by SNK test (P<0.05); CV = Coefficient of variation.

indicating greater FFSB protein degradation, as compared to samples that were not supplemented by the protease.

The increase in methionine availability is paramount. After all, it is the first limiting AA in diets for broilers with primary functions, such as donor of methyl groups, and together with cystine and cysteine, proper feathering (Baker et al., 1996). Methionine was detected at lower concentration of the total AA content in samples of FFSB analyzed in this study, and the role of protease was decisive for the increase of its respective coefficient up to 3.5%. Furthermore, the concentration of methionine is influenced by the levels of lysine and arginine (Oliviera Neto et al., 2007), which also had their coefficients satisfactorily increased with the inclusion of 1.71 and 1.67% of protease, respectively.

The inclusion of monocomponent protease increased the CP digestibility of FFSB samples up to 4.35% (Table 7). Similar results were observed by preliminary studies working with monocomponent protease (Angel et al., 2011; Oxemboll et al., 2011; Freitas et al., 2011). This indicates greater degradation of anti-nutritional factors, improving other nutrients metabolism, increasing metabolizable of the diets. This was also found in in this study.

The mean true ileal digestibility coefficient of nonessential AA of FFSB was 84.34% higher than that found by Ravindran et al. (2014) (77.90%). The inclusion of protease increased the digestibility of nonessential AA evaluated in this study ($P < 0.05$) (Table 7). Of those, cystine, glycine and serine had their ratios increased by 6.15, 4.29 and 3.23%, respectively.

Cystine is a sulfur AA that is present in pancreatic secretions and, the protease supplementation contribute to reduce cystine endogenous losses. Probably, this fact increases cystine digestibiliy coefficients. Also, glycine showed higher digestibility coefficient. The glycine composes mucoglicoproteins and participates in the metabolism of threonine (Ospina-Rojas et al., 2013). Thus, protease supplementation also reduced glycine endougenous losses, which increased its digestibility coefficientes. A similar response was observed by Angel et al. (2011) revealing that supplementation of a monocomponent protease improves endogenous enzymes activty, mainly peptidases. According Smith (2011) the supplementation of diets for broilers with serine-proteases from Bacillus lincheniformis, as in this study, complements the action of endogenous enzymes such as

Table 5: Amino acid composition of toasted full-fat soybean samples (% natural matter).

Amino acid	Toasted full-fat soybean samples								
	1	2	3	4	5	6	7	8	9
Lys	2.15	2.27	2.44	2.47	2.17	2.12	2.44	2.11	2.27
Thr	1.23	1.84	1.34	1.38	1.21	1.22	1.32	1.28	1.31
Met	0.29	0.15	0.37	0.35	0.33	0.31	0.35	0.34	0.34
Arg	2.81	1.40	3.14	3.25	2.84	2.82	3.38	2.92	3.06
His	0.93	0.99	1.02	1.07	0.91	0.88	1.15	0.91	0.97
Ile	1.56	1.65	1.68	1.75	1.52	1.55	1.69	1.61	1.62
Leu	2.85	3.10	3.24	3.33	2.95	2.93	3.24	3.02	3.11
Phe	1.80	1.97	1.99	2.06	1.82	1.81	1.96	1.89	1.94
Val	1.77	0.39	1.95	2.01	1.77	1.74	1.92	1.88	1.84
Cys	0.46	0.49	0.46	0.57	0.43	0.43	0.55	0.53	0.45
Ala	1.64	2.16	1.82	1.84	1.62	1.64	1.86	1.72	1.75
Asp	3.35	3.62	4.25	3.68	2.80	3.44	3.43	3.41	3.51
Glu	6.37	6.95	7.55	7.19	5.93	6.46	6.89	6.58	6.78
Gly	1.72	1.84	1.92	2.03	1.79	1.79	2.06	1.88	1.89
Ser	1.77	1.98	1.96	1.98	1.76	1.72	1.92	1.79	1.92
Tyr	1.23	1.84	1.34	1.38	1.21	1.22	1.32	1.28	1.31
Pro	2.03	1.33	2.30	2.36	1.11	2.07	2.38	2.20	2.19

pepsin and pancreatic proteases, increasing the solubility of proteins of the various dietary ingredients.

In addition, protease supplementation possibilities greater anti-nutritional factors degradation and higher aviability of AA for growth and muscle deposition. The supplementation of protease combined with diet formulation based on digestible AA may result in lower production costs and lower environmental impacts. According to Oxemboll et al. (2011) environmental pollution impacts can be reduced even with protease supplementation as "on top". However, this benefit is more evident, when the protein amount in the diet are reduced.

An effect of FFSB sample in related to the true digestibility coefficients of the main limiting AA for broilers, among the essential AA, non-essential AA and CP, and the sample 4 had the worst results (Table 8).

This indicates variability of whole soybean, especially related to soil type, cultivar planted, and the lack

Table 6: True ileal digestibility coefficients of essential amino acids of full-fat soybean samples, without or with protease supplementation.

FFSB	E	LYS	THR	MET	ARG	HIS	ILE	LEU	PHE	VAL
1	whitout	91.97	80.16	91.13	93.62	97.24	88.58	89.74	90.45	85.07
	with	93.28	84.82	93.61	94.51	97.70	90.24	90.96	91.17	88.53
2	whitout	92.87	82.42	93.47	93.81	97.45	89.47	91.18	91.40	86.70
	with	94.59	86.68	95.09	95.45	98.41	91.71	93.03	93.11	90.28
3	without	92.32	80.73	88.91	92.35	97.40	87.34	88.55	89.44	83.45
	with	93.81	85.44	91.90	93.86	98.11	90.48	92.24	92.00	87.64
4	without	91.27	74.01	89.33	92.34	93.37	84.65	85.60	87.37	82.29
	with	92.93	78.81	92.41	94.42	95.13	86.83	87.87	89.67	84.90
5	without	91.64	80.34	88.54	91.40	92.86	84.08	86.43	86.10	81.29
	with	93.69	87.68	94.69	93.64	94.48	88.36	89.90	89.57	86.30
6	without	91.25	81.18	93.81	93.01	92.73	88.10	88.55	89.20	85.05
	with	93.07	86.73	97.10	95.36	94.88	91.04	91.31	91.97	88.60
7	without	92.35	80.59	94.75	93.22	94.14	87.11	86.75	88.49	84.43
	with	93.75	84.46	95.76	94.44	94.89	89.25	90.09	90.44	86.93
8	without	84.24	70.04	82.68	88.20	80.70	79.23	85.03	81.68	75.45
	with	85.91	74.23	86.09	89.34	82.82	81.83	87.08	83.88	79.03
9	without	91.78	84.77	92.95	93.92	83.36	88.10	89.22	89.75	84.13
	with	92.99	87.97	93.10	94.98	86.44	89.82	90.43	90.30	87.68
Mean	without	91.08b	79.36b	90.62b	92.43b	92.14b	86.30b	87.90b	88.21b	83.10b
	with	92.67a	84.10a	93.31a	94.00a	93.65a	88.84a	90.33a	90.24a	86.66a
CV(%)		1.36	4.02	2.43	1.25	1.75	2.26	2.04	1.97	2.6
					P – value					
FFSB[1]		<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
E		<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
FFSBxE		0.996	0.886	0.424	0.639	0.446	0.787	0.513	0.460	0.910

FFSB = full-fat soybeans samples; E = enzyme; FFSBxE = interaction of factors FFSB and E; a, b Different letters in the same column differ by SNK test (P<0.05); CV = Coefficient of variation. [1]The marginal mean values of FFSB samples are presented in Table 8.

of standardization in the same type of thermal processing. Therefore, the use of more effective thermal methods can reduce the variability of the quality of FFSB. According Alsaftli et al. (2015) more refined thermal processes such as extrusion of FFSB, favor the use of nutrients by birds, with significant gains in performance and carcass characteristics.

The values of digestible AA of different samples of FFSB in this study were similar to those found by Rostagno et al. (2011) and Ravindran et al. (2014), except for methionine values, which were lower (Table 9). Methionine is the principal limiting AA in broiler diets, and the most expensive AA. Therefore, when using the toasted FFSB replacing soybean meal as a protein source, the larger synthetic methionine supplementation can be needed to meet the broiler nutritional requirements.

The results of this study suggest that protease supplementation is a good alternative to the formulation of diets for poultry. Protease supplementation can be

Table 7: True ileal digestibility coefficients of nonessential amino acids and crude protein of full-fat soybean samples, without or with protease supplementation.

FFSB	E	CYS	ALA	ASP	GLU	GLY	SER	TYR	PRO	CP
1	whitout	71.00	86.61	89.97	92.73	80.87	85.79	88.25	88.00	86.08
	with	73.97	88.18	91.23	93.84	82.09	87.64	89.34	88.13	88.47
2	whitout	74.16	88.24	91.24	93.46	83.30	88.39	89.81	89.53	89.48
	with	77.37	90.90	93.99	95.19	84.96	90.42	91.89	91.21	91.16
3	without	74.39	86.29	90.50	92.19	83.06	86.35	88.13	80.05	88.93
	with	76.74	88.90	92.87	94.17	87.39	91.13	92.02	90.96	90.94
4	without	61.52	83.58	89.87	91.97	76.11	84.15	82.86	81.96	85.47
	with	70.01	86.25	91.75	93.67	81.25	87.53	85.85	86.24	88.17
5	without	84.50	83.59	89.44	91.28	76.96	83.04	85.86	84.33	86.93
	with	91.65	87.29	91.92	93.38	80.74	87.53	90.83	88.78	90.87
6	without	78.94	84.13	90.65	92.07	75.14	86.03	89.24	83.47	87.64
	with	85.66	87.53	92.83	94.04	81.31	89.43	92.18	88.86	90.32
7	without	75.66	84.39	91.40	92.75	77.01	84.49	88.45	85.28	87.70
	with	80.63	86.65	92.53	93.95	80.75	86.04	90.13	87.90	91.15
8	without	58.27	76.05	82.47	86.15	64.89	75.59	83.00	77.39	79.70
	with	61.19	78.14	84.16	87.74	68.14	77.89	84.45	79.47	82.36
9	without	87.99	86.33	91.24	91.97	82.01	88.24	91.99	88.36	88.17
	with	93.02	88.03	93.06	93.04	84.18	89.93	92.65	89.05	91.37
Mean	without	74.05b	84.36b	89.64b	91.62b	77.71b	84.67b	87.51b	85.15b	86.79b
	with	78.91a	86.87a	91.59a	93.22a	81.20a	87.50a	89.93a	87.84a	89.43a
CV(%)		5.20	2.54	2.13	1.41	3,92	2.62	2.14	2.38	2.01
						P – value				
FFSB[1]		<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
E		<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
FFSBxE		0.383	0.937	0.974	0.960	0.497	0.432	0.693	0.973	0.883

FFSB = full-fat soybeans samples; E = enzyme; FFSBxE = interaction of factors FFSB and E; CP = crude protein; a, b Different letters in the same column differ by SNK test (P<0.05); CV = Coefficient of variation. [1]The marginal mean values of the FFSB samples are presented in Table 8.

Table 8: Marginal mean values of the full-fat soybean samples for the true ileal digestibility coefficients of main limiting amino acids, essential amino acid, non-essential and crude protein.

FFSB	MET	LYS	THR	TIDCEA	TIDCNEA	TIDCCP
1	92.37 bc	92.62 ab	82.49 b	91.03 ab	88.93 abc	87.28 bc
2	92.48 ab	93.73 a	84.55 ab	92.26 a	90.94 a	90.32 a
3	90.40 c	93.07 ab	83.08 ab	91.64 ab	90.92 ab	89.94 a
4	84.39 d	85.07 c	72.14 d	82.91 e	80.38 d	81.03 d
5	91.61 c	92.66 ab	84.01 ab	88.76 cd	88.44 bc	88.94 abc
6	95.45 a	92.16 b	83.96 ab	90.45 b	89.01 abc	88.98 ab
7	90.87 c	92.10 b	76.41 c	88.14 d	87.58 c	86.82 b
8	95.25 a	93.05 ab	82.52 ab	89.92 bcd	88.67 bc	89.93 a
9	93.03 abc	92.39 ab	86.37 a	90.16 bc	90.15 ab	89.77 a
P-value	<.001	<.001	<.001	<.001	<.001	<.001
CV (%)	2.73	1.63	3.83	1.58	2.03	2.09

a-b Different letters in the same column differ by SNK test ($P<0.05$); FFSB = full-fat soybeans; TIDCAE = true ileal digestibility coefficient amino acid essential; TIDCANE = true ileal digestibility coefficient amino acid non essential; TIDCCP = true ileal digestibility coefficient of CP; CV = Coefficient of variation.

Table 9: True digestible amino acids (AASD) of toasted full-fat soybean samples (% natural matter).

AASD	Toasted full-fat soybean samples								
	1	2	3	4	5	6	7	8	9
Lys	1.98	2.11	2.25	2.25	1.99	1.93	2.25	1.78	2.08
Thr	1.03	1.45	1.16	1.06	1.05	1.01	1.08	0.94	1.16
Met	0.26	0.14	0.33	0.31	0.29	0.29	0.33	0.28	0.32
Arg	2.63	1.31	2.90	2.99	2.59	2.62	3.15	2.57	2.87
His	0.90	0.96	0.99	0.99	0.84	0.82	1.08	0.73	0.81
Ile	1.38	1.48	1.47	1.48	1.28	1.36	1.47	1.27	1.43
Leu	2.56	2.82	2.87	2.85	2.55	2.59	2.81	2.57	2.77
Phe	1.63	1.80	1.78	1.80	1.57	1.61	1.73	1.54	1.74
Val	1.50	0.34	1.63	1.65	1.44	1.48	1.62	1.42	1.55
Cys	0.33	0.36	0.34	0.35	0.36	0.34	0.42	0.31	0.39
Ala	1.42	1.90	1.57	1.54	1.35	1.38	1.57	1.31	1.51
Asp	3.01	3.30	3.85	3.31	2.50	3.12	3.13	2.81	3.20
Glu	5.91	6.49	6.96	6.61	5.41	5.95	6.39	5.67	6.23
Gly	1.39	1.53	1.59	1.54	1.38	1.34	1.59	1.22	1.55
Ser	1.52	1.75	1.69	1.67	1.46	1.48	1.62	1.35	1.69
Tyr	1.08	1.65	1.18	1.14	1.04	1.09	1.17	1.06	1.20
Pro	1.79	1.19	2.02	1.93	1.78	1.73	2.03	1.70	1.93

used in order to optimize protein and energy utilization and to provide greater availability of AA in broiler diets formulated with FFSB. However, it is necessary further studies to evaluate the values of AMEn and AA of toasted FFSB as well as the possible interactions with exogenous enzymes to increase the availability of nutrients required by the broilers.

CONCLUSIONS

The average values of AMEn and their respective CAMEn of toasted full-fat soybeans were 3207 kcal/kg and 62.57%, respectively. The average coefficients of true ileal digestibility of crude protein, essential and nonessential amino acids were 86.79, 87.90 and 84.34%, respectively. The supplementation with protease in diets for broilers, formulated with toasted full-fat soybean, enhanced the values of AMEn and CAMEn, improved the digestibility of crude protein, and the availability of essential and nonessential amino acids. Thus, it is recommended the supplementation of a monocomponent protease in order to optimize nutrient utilization, particularly proteins and amino acids, and to alleviate the adverse effects of inadequate thermal treatment of toasted full-fat soybeans.

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