ANIMAL SCIENCE (ZOOTECNIA)

Revista Brasileira de Ciências Agrárias
ISSN (on line) 1981-0997
v.20, n.1, e3524, 2025
Recife, PE, UFRPE. www.agraria.pro.br
DOI: 10.5039/agraria.v20i1a3524 - Protocol 3524
Subm. 05/11/2023 • App. 23/12/2024 • Pub. 24/02/2025

Reduction of sulfur amino acid levels with protease supplementation in broiler chicken diets

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ABSTRACT: The objective of this study was to evaluate the dietary reduction of sulfur amino acids, using proteases supplementation, in broiler chicken diets, and its impact on performance, carcass yield, and meat cuts. A total of 744-day-old Cobb 500 chicks were distributed in a completely randomized design with four treatments, including a reference basal diet without sulfur amino acid reduction and without protease supplementation, and three levels of sulfur amino acid reduction (4, 6, and 8%) with protease supplementation, with six replicates and 31 birds per experimental unit. From 1 to 21 days of age, there was no significant effect ($p > 0.05$) of reducing sulfur amino acids on the performance of the birds. However, in the period from 1 to 42 days of age, a significant effect ($p < 0.05$) on feed consumption was observed. Breast yield was affected by the reduction of sulfur amino acid levels in the diet ($p < 0.05$). The reduction of sulfur amino acids, when supplemented with protease, does not adversely influence the performance of the birds, as long as this reduction remains within the 8% limit.

Key words: enzyme; methionine; meat cuts; performance; sulfur amino acids

Redução dos níveis de aminoácidos sulfurosos com suplementação de protease em dietas para frangos de corte

RESUMO: Objetivou-se avaliar a redução dietética de aminoácidos sulfurosos com suplementação de protease sobre o desempenho e o rendimento de carcaça e cortes de frangos de corte. Foram utilizados 744 pintos, com 1 dia de idade, Cobb 500, distribuídos em delineamento inteiramente casualizado com quatro tratamentos, incluindo uma dieta basal de referência sem redução de aminoácidos sulfurados e sem suplementação de protease, e três níveis de redução de aminoácidos sulfurados (4, 6, e 8%) com suplementação de protease, com seis repetições e 31 aves por unidade experimental. No período de 1 a 21 dias de idade, não houve efeito ($p > 0,05$) da redução de aminoácidos sulfurosos sobre o desempenho. No período de 1 a 42 dias de idade, os tratamentos afetaram o consumo de ração ($p < 0,05$). O rendimento de peito foi afetado com a redução dos níveis de aminoácidos sulfurosos na dieta ($p < 0,05$). A redução de aminoácidos sulfurosos com suplementação de protease não afeta o desempenho das aves até o nível de 8%.

Palavras-chave: enzima; metionina; rendimento de cortes; desempenho; aminoácidos sulfurosos

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Associate Editor: Mário de Andrade Lira Júnior

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Introduction

Nutrition is the most financially influential factor in the industrial production of broiler chickens. Therefore, it is essential to reduce costs to increase the profitability of the activity without compromising the birds performance. Among the nutrients in feed, protein is one of the costliest (Saleh et al., 2019). In Brazil, diets are primarily formulated based on corn and soybean meal to meet energy and protein requirements. However, these diets are deficient in certain amino acids, such as methionine, needing the supplementation of synthetic methionine sources to satisfy the nutritional requirements of broiler chickens (Viana et al., 2009; Brumano et al., 2010).

Methionine serves various functions in the birds body, playing a crucial role in protein synthesis and, consequently, contributing to increased meat yield (Walk & Rao, 2018). Additionally, it is a precursor amino acid to cysteine and an important donor of methyl groups that can be used in methylation processes, remethylation, carnitine, and creatine synthesis (Simon, 1999).

Another important consideration is that the presence of antinutritional factors (trypsin inhibitors, lectins, and allergenic proteins) in soybean meal (Rocha et al., 2014) and non-starch polysaccharides (NSPs) and phytate in corn (Troni et al., 2016) can affect the bioavailability of methionine for broiler chickens. Endogenous intestinal proteases are insufficient to achieve complete digestibility of crude protein and amino acids in broiler chicken diets (Bertechini et al., 2020). Therefore, the supplementation of exogenous protease in broiler diets improves protein and amino acid digestibility in feed, consequently leading to improved weight gain and feed conversion in broiler chickens (Kalmendal & Tauson, 2012; Yegani & Korver, 2013; Dalólio et al., 2016; Saleh et al., 2019; Dessimoni et al., 2019; Bertechini et al., 2020).

Supplementing broiler diets with protease increases the standardized ileal digestibility of amino acids from protein sources (Vieira et al., 2013; Dalólio et al., 2016; Bertechini et al., 2020) and reduces nitrogen excretion (Canogullari et al., 2009). This can lead to reduced feed costs due to a decrease in the inclusion of synthetic crystalline amino acids such as methionine. The ideal protein is defined by the proportion and quality of amino acids in a protein source, ensuring a balanced intake of essential amino acids to promote the growth, maintenance, and repair of body tissues according to the requirements of the animal category (Carvalho et al., 2023). Improving the balance between amino acids and total protein is crucial for optimizing the nutritional benefits of protein, highlighting the importance of selecting high-quality sources and combining different sources in the diet to achieve adequate nutrition.

In this context, the objective of this study was to evaluate the reduction of sulfur amino acids with and without protease supplementation in the diet concerning the performance and carcass and cut yield of broiler chickens.

Materials and Methods

The experiment was conducted in the experimental poultry house of the Poultry Technology Research Center (CPTA), located at BR 265 - km 144, in the municipality of Lavras, MG, Brazil, and carried out following the ethical principles for animal experimentation established by the Brazilian College of Animal Experimentation (Cobea, 1991). This research followed the ethical principles of animal experimentation and utilized the minimum number of animals required to obtain precise results in accordance with the Ethical Principles in Animal Experimentation and was approved by the Ethics Committee (Number 017/2023). A total of 744 broiler chicks, with an average weight of 45 g, from the Cobb 500 lineage, were used, from one day old until 42 days of age.

The birds were distributed in a completely randomized design with four treatments, including a reference basal diet without sulfur amino acid reduction and without protease supplementation (T1), and three levels of sulfur amino acid reduction (4, 6, and 8%) (T2, T3, and T4) with protease supplementation, with six replicates and 31 birds per experimental unit.

The protease used was RONOZYME® ProAct, which is the result of the fermentation of *Bacillus licheniformis* containing genes from *Nocardiopsis prasina*. The activity level of this enzyme is defined by the amount of product required 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 enzyme used contains 75,000 PROT/gram of enzyme. A quantity of 200 g ton^{-1} of protease was used, following the manufacturers recommendation in all treatments except the basal diet (T1).

The birds were housed in a masonry shed with cement flooring and asbestos roofing, built in the east-west orientation and divided into 24 pens measuring 2.0 × 1.5 m, which were prepared to receive the chicks. Each pen contained a lamp with a 150W bulb, a pendulum drinker, and a tubular feeder. Maximum and minimum temperatures and relative humidities were recorded throughout the experimental period using a thermo-hygrometer located in the center of the shed. The data obtained for maximum, average, and minimum air temperatures, as well as maximum, average, and minimum relative humidity during the entire experimental period, were, respectively, 26.43 °C; 21.55 °C; 16.67 °C and 81.5%; 63.3%; 45.1%.

The birds had ad libitum access to water and feed throughout the experimental period. The chicks were vaccinated against Mareks disease in the hatchery, received coccidiosis and Gumboro vaccines on the second day of life, and a booster was administered on the 18th day of life.

The experimental diets were formulated following the recommendations of Rostagno et al. (2017) and divided into three rearing phases: starter (1-21 days of age) (Table 1), grower (22-35 days of age) (Table 2), and finisher (36-42 days of age) (Table 3).

Table 1. Percentage and calculated composition of diets in the period from 1 to 21 days.

Ingredients (kg)	Reduction of sulfur amino acid levels (%)			
	0	4	6	8
Corn	56.498	56.498	56.498	56.498
Soybean meal	37.659	37.659	37.659	37.659
Soybean oil	1.929	1.929	1.929	1.929
Limestone	0.903	0.903	0.903	0.903
Dicalcium phosphate	1.816	1.816	1.816	1.816
Common salt	0.502	0.502	0.502	0.502
L-lysine H-Cl	0.141	0.141	0.141	0.141
DL-Methionine	0.244	0.210	0.193	0.175
L-Threonine	-	-	-	-
Suppl. Mineral[1]	0.100	0.100	0.100	0.100
Suppl. Vitamin[2]	0.100	0.100	0.100	0.100
Inert	0.070	0.150	0.190	0.240
Protease[3]	-	0.020	0.020	0.020
Zinc bacitracin	0.025	0.025	0.025	0.025
Choline chloride	0.013	0.013	0.013	0.013
Salinomycin	-	-	-	-
Total	100.000	100.000	100.000	100.000
Nutrient – Calculated composition				
Metabolizable energy (Kcal kg^{-1})	2950	2950	2950	2950
Crude protein (%)	22.0	22.0	22.0	22.0
Ca (%)	0.899	0.899	0.899	0.899
P available (%)	0.499	0.499	0.499	0.499
Methionine + Cystine dig (%)	0.844	0.810	0.794	0.776
Lysina dig. (%)	1.189	1.189	1.189	1.189
Threonine dig. (%)	0.773	0.773	0.773	0.773
Na (%)	0.218	0.218	0.218	0.218

1 – Suppl. Mineral - Levels per kg of the product: Zinc (110,000 mg), Selenium (360 mg), Iodine (1,400 mg), Copper (20,000 mg), Manganese (15,6000 mg), Iron (96,000 mg). 2 - Suppl. Vitamin K3 (6,000 mg), Vitamin B12 (40,000 mg), Niacin (75,000 mg), Vitamin B1 (5,000 mg), Folic Acid (3,000 mg), Pantothenic Acid (30,000 mg), Biotin (150 mg), BHT (100 mg), Vitamin B6 (8,000 mg), Vitamin A (25,000,000 IU), Vitamin D3 (6,000,000 IU), Vitamin E (45,000 IU), Vitamin B2 (12,000 mg). 3 - Ronozyme Proact protease enzyme (200 g ton^{-1}). Dig: digestible. P: Phosporo.

Table 2. Percentage and calculated composition of diets in the period from 22 to 35 days.

Ingredients (kg)	Reduction of sulfur amino acid levels (%)			
	0	4	6	8
Corn	62.441	62.406	62.389	62.370
Soybean meal	30.765	30.816	30.841	30.870
Soybean oil	3.141	3.159	3.168	3.178
Limestone	0.857	0.851	0.850	0.850
Dicalcium phosphate	1.652	1.652	1.652	1.652
Common salt	0.470	0.469	0.469	0.469
L-lysine H-Cl	0.200	0.200	0.200	0.200
DL-Methionine	0.230	0.198	0.182	0.166
L-Threonine	0.046	0.046	0.046	0.046
Supplement Mineral[1]	0.050	0.050	0.050	0.050
Supplement Vitamin[2]	0.045	0.045	0.045	0.045
Inert	0.020	-	-	-
Protease[3]	-	0.020	0.020	0.020
Zinc bacitracin	0.025	0.025	0.025	0.025
Choline chloride	0.013	0.013	0.013	0.013
Salinomycin	0.050	0.050	0.050	0.050
Total	100.000	100.000	100.000	100.000
Nutrient – Calculated composition				
Metabolizable energy (Kcal kg^{-1})	3100	3100	3100	3100
Crude protein (%)	19.4	19.4	19.4	19.4
Ca (%)	0.824	0.824	0.824	0.824
P available (%)	0.411	0.411	0.411	0.411
Methionine + Cystine dig (%)	0.773	0.742	0.727	0.712
Lysina dig. (%)	1.073	1.073	1.073	1.073
Threonine dig. (%)	0.697	0.697	0.697	0.697
Na (%)	0.205	0.205	0.205	0.205

1 – Suppl. Mineral - Levels per kg of the product: Zinc (110,000 mg), Selenium (360 mg), Iodine (1,400 mg), Copper (20,000 mg), Manganese (156,000 mg), Iron (96,000 mg). 2 - Suppl. Vitamin K3 (6,000 mg), Vitamin B12 (40,000 mg), Niacin (75,000 mg), Vitamin B1 (5,000 mg), Folic Acid (3,000 mg), Pantothenic Acid (30,000 mg), Biotin (150 mg), BHT (100 mg), Vitamin B6 (8,000 mg), Vitamin A (25,000,000 IU), Vitamin D3 (6,000,000 IU), Vitamin E (45,000 IU), Vitamin B2 (12,000 mg). 3 - Ronozyme Proact protease enzyme (200 g ton^{-1}). Dig: digestible. P: Phosporo.

The evaluated performance characteristics were: body weight, weight gain, feed consumption, and conversion at 21 and 42 days of age. The initial and final average weights were obtained by weighing all birds in each treatment and dividing the total weight by the number of birds. The average weight gain was calculated by subtracting the final average weight from the initial average weight.

The average feed consumption was determined by the difference between the amount of feed provided and any remaining feed, divided by the number of birds. In cases of mortality, the bird's feed consumption until the day of its death was considered, and subsequent adjustments were made for feed conversion calculation, following Sakomura & Rostagno (2015).

At 42 days of age, two birds from each experimental plot were selected to represent the average weight of that plot. These birds were fasted for 8 hours and then slaughtered to evaluate carcass yield, breast yield, and abdominal fat. Carcass yield was calculated as a percentage of live weight before slaughter: %RC = (carcass weight × 100 / live weight). The yield of carcass parts and breast was calculated as a percentage of carcass weight: %RP = (part weight × 100 / carcass weight).

The data were analyzed using analysis of variance, employing the GLM procedure of SAS (SAS, 2002). To check the statistical assumptions of the normality of residuals, the Shapiro-Wilk test was used, and the homogeneity of variances was assessed using the Levene test. For the levels of reduction in sulfur amino acids with protease supplementation, a polynomial regression analysis was conducted using the PROC REG procedure in the SAS program (SAS, 2002).

Results and Discussion

There was no effect of the reduction in sulfur amino acids ($p > 0.05$) on the performance of broiler chickens during the 1 to 21 days and 1 to 42 days of age periods (Table 4).

This can be explained by the protease having a higher reactivity when diets are formulated with higher levels of crude protein, as also observed by Angel et al. (2011). It's important to note that the nutritional recommendations of

Table 3. Percentage and calculated composition of diets in the period from 36 to 42 days.

Ingredients (kg)	Reduction of sulfur amino acid levels (%)			
	0	4	6	8
Corn	61.054	61.080	61.094	61.108
Soybean meal	31.589	31.585	31.582	31.580
Soybean oil	4.069	4.076	4.080	4.084
Limestone	0.796	0.796	0.796	0.796
Dicalcium phosphate	1.482	1.482	1.482	1.482
Common salt	0.442	0.442	0.442	0.442
L-lysine H-Cl	0.107	0.107	0.107	0.107
DL-Methionine	0.183	0.154	0.138	0.123
Supplement Mineral[1]	0.050	0.050	0.050	0.050
Supplement Vitamin[2]	0.045	0.045	0.045	0.045
Inert	0.095	0.075	0.075	0.075
Protease[3]	-	0.020	0.020	0.020
Zinc bacitracin	0.025	0.025	0.025	0.025
Choline chloride	0.013	0.013	0.013	0.013
Salinomycin	0.050	0.050	0.050	0.050
Total	100.000	100.000	100.000	100.000
Nutrient – Calculated composition				
Metabolizable energy (Kcal kg^{-1})	3150	3150	3150	3150
Crude protein (%)	19.0	19.0	19.0	19.0
Ca (%)	0.763	0.763	0.763	0.763
P disponible (%)	0.380	0.380	0.380	0.380
Methionine + Cystine dig (%)	0.732	0.703	0.688	0.673
Lysina dig. (%)	1.017	1.017	1.017	1.017
Threonine dig. (%)	0.661	0.661	0.661	0.661
Na (%)	0.194	0.194	0.194	0.194

1 – Suppl. Mineral - Levels per kg of the product: Zinc (110,000 mg), Selenium (360 mg), Iodine (1,400 mg), Copper (20,000 mg), Manganese (156,000 mg), Iron (96,000 mg). 2 - Suppl. Vitamin K3 (6,000 mg), Vitamin B12 (40,000 mg), Niacin (75,000 mg), Vitamin B1 (5,000 mg), Folic Acid (3,000 mg), Pantothenic Acid (30,000 mg), Biotin (150 mg), BHT (100 mg), Vitamin B6 (8,000 mg), Vitamin A (25,000,000 IU), Vitamin D3 (6,000,000 IU), Vitamin E (45,000 IU), Vitamin B2 (12,000 mg). 3 - Ronozyme Proact protease enzyme (200 g ton^{-1}).

Rostagno et al. (2017) are higher than those recommended by Cobb-Vantress Guidelines (2008), and in this study, the diets were formulated following the recommendations of Rostagno et al. (2017).

Studies also demonstrate that supplementation with monocomponent protease improves the ileal digestibility of amino acids (Dalólio et al., 2016; Bertechini et al., 2020). However, this improvement in standardized ileal amino acid digestibility may not necessarily translate into improved performance of broiler chickens, despite reducing production costs. The supplementation with protease in the present study likely did not generate a significant response in the digestibility of the limiting amino acid (Liu et al., 2013).

Based on these findings, it can be inferred that there was a greater response from the protease as the levels of sulfur amino acids in the diet were reduced. According to Dessimoni et al. (2019), ileal amino acid digestibility in corn and soybean meal-based diets for broiler chickens can increase by up to 40% with the supplementation of monocomponent protease, regardless of the level of crude protein and synthetic crystalline amino acids used in different nutritional plans.

Table 4. Mean values of body weight (BW, g bird^{-1}), weight gain (WG, g bird^{-1}), feed intake (FI, g bird^{-1}) and feed conversion (FC, g g^{-1}) of broilers fed diets containing different levels of sulfur amino acids and protease supplementation at 21 days to 42 days of age.

Reduction of sulfur amino acids (%)	BW (g bird^{-1})	WG (g bird^{-1})	FI (g bird^{-1})	FC (g g^{-1})
	21 days			
0	595	550	874	1,62
4	594	549	875	1.62
6	584	539	874	1.63
8	591	546	893	1.68
Average	591	546	879	1.64
Regression	ns	ns	ns	ns
R^2	-	-	-	-
CV(%)	3.63	3.91	3.48	5.12
	42 days			
0	2252	2208	3750	1.79
4	2237	2193	3831	1.80
6	2266	2221	3814	1.82
8	2293	2248	3906	1.83
Average	2262	2217	3825	1.81
Regression	ns	ns	Q^2	ns
R^2	-	-	0.998	-
CV(%)	3.92	4.00	4.07	4.24

ns - Not significant. CV (%) = Coefficient of variation. Q^2 = Quadratic effect.

Feed consumption showed a quadratic effect ($p < 0.05$) during the 1 to 42 days of age period when different levels of sulfur amino acids were supplemented with protease (Figure 1).

The increase in feed intake as sulfur amino acids in the diet were reduced in association with protease supplementation suggesting that the reduction of methionine was the most limiting nutrient in the diets, and the birds increased their intake. According to Walk & Rao (2018), the increase in feed intake by birds occurs to meet their nutritional needs. Regarding carcass and cut yield, only the live weight at slaughter presented a significant difference ($p < 0.05$) according to Table 5.

The results found in this study are in line with what was observed by Kamel et al. (2015) and Dessimoni et al. (2019). The increase in the inclusion of essential amino acids

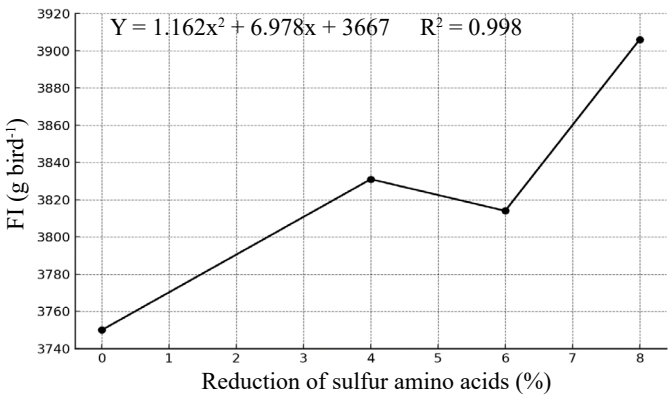

Figure 1. Feed intake (FI, g bird^{-1}) of broilers fed diets containing different levels of sulfur amino acids and protease supplementation at 42 days of age.

Table 5. Body weight at slaughter (BW), carcass yield (CY), breast yield (BY), thigh + drumstick yield (TYD), wing yield (WY), and abdominal fat yield (AFY) of broiler chickens slaughtered at 42 days of age.

Reduction of sulfur amino acids in the diet (%)	BW (g)	CY (%)	BY (%)	TYD (%)	WY (%)	AFY (%)
0	2290	70.87	38.28	28.37	11.96	1.80
4	2208	68.87	36.94	29.68	12.36	2.21
6	2203	68.20	35.16	30.51	12.18	1.96
8	2252	68.83	36.04	29.24	12.02	1.97
Average	2238.75	69.20	36.60	29.45	12.13	1.98
Regression	ns	ns	L[1]	ns	ns	ns
R^2	ns	ns	0.893	ns	ns	ns
CV (%)	3.21	2.48	5.40	4.12	4.76	28.68

* Means followed by different letters in the same column differ by the F ($p < 0.05$ test), ns - Not significant; ASA - Sulfurous amino acids. 1 - Linear effect ($p < 0.05$).

(methionine, lysine, and threonine) combined with protease supplementation in broiler chicken diets are the main factors that can affect carcass characteristics, regardless of the level of metabolizable energy and crude protein; however, there is no change in carcass and cut yield (Kamel et al., 2015). However, in this study, breast yield was affected by the reduction of sulfur amino acid levels with protease supplementation, showing a significant linear effect ($p < 0.05$) (Table 5). The reduction of sulfur amino acid levels reduced breast yield (Figure 2).

It has been reported that diets with protease supplementation promote various benefits in broiler chicken performance, such as an increase in the weight of prime cuts and a reduction in abdominal fat (Tactacan et al., 2016; Saleh et al., 2019). In addition, protease optimizes nutrient utilization, reduces nitrogen excretion, and increases the bioavailability of amino acids due to the breakdown of anti-nutritional factors (Hajati et al., 2009; Dalólio et al., 2016; Bertechini et al., 2020).

Therefore, it was expected in this study that diets with protease supplementation could improve breast muscle deposition, even with reduced levels of sulfur amino acids. Angel et al. (2011) stated that the requirements for essential amino acids, especially sulfur amino acids (methionine+cysteine) and lysine, are critical for maximum breast muscle deposition.

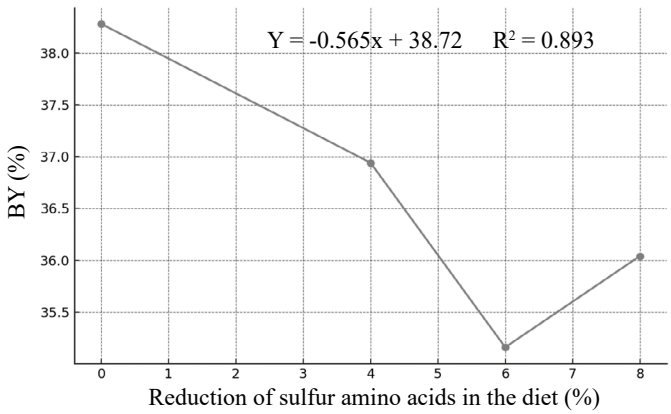

Figure 2. Percentage reduction in breast yield of male broiler chickens as a function of sulfur amino acid levels in the diet at 42 days of age.

The requirement for digestible methionine decreases with age, while cysteine increases (Rostagno et al., 2017). This phenomenon can be attributed to keratin synthesis, which replaces down feathers with feathers (Mitchel, 1964). In a study conducted by Pacheco et al. (2018), the results indicated that the ideal ratio of methionine to cysteine is 52:48 to achieve maximum performance, and beyond this point, the conversion of methionine to cysteine is prioritized via the transsulfuration pathway.

The requirements for sulfur-containing amino acids in the diet of broiler chickens still yield contradictory results regarding the reduction or not of crude protein levels in the diet, as well as the association of DL-methionine and cystine supplementation as sources of these amino acids (Fialho, 2023).

Thus, it can be inferred that the reduction in sulfur amino acid levels was significant, and protease supplementation was not sufficient to increase breast muscle deposition in broiler chickens.

Conclusion

The reduction of sulfur amino acids by up to 8%, accompanied by protease supplementation, does not impact the overall performance of broiler chickens throughout the entire rearing phase. However, even with protease supplementation, lowering sulfur amino acid levels leads to a decrease in breast meat yield by the 42nd day of age.

Acknowledgments

The authors would like to express their gratitude to DSM and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) for their financial support and scholarship grants.

Compliance with Ethical Standards

Author contributions: Conceptualization: JM, AGB; Data curation: JM, EFA, FSD, GCS; Formal analysis: JM, EFA, FSD, GCS; Investigation: JM, EFA, FSD, GCS; Methodology: JM,

EFA, FSD, GCS; Project administration: AGB; Supervision AGB; Validation: AGB; Writing – original draft: FSD, JM; Writing – review & editing: JKV; FSD.

Conflict of interest: The authors declare no conflict of interest.

Funding source: The Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) - Financing Code 001, and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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