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Original Article

Digestible Methionine + Cysteine: Digestible Lysine Ratio in Diets for Broilers Submitted to Inflammatory Challenge

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ABSTRACT

Methionine (Met) and cysteine (Cys) are nutrients in broiler diets, responsible for strengthening protein synthesis, immunity, and metabolic regulation. To estimate the ideal digestible Met + Cys:digestible Lysine (Lys) ratio for broilers under a lipopolysaccharide (LPS) inflammatory challenge, 384 male broilers were distributed in a completely randomized 4×2 factorial design, with four ratios of dig. Met + Cys:dig. Lys (0.69, 0.73, 0.77, and 0.81) and two conditions (with or without challenge). Each treatment had eight replicates, with six birds per experimental unit (EU). The evaluated parameters included broilers' weight gain (WG), feed intake (FI), and feed conversion ratio (FCR); jejunum mRNA transcript levels of nuclear factor kappa-B (NF-Kb), glutathione peroxidase (GPX), superoxide dismutase (SOD), glutathione synthetase (GSS), and methionine adenosyltransferase 2 (MAT2); relative weights of liver and spleen, and fat mass (%) and lean mass (%). A linear regression model would estimate the ideal ratio if an effect had occurred. No interaction (p>0.05) was observed between the factors for all the data, nor did the different ratios had any effect (p>0.05) either. LPS-administered exhibited reduced performance, heavier liver and spleen, and lower GSS expression. Hence, the lowest dig Met + Cys:dig Lys ratio (0.69) was sufficient to maintain the performance parameters, the relative weight of lymphoid organs, fat and lean mass, and NF-Kb, GPX, SOD, GSS, MAT2, and CBS mRNA transcript levels in the jejunum.

INTRODUCTION

Currently, in intensive production, broilers are subject to chronic inflammatory challenge that triggers immune responses and impairs immunological homeostasis (Zhang *et al*., 2019a) due to pathogenic microorganisms present in the production system, vaccinations with excessive dosages, and abuse or lack of chemotherapy with growth-promoting antibiotics (Liu *et al*., 2015). This challenge prevents poultry from expressing their maximum genetic and economic potential (Li *et al*., 2015).

In experimental trials on inflammation in broiler chickens, inoculation of bacterial LPS is a technique used to increase the activation of chickens' inflammatory response without submitting them to potentially pathogenic agents, while also simulating a condition closer to the one found in commercial sheds (Beutler & Rietschel, 2003; Nunes *et al*., 2020). When they are inoculated and perceived by the immune system, there is an increase in the production of the NF-Kb transcription factor that stimulates the expression and synthesis of inflammatory genes (Liu *et al*., 2022).

The sulfur amino acids (SAA) methionine (Met) and cysteine (Cys) are involved in functions that fortify broilers' immune system, including

regulation of the innate immune system, lipid metabolism, digestive functions, protein synthesis, and defense against oxidative stress (OS; Martínez *et al*., 2017). The leading cause of OS is the imbalance between the production of free radicals, such as reactive oxygen species (ROS), and antioxidant enzymes (Nawaz & Zhang, 2021). Adding high levels of SAA to feed increases the synthesis and activity of GPX and SOD (Wen *et al*., 2016), thereby decreasing the presence of free radicals that damage macromolecules.

An increase in Met transsulfuration mitigates the effects of OS and maintains the demand of Cys for cellular reduced glutathione (GSH) synthesis (Del Vesco *et al*., 2015; Bortoluzzi *et al*., 2019). Moreover, SAA participates in glutathione synthetase (GSS) gene expression, resulting in the effective synthesis of potent antioxidants that contribute to the removing system's ability of ROS to alleviate the OS effect (Lai *et al*., 2018; Lugata *et al*., 2022).

Therefore, additional SAA supplementation may improve performance by reducing OS and, consequently, the inflammatory challenge. One strategy to mitigate the adverse effects of inflammation is to increase the intake of dig SAA through the ratio dig SAA:dig lysine (Lys), such as dig Met + Cys. However, few studies have estimated the ideal dig Met + Cys to dig Lys ratio in diets for broiler chickens under inflammatory challenge.

Thus, it was hypothesized that the ideal ratio of dig Met + Cys:dig Lys in broiler diets is increased in immunologically challenged broilers. The objective of this study was therefore to estimate the ideal ratio of dig Met + Cys:dig Lys for broilers submitted or not to LPS inflammatory challenge.

MATERIALS AND METHODS

Ethics Committee

The Animal Care and Use Committee of the Federal University of Viçosa, Brazil, approved all animal procedures conducted in this study (protocol no. 70/2021). The experiment was conducted according to the experimental protocols for using live broilers from the National Council for Experimentation Animal Control (CONCEA, 2008) in the municipality of Viçosa (20°45'14" S, 42°52'53" W, altitude 648.74 m), in the state of Minas Gerais, Brazil.

Birds and experimental design

One-day-old male broiler chickens (Cobb 500) were obtained from a commercial hatchery. The poultry were reared on the floor according to lineage management recommendations until the beginning of the experiment. They had free access to water and were fed a corn/soybean meal-based standard diet in mashed form (*ad libitum*). At the thirteenth day of age, based on their body weight, a total of 384 male broilers (514 ± 51.4 g) were distributed in a 4 x 2 (four ratios of dig Met + Cys:dig Lys x with or without challenge) completely randomized factorial design, with eight replicates per treatment, and six chickens per experimental unit (EU). The birds were housed in 64 EUs consisting of wire floor cages (667 cm²/broiler) in a two-level battery equipped with a trough feeder and a nipple drinker. The temperature was maintained according to lineage management recommendations, and the broilers were exposed to 18 hours of continuous light daily during the experimental period. To simulate a repeated exposure inflammatory challenge, all birds were weighted individually so that treatments 5, 6, 7, and 8 received an intraperitoneal application of 1mg of LPS / kg of body weight at 14, 16, 18, and 20 days old. The birds from treatments 1, 2, 3, and 4 were individually weighed and received a comparable amount of saline solution (SS) at a similar location to the administration of LPS in treatments 5, 6, 7, and 8, ensuring a consistent induction of stress.

Diets

The experimental diets (Table I) were based on corn and soybean meal, and were formulated according to the nutritional recommendations by Rostagno *et al*. (2017) for the 8 to 21 days old phase, except for the levels of dig Met + Cys, which varied per treatment. The ratios of dig Met + Cys:dig Lys tested were 0.69, 0.74, 0.79, and 0.84. 95% of the recommended level of dig lysin was used. The suboptimal level aimed to ensure that the chickens consumed all dig Lys and that the ideal dig Met + Cys:dig Lys ratio was not underestimated. Samples of experimental diets were collected for analysis of crude protein and amino acids (Aas) to correct the formulas before preparing the experimental diets (Table I). All experimental diets had an essential nitrogen:total nitrogen ratio that was lower than 0.50, as recommended by Maia *et al*. (2021).

Performance and sample collection

The broilers' performance was evaluated at 21 days of age by determining the weight gain (WG), feed intake (FI), and feed conversion ratio (FCR). At 20 days, the bird with the weight closest to the average weight of the EU was selected for sample collection. Then, 24

Table 1 – Ingredients and nutrient composition of experimental diets, as fed basis.

Ingredients, g/kg				
	Ratio 0.69	Ratio 0.74	Ratio 0.79	Ratio 0.84
[3] Corn	519.30	519.30	519.30	519.30
[4] Soybean meal	406.40	406.40	406.40	406.40
Soybean oil	34.50	34.50	34.50	34.50
Dicalcium phosphate	16.30	16.30	16.30	6.30
Limestone	8.60	8.60	8.60	8.60
Salt	5.10	5.10	5.10	5.10
DL-Methionine, 990 g/kg	2.10	2.70	3.30	3.90
Starch	1.80	1.20	0.60	0.00
Mineral Premix [1]	1.30	1.30	1.30	1.30
Vitaminic Premix [2]	1.30	1.30	1.30	1.30
L-Threonine, 985 g/kg	1.00	1.00	1.00	1.00
Choline chloride, 600 g/kg	1.00	1.00	1.00	1.00
L-Triptofano, 980 g/kg	0.50	0.50	0.50	0.50
L-Lysine HCl, 780 g/kg	0.40	0.40	0.40	0.40
Total	100.00	100.00	100.00	100.00
Calculed composition				
Nutrients	Ratio 0.69	Ratio 0.74	Ratio 0.79	Ratio 0.84
Metabolisable Energy, MJ/Kg	12.77	12.77	12.77	12.77
Crude Protein, g/kg	230.00	230.00	230.00	230.00
Calcium, g/kg	8.70	8.70	8.70	8.70
Available Phosphorous, g/kg	4.10	4.10	4.10	4.10
Sodium, g/kg	2.10	2.10	2.10	2.10
Digestible Lys, g/kg	11.90	11.90	11.90	11.90
Digestible Met + Cys, g/kg	8.20	8.80	9.40	10.00
Digestible Valine, g/kg	9.30	9.30	9.30	9.30
Digestible Threonine, g/kg	8.80	8.80	8.80	8.80
Digestible Tryptophan, g/kg	2.10	2.10	2.10	2.10
Analyzed composition: liquid -chromatography of amino-acids				
Nutrients, g/kg	Ratio 0.69	Ratio 0.74	Ratio 0.79	Ratio 0.84
Crude Protein	224.10	239.30	234.0	238.30
Lys	13.20	13.50	13.50	13.70
Met + Cys	6.20	7.40	8.70	8.90
Valine	10.80	10.80	10.50	10.60
Threonine	9.60	9.60	9.40	9.80
Tryptophan	1.50	1.50	1.50	1.50

[1] Trace mineral premix provided per kg of diet: Mn, 58.36 g; Fe, 41.68 g; Zn, 54.21 g; Cu, 8.31 g; I, 0.84 g; Se, 0.25 g.

[2] Vitamin premix provided per kg of diet: vitamin A, 9,638,000 IU; vitamin D3, 2,410,000 IU; vitamin E, 36,100 IU; vitamin B1, 2.60 g; vitamin B2, 6.45 g; vitamin B6, 3.61 g; vitamin B12, 15.9 mg; vitamin K3, 1.94 g; pantothenic acid, 12.95 g; nicotinic acid, 39.20 g; folic acid, 0.90 g; biotin, 89.80 mg.

[3] Corn analyzed composition (as fed basis): 89.81% dry matter, 7.44% crude protein, 0.16% methionine, 0.11% cysteine, 0.24% lysine, 0.26% threonine, 0.33% valine, 0.07% tryptophan, 0.03% hydroxyproline, 0.31% phenylalanine, 0.77% leucine, 0.24% isoleucine, 0.24% tyrosine, 0.66% proline, 0.52% alanine, 0.36% arginine, 0.03% taurine, 0.19% histidine, 0.32% glycine, 0.34% serine, 1.27% glutamic acid, 0.50% aspartic acid, 6.69% total amino acids sum.

4 Soybean meal analyzed composition (as fed basis): 88.40% dry matter, 46.37% crude protein, 0.63% methionine, 0.70% cysteine, 2.87% lysine, 1.88% threonine, 2.19% valine, 0.35% tryptophan, 0,17% hydroxyproline, 2.34% phenylalanine, 3.43% leucine, 2.08% isoleucine, 1.71% tyrosine, 2.44% proline, 2.24% alanine, 3.43% arginine, <0.01% taurine, 1.18% histidine, 2.23% glycine, 2.48% serine, 8.45% glutamic acid, 5.41% aspartic acid, 46,21% total amino acids sum.

hours after the last application of LPS, the bird was euthanized and slaughtered. 2 cm of the jejuni were collected, stored individually in cryogenic tubes, and kept in an ultra-freezer until analyzed to measure mRNA transcript levels. At 21 days of age, when one bird per EU was slaughtered for sample collection, the leftover feed was weighed, and the feed intake per bird from 13 to 21 days of age was calculated. The total feed intake per bird and the final weight were used to determine the feed conversion ratio. The liver and spleen of the sampled broiler were removed and weighed separately to determine their relative weights. The relative weights were determined in relation to the animal's live weight (%).

Determination of mRNA transcript level

The total RNA transcript level of the samples from the jejunum was extracted using a Trizol® reagent (In-

vitrogen, Carlsbad, California, 279 USA), according to the manufacturer's instructions, in the proportion of 1 mL for each 80 mg of tissue. This process measured mRNA expression for nuclear factor kappa-B (NF-Kb; F: GTGTGAAGAAACGGGAACTG; R: GGCACG-GTTGTCATAGATGG), glutathione peroxidase (GPX) (F: GACCAACCCGCAGTACATCA; R: GAGGTGCGG-GCTTTCCTTTA), superoxide dismutase (SOD) (F: AG-GGGGTCATCCACTTCC; R: CCCATTTGTGTTGTCTC-CAA), GSS (F: GTGCCAGTTCCAGTTTTCTTATG; R: TCCCACAGTAAAGCCAAGAG) and methionine ade-nosyltransferase 2 (MAT2; F: CTTCCCAGCAGCCACTT-GAG; R: GCAGTCAAGCTGAGCGTTCC). RNA integrity was assessed on a 1% agarose gel stained with ethid-ium bromide (10 mg/mL) and visualized under ultravi-olet light. For real-time PCR, the fluorescent dye SYBR GREEN (SYBR® GREEN PCR Master Mix, Applied Bio-systems, USA) was used. The amplification conditions in the thermocycler were initially denatured at 95ºC for 10 min, followed by 40 cycles of denaturation at 95ºC for 15 seconds, and annealing at 60ºC for 1 min. The melting curves were performed to guarantee the specificity of the PCR products. The β-actin gene (F: ATTGTCCACCGCAAATGCTTC; R: AAATAAAGCCAT-GCCAATCTCGTC) was used as an endogenous con-trol. The data were generated following the 2–ΔΔCT method (Livak & Schmittgen, 2001).

DEXA estimated fat mass (%) and lean mass (%)

Chicken body composition was measured using a Prodigy Advance DEXA scanner (GE Medical Systems Ultrasound & Primary Care Diagnostics LLC 3030 Ohmeda Drive, Madison, WI, 53718, USA) with encore software version 18. A complete body scan was performed and analyzed in the small animal body mode. At the start of each scanning day, a quality assurance program was performed using a phantom standard to ensure the accurate calibration of the scanner. Eight euthanized chickens per treatment were placed in a dorsal position with spread wings and stretched legs to avoid extensive overlap of body parts. Subsequently, the lines defining the regions of interest were corrected for the body. Since these lines are fixed at specific intersections, a calculated compromise was consistently applied. Based on the attenuation of the 2 X-ray beams by different absorbing materials, the software calculated the estimated values for total tissue, lean and fat tissue. These values were obtained for the whole body region. After that, equations developed by Schallier *et al*. (2019) were used to correct the values of total fat mass [1] and total lean mass percentage [2]:

Total Body Fat Percentage = -1.288(±2.597)+0.806 (±0.159)×Fat Percentage DEXA (%) [1]

Table 2 – Growth performance, relative organ weights, mRNA transcript level and DEXA estimated fat mass and lean mass of broiler chickens at 21 days of age.

	Control					Challenged						*p*-value		
	0.69	0.74	0.79	0.84	Mean	0.69	0.74	0.79	0.84	Mean	[2] SEM	Rel	LPS	Rel x LPS
Growth performance.														
[1] WG	0.62	0.62	0.63	0.63	0.63	0.59	0.57	0.57	0.60	0.58	<0.01	0.53	<0.01	0.18
[1] FI	0.78	0.78	0.78	0.78	0.78	0.76	0.74	0.73	0.75	0.75	<0.01	0.07	<0.01	0.88
[1] FCR	1.26	1.25	1.23	1.23	1.24	1.28	1.30	1.28	1.26	1.28	<0.01	0.19	<0.01	0.47
Relative organ weights.														
Liver	2.38	2.45	2.36	2.37	2.39	2.46	2.81	2.57	2.62	2.62	0.04	0.32	<0.01	0.68
Spleen	0.09	0.09	0.08	0.08	0.08	0.12	0.12	0.12	0.12	0.12	<0.01	0.98	<0.01	0.97
mRNA transcript level of NF-κB, GPX, SOD, GSS and MAT.														
[3] NF-Kb	1.58	1.66	1.71	1.54	1.62	1.26	1.43	1.71	1.54	1.49	0.11	0.79	0.77	0.84
[3] GPX	1.26	1.29	1.84	1.23	1.41	1.29	0.97	1.15	1.10	1.13	0.10	0.63	0.19	0.68
[3] SOD	1.61	1.71	1.98	1.61	1.73	1.43	1.58	1.78	1.62	1.60	0.08	0.55	0.49	0.97
[3] GSS	0.93	0.58	0.85	0.32	0.67	0.09	-0.08	0.74	-0.02	0.18	0.12	0.21	0.04	0.69
[3] MAT2	1.59	1.92	2.07	1.50	1.77	1.28	1.41	1.73	1.53	1.49	0.09	0.36	0.14	0.78
DEXA estimated fat mass (%) and lean mass (%).														
Fat mass	7.62	8.62	8.97	9.60	8.70	8.95	8.98	9.22	9.20	9.09	0.15	0.05	0.17	0.21
Lean mass	91.71	90.57	90.16	89.57	90.50	90.24	90.20	89.96	90.37	90.19	0.16	0.10	0.31	0.08

[1] WG: weight gain (kg/Bird); FI: feed intake (kg/Bird); FCR: feed conversion ratio (kg of gain/kg of feed).

[2] Standard error of the mean.

[3] NF-Kb = nuclear factor kappa-B; GPX = glutathione peroxidase; SOD = superoxide dismutase; GSS = glutathione synthetase; MAT2 = methionine adenosyltransferase 2 beta.

Total Body Lean Percentage = 19.95(±13.47)+0.805(±0.163)×Lean Percentage DEXA (%) [2]

Statistical analyses

The statistical analysis was conducted using a 4 × 2 factorial arrangement of treatments to investigate the response of (challenged and unchallenged) broilers to 4 ratios of digestible Methionine + Cysteine:digestible Lysine in the diets. Data were analyzed using the GLM procedure of SAS 9.4 (SAS Institute Inc., Cary, NC, USA). The cage served as the experimental unit for growth performance, and one bird served as the experimental unit for determining mRNA transcript levels, relative weights of liver and spleen, and DEXA estimated fat and lean mass. Differences were considered significant at an alpha <0.05. The mean comparison method was used, and a linear regression model would estimate the ideal ratio if an effect had occurred. The assumptions of analysis of variance, regarding the normality of residuals and the homogeneity of variances, were evaluated using the Shapiro-Wilk and Hartley tests, respectively. The statistical model used for the analysis consisted of $Yijk=\mu+Ri+Cj+(R \times C)ij+\varepsilon ijk$, where *Yijk* is the measured dependent variable, μ is the overall mean, R is the effect of the different ratios, C effect of the challenge, R x C is the effect of the interaction, and εijk the random error.

RESULTS

Performance

No mortality was observed during the experiment. There was no interaction ($p>0.05$) between the inflammatory challenge and the increase of dig Met + Cys:dig Lys ratio for WG, FI, and FCR. The different ratios of dig Met + Cys:dig Lys had no significant effect ($p>0.05$) on the same data observed. However, there were significant differences ($p>0.05$) between challenged and unchallenged animals. Chickens that received the intraperitoneal application of LPS all had lower performance parameters than those that received a SS application (Table II).

Organs relative weight

There was no interaction ($p>0.05$) between the inflammatory challenge and the increase of the dig Met + Cys:dig Lys ratio on the relative weight of the liver and spleen. The different ratios of dig Met + Cys:dig Lys had no significant effect ($p>0.05$) on these same indices. However, there was a significant difference ($p<0.05$) between challenged and non-challenged animals. The chickens that received an intraperitoneal application of LPS had a heavier liver and spleen than those that received the application of SS (Table II).

mRNA transcript level

There was no interaction ($p>0.05$) between the inflammatory challenge and the increase in the dig Met + Cys:dig Lys ratio for the mRNA transcript level of NF-Kb, GPX, SOD, GSS, MAT2, and CBS in the jejunum. Furthermore, the different ratios of dig Met + Cys:dig Lys had no significant effect ($p>0.05$) on the mRNA transcript level of the same target genes. However, there was a significant difference ($p<0.05$) between challenged and non-challenged animals for GSS mRNA transcript level. Broilers that received an intraperitoneal application of LPS had lower expression than those that received SS (Table II).

DEXA estimated fat and lean mass

There was no interaction ($p>0.05$) between the inflammatory challenge and the increase of dig Met + Cys: dig Lys ratio for lean and fat mass. The different ratios of dig Met + Cys: dig Lys also had no significant effect ($p>0.05$) on the same body compositions. Furthermore, there were no significant differences ($p>0.05$) between challenged and non-challenged animals for DEXA estimated fat and lean mass (Table II).

DISCUSSION

The application of LPS is commonly used to experimentally stimulate the inflammatory response of broilers (Chen *et al*., 2020; Kreuz *et al*., 2020; Qiu *et al*., 2022). This was again confirmed by this study's results, with worsening performance, lymphoid organ hypertrophy, and mRNA transcript level of GSS. Additionally, the transsulfuration of Met into Cys increases under challenge conditions, and the amount of Met will be deficient for production. Thus, less Met will be acquired and retained in tissues linked to growth performance (Conde-Aguilera *et al*., 2013), potentially impacting the quality of prime meats, such as breast muscle (Conde-Aguilera *et al*., 2016).

This lower performance can be attributed to LPS-induced inflammation that deteriorates the intestinal barrier function (Wang *et al*., 2022a), and absorption and route change of nutrients to support homeostatic activities compensating the deficiency (Korver & Klasing, 1997; Zhang *et al*., 2013). The compromise in the use of Aas by the animal (Li *et al*., 2007; Liu *et al*., 2015) alters the nutritional requirement of these nutrients. In this study, it was expected that

changing the optimal dig Met + Cys:dig Lys ratio in broilers submitted or not to inflammatory challenge would improve growth performance. However, this hypothesis was not confirmed, possibly because the level of these Aas in the basal diet was suboptimal, as found by Savaram *et al*. (2022).

Metabolites of Aas can regulate OS and anti-inflammatory activity (Del Vesco *et al*., 2015; Zhang *et al*., 2020). This is justified by the increased liver and spleen hypertrophy with incremental levels of dietary Aas due to the rise in protein biosynthesis and liver activity (Jahanian & Khalifeh-Gholi, 2018), and by the associated enhanced immune cell replication and proliferation (Barekatain *et al*., 2019). Therefore, one strategy to mitigate the adverse effects of bacterial LPS-induced inflammation is to increase the intake of dig Aas in the diet by increasing the dig Aas:dig Lys ratio (Star *et al*., 2012; Abdaljaleel *et al*., 2018; Lisnahan *et al*., 2022) through Met + Cys addition. However, the results confirm that altering the ideal dig Met + Cys:dig Lys ratio does not reduce oxidative and inflammatory stress by altering the relative weight of the liver and spleen. Nonetheless, animals that received the application of LPS had higher relative weight of the liver and spleen than the non-challenged animals (SS application), which agrees with Lieboldt *et al*. (2016) and Wang *et al*. (2022b).

LPS can increase NF-Kb production, which stimulates the expression of inflammatory genes (Yang *et al*., 2019; Liu *et al*., 2022). Adding high levels of Met to feed can strengthen the antioxidant system by increasing SOD activity in the proximal intestine (Wang *et al*., 2019), protecting and repairing cells (Roushdy *et al*., 2018). Powerful negative radicals are formed in tissues through metabolism or cellular reactions, such as superoxide or singlet oxygen. The SOD enzyme can catalytically convert these free radicals into molecular oxygen (O2) and hydrogen peroxide (H2O2), which are less harmful. However, the exaggerated accumulation of H2O2 is toxic to tissues or cells (Ighodaro & Akinloye, 2017).

Met is a precursor of GSH (Gasparino *et al*., 2017), and its supplementation increases GPX expression (Del Vesco *et al*., 2015; Zhang *et al*., 2019b). It plays a vital role in antioxidant defense, indirectly breaking down H2O2 to water, using H2O2 as a substrate for GSH oxidation (Ighodaro & Akinloye, 2017), and stopping lipid peroxidation (Chen *et al*., 2017). Thus, GPX contributes to the protection of all cells that utilize oxidative metabolism by eliminating increased reactive species during the inflammatory process. In this study, it was expected that altering the optimal dig Met + Cys:dig Lys ratio for broilers subjected to inflammatory challenge or not would improve the mRNA transcript level of NF-Kb, GPX, and SOD in the jejunum. However, this hypothesis was not confirmed.

According to Li *et al*. (2017), SOD activity can be improved by S-adenosylmethionine, a vital methyl donor. It is synthesized from Met and adenosine triphosphate through the upregulation of MAT2 (Faraji *et al*., 2018). S-adenosylmethionine acts in methylation and biosynthesis processes of molecules that increase energy availability for protein deposition, such as the methylation of guanidinoacetic acid to form creatine (Mousavi *et al*., 2013). This study hypothesized that altering the optimal dig Met + Cys:dig Lys ratio for broilers subjected or not to inflammatory challenge would improve the mRNA transcript level of MAT2 in the jejunum. However, this was not confirmed.

Reverse transsulfuration is a Cys biosynthetic pathway characterized by the cleavage of cystathionine. It starts with CBS separating the homocysteine complex (HCy) and serine to form cystathionine (Kruger, 2017). In addition to this role, HCy can regenerate Met via remethylation (Wan *et al*., 2017). This study expected that altering the optimal dig Met + Cys:dig Lys ratio for broilers subjected or not to inflammatory challenge would improve CBS's mRNA transcript level in the jejunum. However, this hypothesis was not confirmed.

GSS is an enzyme that acts in the second step of the formation of GSH by catalyzing the reaction of dipeptide gamma-glutamylcysteine with glycine (Lu, 2013). GSH is present in the intestinal epithelium, where it can improve intestinal morphology characteristics (Song *et al*., 2018). However, when chickens go through LPS-induced inflammation, there is a lower production of GSH (Sun *et al*., 2014). Since GHS depends on the GSS enzyme activity for their formation, it can be stated that there was a reduction in the expression of GSS as well. This study predicted that the alteration of the ideal dig Met + Cys:dig Lys ratio for broilers submitted or not to inflammatory challenge would improve the mRNA transcript level of GSS. However, this hypothesis was not confirmed. On the other hand, findings show that broilers that received LPS-induced inflammation had lower mRNA transcript level of GSS in the jejunum.

SAAs stand out in the protein and lipid metabolism of poultry (Majdeddin *et al*., 2019). These Aas participate in pathways that give rise to polyamines, creatine, melatonin, and epinephrine (Zhou *et al*., 2020). In addition, SAAs participate in metabolic pathways of methyl group donation, such as choline and betaine

(Alagawany *et al*., 2022). Regarding lipid metabolism, SAAs interfere with the oxidative catabolism of fat tissue (Elsharkawy *et al*., 2021) (like carnitine biosynthesis (Ringseis *et al*., 2018) that stimulates fatty acid oxidation for better energy production), growth performance (Golrokh *et al*., 2016), and improves carcass quality, as reported by Asadi *et al*. (2016). Thus, this study hypothesized that altering the ideal dig Met + Cys:dig Lys ratio for broilers submitted or not to inflammatory challenge would change the percentage of lean and fat mass. However, results revealed that these variables were not influenced.

CONCLUSIONS

It is therefore inferred that the lowest dig Met + Cys:dig Lys ratio, that is 0.69 (1.193% dig Lys: 0.823% dig Met + Cys), was sufficient to maintain the performance parameters, the relative weight of lymphoid organs, fat and lean mass, and the NF-Kb, GPX, SOD, GSS, MAT2, and CBS mRNA contents of the jejunum.

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Author contributions

Conceptualization, SOB, MSD, LFTA and AAC; methodology, SOB, AAC, COB and RAN; software, AAC and SOB; validation, SOB, AAC and HRS; formal analysis, SOB, RAN, RDB and AAC; investigation, SOB, LPC and AAC; resources, AAC; data curation, AAC and SOB; writing—original draft preparation, SOB and JVSM; writing—review and editing, SOB, RVN and AAC; visualization, SOB and AAC; supervision, AAC nad LFTB; project administration, AAC; funding acquisition, AAC. All authors have read and agreed to the published version of the manuscript.

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Data availability statement

The data will be available upon request.

Conflicts of interest

We declare that we have no conflicts of interest.

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