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Research article

Optimal methionine: cystine ratio for performance and nitrogen balance in growing chickens fed low protein diet

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ABSTRACT: This study aimed to determine the optimal methionine:cystine (Met:Cys) ratio in diets with reduced crude protein (CP). Two trials were carried throughout14 to 28 days. In trial 1, 1200 broiler chicks were distributed under a random design of ten treatments in a 2 × 5 factors of variability arrangement with two protein levels (21.5 and 19.5 % CP) and five digestible Met:Cys ratios (42:58, 46:54, 50:50, 54:46, and 58:42). Each treatment consisted of six replicates each, with 20 birds per experimental unit. In trial 2, 36 birds were selected and randomly allocated in six treatments with three replications each to carry out a nitrogen (N) balance study. The treatments were designed in a 2 × 3 factorial arrangement with two levels of protein (21.5 and 19.5 % CP) and three Met:Cys ratios (42:58, 50:50, and 58:42). Statistical analyses were performed as two-way analysis of variance (ANOVA) with interactions and main effects compared by Tukey's test. All statistical procedures were performed using the PROC GLM, and PROC NLIN was used to fit the quadratic broken line. No interaction was observed for weight gain, but there was a significant effect of the Met:Cys $p < 0.05$. There was a significant interaction between Met:Cys and CP level for feed intake (FI) and feed conversion rate $p < 0.05$. The CP level did not affect N retention (NR) $p > 0.05$ but significantly affected N intake (N_{in}), excretion (N_{exc}), and utilization (k_N) efficiency. Therefore, an average Met:Cys ratio of 52:48 promoted a better performance response, improved in k_N, and even CP reduction.
Keywords: nitrogen excretion, protein reduction, sulfur amino acids

Introduction

The dietary protein reduction in poultry feed formulation has gained considerable attention as regards the economic, welfare, environmental, and sustainable aspects (Baker, 2009). Consequently, nutritionists have been exploring ways to reduce dietary crude protein (CP) by supplementing crystalline amino acids to precisely meet the requirements of the birds (Baker, 2009), leading to reduced N excretion (N_{exc}) (Dersjant-Li and Peisker, 2011). A recent study conducted by Lambert et al. (2023) has shown that reducing dietary CP by up to 17.7 % in grower and 16.5 % in finisher diets while maintaining an adequate level of crystalline amino acid can lead to similar or improved production performance, reduced water intake, feed conversion rate, excreted nitrogen (N_{out}), litter moisture, and reduced footpad injury.

Diets based on the corn-soybean meal are often lacking in sulfur amino acids (SAA), particularly cystine (Cys) (Ahmed and Abbas, 2011; Faridi et al., 2016). Methionine (Met) is an essential amino acid required for protein synthesis and is mainly found in lean tissue (Geltink and Pearce, 2019). On the other hand, Cys can be considered a conditionally essential amino acid as it is biosynthesized from Met and plays an important role in developing various tissues, including feathers.

Since only Met is supplemented in the diet and Cys is not, the Met requirement may differ in low CP diets. Thus, understanding the metabolic pathway of Met and considering the Cys requirement can further improve methionine:cystine (Met:Cys) recommendations (Kalinowski et al., 2003), especially in reduced CP diets. There is also a lack of information in the literature about studies showing a difference in the recommendation of Met:Cys ratio in low-protein diets. A number of recommendations suggest a Met:Cys around 53:47 (Baker et al., 1996; Pacheco et al., 2018) and 55:45 (Rostagno et al., 2017) for growing broilers. However, the values were not determined for low protein diets. Therefore, it is hypothesized that with CP reduction, it would be necessary to adjust the Met:Cys ratio to improve nitrogen retention (NR) and broiler performance.

In this study, we investigated the effect of varying the Met:Cys ratio in diets characterized by reduced CP on the performance of broiler chickens and nitrogen utilization (k_N), with the goal of providing practical recommendations for breeding chickens.

Materials and Methods

Animal ethics statement

The study was carried out in the Poultry Science Laboratory at the Faculdade de Ciências Agrárias e Veterinárias of Universidade Estadual Paulista (UNESP), located at 21°15′17″ S, 48°19′20″ W, altitude 607 m. The Comitê de Ética no Uso de Animais approved all experimental procedures and animal management were approved by under protocol number 2053/21.

Birds and management

A total of 1380 male Cobb 500 broiler chickens were obtained from a local hatchery and accommodated in a poultry house with controlled environmental conditions following management guidelines (Cobb-Vantress, 2018). The broilers were reared in pens measuring 1.4 × 3.0 m. Each pen was equipped with nipple drinkers and a single tubular feeder to secure optimal access to water and feed. The light program implemented consisted of 18L:6D h during the rearing phase. During the initial 13 days of post-hatch, all broilers received a common diet to meet or exceed the nutritional recommendations proposed by Rostagno et al. (2017). Both water and mash-type feed were available *ad libitum* for the birds. Temperature and humidity during the experiment followed those recommended by the strain manual (Cobb-Vantress, 2018).

Experimental design in the performance trial

At 14 days of age, 1200 broilers with an average body weight of 651 ± 5 g were selected. Subsequently, the chicks were assigned randomly to 60 pens with 20 birds each. The treatments were designed in a 2 × 5 factorial arrangement with two protein levels (21.5 and 19.5 % CP) and five Met:Cys ratios (42:58, 46:54, 50:50, 54:46, and 58:42), resulting in ten treatments with six replicates each. The experimental diets were formulated to meet or exceed the nutritional recommendations by management guidelines (Cobb-Vantress, 2018), except the digestible Met + Cys content, which was maintained at 0.62 % standardized ileal digestibility to ensure that these amino acids were the limiting nutrient across all experimental diets. To achieve the distinct Met:Cys ratios as expected for each treatment (Table 1), DL-methionine and L-cystine (L-Cys) were supplied in accordance with the respective treatment.

Table 1 – Ingredients and nutritional composition of the experimental diets.

	Dietary crude protein (% CP) / Met:Cys									
Ingredients, (g kg^{-1})	21.5	21.5	21.5	21.5	21.5	19.5	19.5	19.5	19.5	19.5
	42:58	46:54	50:50	54:46	58:42	42:58	46:54	50:50	54:46	58:42
Corn (7.86 %)	530.00	531.00	529.00	529.00	529.00	630.00	627.00	629.00	628.00	627.00
Soybean meal (45 %)	218.00	221.00	217.00	217.00	217.00	279.00	260.00	267.00	266.00	261.00
Peanut meal (40.75 %)	157.00	153.00	158.00	158.00	158.00	153.00	38.00	30.00	32.00	37.00
Oil	54.00	54.00	54.00	54.00	54.00	32.00	33.00	32.00	32.00	33.00
Dicalcium Phosphate	18.00	19.00	18.00	18.00	18.00	19.00	19.00	19.00	19.00	19.00
Limestone	7.90	7.90	7.90	7.90	7.90	7.80	7.80	7.80	7.80	7.80
Salt	1.90	2.00	1.90	1.90	1.90	1.90	1.90	1.90	1.90	1.90
Sodium bicarbonate	2.40	2.40	2.40	2.40	2.40	2.60	2.60	2.60	2.60	2.60
Vitamin and Mineral premix[1]	1.60	1.60	1.60	1.60	1.60	1.60	1.60	1.60	1.60	1.60
DL-Methionine (98 %)		0.20	0.50	0.70	0.90		0.20	0.50	0.70	0.90
L- Lysine (99 %)	3.80	3.70	3.80	3.80	3.80	3.60	3.80	3.70	3.70	3.80
L-Threonine (98 %)	1.30	1.30	1.30	1.30	1.30	1.40	1.50	1.40	1.40	1.50
L-Valine (99 %)	0.60	0.60	0.60	0.60	0.60	1.10	1.20	1.10	1.10	1.20
L- Cystine (100 %)	0.90	0.70	0.50	0.20		0.90	0.70	0.50	0.20	
L- Isoleucine (100 %)						0.20	0.30	0.30	0.30	0.30
Choline chloride (60 %)	1.60	1.50	1.60	1.60	1.60	1.10	1.20	1.10	1.10	1.20
Mycotoxin adsorbent	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	Nutritional composition									
Metabolizable energy, Mcal kg^{-1}	3.08	3.08	3.08	3.08	3.08	3.08	3.08	3.08	3.08	3.08
CP, %	21.5 (22.3)	21.5 (22.8)	21.5 (22.0)	21.5 (22.2)	21.5 (22.3)	19.5 (19.7)	19.5 (19.5)	19.5 (19.9)	19.5 (19.7)	19.5 (20.0)
SID Met+Cys, %	0.62 (0.62)	0.62 (0.62)	0.62 (0.62)	0.62 (0.61)	0.62 (0.60)	0.62 (0.60)	0.62 (0.59)	0.62 (0.60)	0.62 (0.60)	0.62 (0.59)
SID Methionine, %	0.26 (0.25)	0.29 (0.27)	0.31 (0.29)	0.33 (0.31)	0.36 (0.33)	0.26 (0.24)	0.29 (0.26)	0.31 (0.29)	0.33 (0.31)	0.36 (0.32)
SID Cystine, %	0.36 (0.37)	0.33 (0.34)	0.31 (0.32)	0.29 (0.29)	0.26 (0.27)	0.36 (0.36)	0.33 (0.33)	0.31 (0.31)	0.29 (0.29)	0.26 (0.27)
SID Lysine, %	1.12	1.12	1.12	1.12	1.12	1.12	1.12	1.12	1.12	1.12
SID Threonine, %	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73
SID Valine, %	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90
SID Isoleucine, %	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78
SID Met:Cys from analyzed values	(40:60)	(44:56)	(47:53)	(51:49)	(54:46)	(40:60)	(44:56)	(48:52)	(51:49)	(55:45)

[1]Content (per kg of diet) - vitamin A = 10,575 UI; vitamin D3 = 2554 UI; vitamin K = 1.80 mg; vitamin E = 14.87 mg; vitamin B1 = 2.00 mg; vitamin B2 = 4.50 mg; vitamin B6 = 2.50 mg; vitamin B12 = 2.00 mg; niacin = 30.00 mg; folic acid = 0.75 mg; calcium pantothenate = 11.74 mg; biotin = 0.01; Fe = 43.44 mg; Zn = 43.35 mg; Cu = 8.56 mg; Mn = 56.00 mg; I = 0.56 mg; Se = 0.34 mg; antioxidant 4.20 mg; salinomycin sodium 12 %; butil hidroxy toluene; adsorbente 0.001 %. Values in parentheses indicate analyzed dietary concentration of the amino acids (AMINODat 6.0). CP = crude protein; SID = standard ileal digestibility; Met:Cys = methionine to cystine rate.

Body weight was recorded at 14 days and at the end of the experimental period (28 days) to calculate body weight gain (BWG), while FI data was collected throughout the duration of the experiment. Daily mortality was recorded and used to correct the feed conversion ratio (FCR).

Experimental design in the nitrogen balance trial

The N balance trial was conducted using 36 broiler chickens selected at 9 days of age and individually housed within metabolic cages measuring 50 × 50 × 50 cm. The birds were confined to the cages for five days, from 9 to 13 days of age, and subsequently fed with the experimental diets from 14 to 28 days of age. The treatments were designed in a 2 × 3 factorial arrangement with two protein levels (21.5 and 19.5 % CP) and three Met:Cys ratios (42:58, 50:50, and 58:42), totaling six treatments with six replications each. The composition of the experimental diets was identical to that of the performance trial (Table 1).

During the experimental period, daily excreta were collected and their respective weights were recorded. The collected excreta were preserved at –4 °C in plastic bags. Subsequently, they were homogenized and dried in an airflow oven at 50 °C for 72 h. The dried diet and excreta samples were ground in preparation for subsequent laboratory analyses. Dry matter content was determined following the AOAC method 992.23 (AOAC, 2005) using an oven drying process at 105 °C for 6 h. The measurement of N content was achieved utilizing the Kjeldahl AOAC method 988.05 (AOAC, 2005).

The N balance was calculated as follows:

$$NR = N_{in} - N_{out}$$

$$N_{in} = FI \times N_{feed}$$

$$N_{out} = Exc \times N_{exc}$$

$$k_N = \frac{NR}{N_{in}}$$

In which N_{in} (g d^{-1}) is the nitrogen intake, N_{feed} and N_{exc} (%), the nitrogen content on the feed and excreta, respectively; N_{out} (g d^{-1}), the excreted nitrogen, Exc (g d^{-1}) denotes excreta production, and NR (g d^{-1}) the nitrogen retention. The efficiency of nitrogen utilization is represented by k_N.

Statistical analyses

A two-way analysis of variance (ANOVA) was performed to evaluate the main effects and interactions between protein levels and Met:Cys ratios on performance and N balance results. Using the Tukey test, significance was considered at a 5 % probability level.

A segmented model (quadratic broken-line) was used to determine the optimum Met:Cys ratio. This model expresses the performance result (BWG or FCR) as a function of the Met/Met + Cys (analyzed values according to the digestibility coefficient in AMINODat 6.0). The structure of the model is described as follows:

$$Y = \begin{cases} L + U \times (R - X)^2, X < R \\ L, X \geq R \end{cases}$$

In this model, Y is the response variables (BWG or FCR), X represents the Met/Met + Cys, L corresponds to the plateau value, U represents the slope below the point R, and R designates the optimal level of Met/Met + Cys at the breaking point.

The statistical procedures were carried out using the PROC GLM (ANOVA) and PROC NLIN (non-linear regression analysis) procedures from SAS software (Statistical Analysis System, ODA version, 2022). Statistical significance was determined at a threshold of $p < 0.05$.

Results

Performance responses

The performance outcomes of broiler chickens fed diets varying in protein levels and Met:Cys ratios are presented in Table 2. There was no interaction between protein levels and Met:Cys ratios for BWG $p > 0.08$. While evaluating the main effects, it was observed that increasing the Met:Cys ratio resulted in a better BWG $p < 0.05$ with the maximum at 54:46. The reduction in CP did not impact BWG $p > 0.12$. Interactions between protein levels and Met:Cys ratios were observed for FCR and FI $p < 0.05$. Additionally, it was observed that FCR at both the lowest and highest Met:Cys ratios (42:58 and 58:42, respectively) are particularly high in 19.50 % CP diets compared to 21.5 % CP diets. On the other hand, a reduction in $p < 0.05$ in FI was observed at the lowest Met:Cys ratio (42:58) in the standard CP diet compared to the lower CP diet.

Optimum Met/Met + Cys for the CP levels

This regression analysis was conducted for each level of CP. The quadratic broken line model fitted the data in both cases $p < 0.01$, allowing us to determine the optimum ratio (Figure 1A and B).

In diets with 21.5 % CP, the optimal BWG (1.34 kg) was obtained at a Met:Cys ratio of 51:49. In contrast, in diets with 19.5 % CP, the optimal BWG (1.31 kg) was achieved at a Met:Cys ratio of 53:47. The rate at which the BWG increased before reaching the breakpoint exhibited a similar pattern for both CP levels (approximately $U = 0.001$). The FCR improved as the Met:Cys ratio increased with a similar pattern across

Table 2 – Effect of the dietary treatments on body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) in broiler chickens from 14 to 28 days.

CP	Met:Cys	BWG	FI	FCR
%		---------------- g d^{-1} ----------------		g:g
21.50	42:58	1075	1796^{D}	1.67^{B}
	46:54	1241	1964^{BC}	1.58^{CD}
	50:50	1297	2005^{AB}	1.55^{DE}
	54:46	1338	2043^{AB}	1.53^{EF}
	58:42	1357	2011^{AB}	1.49^{F}
19.50	42:58	1108	1912^{C}	1.73^{A}
	46:54	1230	1976^{ABC}	1.61^{C}
	50:50	1259	1989^{ABC}	1.58^{CD}
	54:46	1327	2031^{AB}	1.53^{EF}
	58:42	1301	2050^{A}	1.57^{CDE}
SEM		0.013	0.011	0.009
Main factors effect				
CP	21.50	1262	1964	1.56
	19.50	1247	1992	1.60
Met:Cys	42:58	1092^{D}	1854	1.70
	46:54	1236^{C}	1970	1.59
	50:50	1278^{B}	1997	1.56
	54:46	1333^{A}	2037	1.53
	58:42	1333^{A}	2031	1.53
Probability				
CP		0.122	0.018	< 0.001
Met:Cys		< 0.001	< 0.001	< 0.001
Interaction		0.08	0.014	0.014

Different letters on the same column represent statistical difference for Tukey's test at 5 % probability level. CP = crude protein; Met:Cys = methionine to cystine rate; SEM = standard error of the mean.

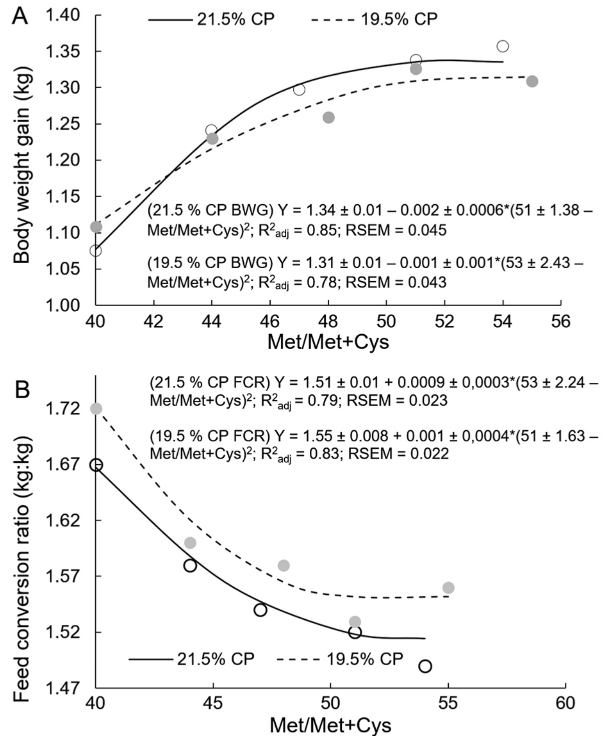

Figure 1 – Quadratic broken line model fit to A) body weight gain (BWG) and B) feed conversion ratio (FCR) in the function of Methionine to Methionine+Cystine (Met/Met + Cys) ratio for 21.5 % of crude protein (CP) (continuous line, observed values ○) and 19.5 % (dash line, observed values ●). The fit of each non-linear regression was significant (p < 0.05). R^2_{adj} = adjusted R-square; RSEM = root mean square error.

both CP levels. The rate at which FCR declined also exhibited similarity for both CP levels (approximately U = 0.001). The plateau for FCR was achieved at 1.55 in diets with 19.5 % CP and a breakpoint of Met:Cys at 51:49, whereas in diets with 21.5 % CP, the plateau for FCR was achieved at 1.51 with a breakpoint of Met:Cys at 53:47.

Nitrogen balance

The outcomes of the N balance trial are presented in Table 3. There were no interactions between CP level and Met:Cys ratios for any of the parameters evaluated at $p > 0.05$. As regards the main effects, there was an impact of CP level on N_{in}, N_{out}, and k_N $p < 0.05$. Reducing dietary CP resulted in lower N_{in} $p < 0.05$, consequently decreasing N_{out} $p < 0.05$. Interestingly, k_N was higher with the CP reduction $p > 0.05$. The reduction in dietary CP level had no impact on NR where $p > 0.05$. The ratios of Met:Cys affected both NR and k_N. The lowest Met:Cys ratio (42:58) resulted in lower NR, whereas a Met:Cys ratio of 50:50 and above had no impact on NR where $p > 0.05$. On the other hand, the highest Met:Cys ratio (58:42) exhibited a higher k_N.

Table 3 – Effect of the dietary treatments on nitrogen intake (N_{in}), excretion (N_{out}), retention (NR) and efficiency of nitrogen utilization (k_N) of broiler chickens from 14 to 28 days.

Crude protein	Met:Cys	N_{in}	N_{out}	NR	k_N
%		---------------- g d^{-1} ----------------			%
21.50	42:58	5.61	1.90	3.71	0.661
	50:50	5.92	1.89	4.03	0.680
	58:42	6.12	1.75	4.37	0.714
19.50	42:58	5.07	1.58	3.49	0.690
	50:50	5.59	1.62	3.99	0.712
	58:42	5.61	1.48	4.13	0.737
SEM		0.104	0.046	0.078	0.005
Main factors effect					
Crude protein	21.50	5.89^{a}	1.84^{a}	4.04	0.685^{b}
	19.50	5.42^{b}	1.56^{b}	3.87	0.713^{a}
Met:Cys	42:58	5.34	1.74	3.60^{B}	0.676^{B}
	50:50	5.76	1.76	4.01^{A}	0.696^{B}
	58:42	5.87	1.62	4.25^{A}	0.725^{A}
Probability					
Crude protein		0.021	0.001	0.215	< 0.001
Met:Cys		0.075	0.321	0.001	< 0.001
Interaction		0.884	0.961	0.796	0.852

Different letters on the same column represent statistical difference for Tukey's test at 5 % probability level. SEM = standard error of the mean. Met:Cys = methionine to cystine rate.

Discussion

Our findings indicate that broiler chicken performance is subject to the diet's interplay of Met:Cys ratio and CP level. Diets with high Met:Cys ratios demonstrated superior BWG and FCR outcomes, while diets with lower Met ratios adversely affected FI. These insights hold implications for formulating of broiler chicken diets aiming to optimize performance and enhance overall production efficiency. Diets formulated based solely on corn and soybean meal generally do not meet the requirements of Met + Cys, requiring supplementation with crystalline Met, particularly given the limited availability of L-Cys. Adjustment of standard ileal digestibility (SID) of Cys levels is possible with alternative ingredients, such as peanut meal (0.47 % SID Cys), which has a high concentration of this amino acid. Among these options are ingredients such as wheat gluten (1.52 % SID Cys), feather meal (ranging from 1.78 to 2.17 % SID Cys), canola (0.72 % SID Cys), cottonseed (0.54 % SID Cys), and certain algae species, such as *Spirulina platensis* (J) Leonard (1.02 % SID Cys) according to Rostagno et al. (2017). However, some of these ingredients contain antinutritional factors, and the amino acid profile needs to be well characterized. This make manipulating the Met:Cys ratio solely through ingredients challenging, requiring a careful approach in diet formulation to meet the nutritional needs of animals.

It was observed that a 2 % reduction in CP did not have an impact on broiler BWG, but there was an effect on FI and FCR associated with the Met:Cys ratios. Notably, the effect of CP reduction on FCR was particularly pronounced when birds were subjected to a reduction in the Met:Cys ratio. However, birds fed with a Met:Cys of 54:46 exhibited performance similar to those maintained on the normal CP level. Considering the recalculated Met:Cys ratio of 51:49 using the values analyzed (Table 1), the value is not far from what has been observed in other studies. For instance, some studies observed that Cys can contribute with 48 % of Met + Cys in birds from two to four weeks of age (Pacheco et al., 2018). Moreover, the optimal Met:Cys ratio determined by the quadratic broken line ranged from 51:49 to 53:47 for both performance parameters evaluated (BWG and FCR) and protein level. Interestingly, when the average is calculated the resulting value corresponds to the same Met:Cys ratio of 52:48 for the same period evaluated (Pacheco et al., 2018).

It is essential to consider that physiological changes in birds are an important factor in determining Met + Cys requirements (Wheeler and Latshaw, 1981). As the birds grow, the requirements of Met decrease, although this behavior may differ for Cys (Heger et al., 2002). Other studies have concluded that 50 % Cys is recommended for broilers from 6 to 8-week-olds, which is higher than the value we found in the younger birds (Suzuki et al., 2020). Moreover, one of the first studies suggested that Cys can satisfy needs at 52 % in birds between 3 to 6-weeks-old which is even higher than 50 % (Baker et al., 1996). Although we did not evaluate the Met:Cys ratio in older birds, there is a clear indication that age is an important factor to consider in future studies.

Another aspect to mention as regards the differences observed in the Met:Cys ratio with the bird's age when the feather is developing. Notable insights into this phenomenon emerge from studies that draw attention to the potential increase in Cys requirements during the transition from plumage to feathers (Brede, 2018). This transition during feather growth can affect the ideal Met:Cys ratio, especially compared to older and fully feathered birds. This divergence in the optimal Met:Cys ratio stems from the substantial SAA composition observed in feather proteins in turkeys that was previously described (Ferket et al., 1997). Another investigation found that a considerable 25 % of Cys and 2 % of dietary Met are exclusively channeled into the synthesis of keratin, a foundational protein of feathers (Leeson and Walsh, 2004).

There is also a key point to consider from the N balance study. A recent meta-analysis has shown that one percentage point in CP decreased daily N_{out} by 10.4 %, whereas NR was unaffected by CP (Rauglaudre et al., 2023). Similarly, in the current study, the NR was unaffected by CP, with a one percentage point reduction in CP resulting in 7.61 % N_{out}. As regards the Met:Cys, the NR was significantly improved when Met provided at least 50 % of Met + Cys, with the highest efficiency observed at 58 %. Another interesting study provided evidence supporting the metabolic priority of converting Met to Cys, regardless of supplementation of L-Cys (Pacheco et al., 2018). Therefore, a depreciation in NR would not be expected if the Met meets at least 50 % of requirements.

Our investigation described the main role of the Met:Cys ratio in influencing key N balance parameters, specifically NR and the k_N. Notably, a lower Met:Cys ratio detrimentally impacted NR, while conversely, a higher ratio exhibited improved k_N.

In conclusion, the Met:Cys ratio was independent of protein reduction level, with the metabolic dependence of the Cys synthesis from Met being a more critical factor. Thus, maintaining a higher Met:Cys ratio is crucial to ensuring better productive performance, with a recommended ratio of 52:48. This relationship was supported by improvement in the NR and k_N observed with a Met:Cys rate in excess of 50:50, even in diets with reduced CP levels.

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Authors' Contributions

Conceptualization: Fialho ATS, Dorigam JCP. **Data curation:** Fialho ATS, Riveros RL. **Formal analysis:** Fialho ATS, Riveros RL. **Funding acquisition:** Sakomura NK, Dorigam JCP. **Investigation:** Fialho ATS. **Methodology:** Fialho ATS, Riveros RL, Pacheco LG. **Project administration:** Sakomura NK, Dorigam JCP. **Resources:** Sakomura NK, Dorigam JCP. **Supervision:** Sakomura NK. **Writing – original draft:** Fialho ATS. **Writing – review & editing:** Riveros RL, Leme BB, Silva RL.

Conflict of interest

The authors declare that this study has no financial or personal conflicts of interest that could have directly or indirectly influenced the work reported.

Data availability statement

The authors confirm that the data supporting the findings of this study will be available after a formal request to the corresponding author in accordance with institutional politics.

Declaration of use of AI Technologies

This study utilized Artificial Intelligence (AI) tools exclusively for grammar and spelling checking. AI was not employed in the writing of the original draft, data interpretation, analyses, discussions or synthetic data generation.

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