





Using different statistical models to estimate the optimal methionine+cysteine to lysine ratio for male Cobb500 broilers: Growth performance, body composition, and meat yields[☆]

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ARTICLE INFO

Keywords:

Ideal protein
Sulfur amino acids
Productive performance
Poultry

SUMMARY

Methionine (Met) is a vital sulfur amino acid essential for the survival, growth, and reproduction of poultry, - one which participates on multiple metabolic processes than exclusively protein synthesis. As feed ingredients naturally contains insufficient amounts of Met and cysteine (Cys), synthetic Met sources are commonly supplemented in feeds. The proper balance of Met and Cys is important not only for protein synthesis but also for improving feed efficiency and reducing environmental impacts, such as nitrogen emissions and water consumption. This study explored the effects of different digestible methionine + cysteine to lysine (Met+Cys:Lys) ratios on male Cobb500 broiler chicken performance across three growth phases: starter, grower, and finisher. The findings revealed that as birds matured, their Met+Cys:Lys ratios initially increased and then slightly decreased. Optimizing this ratio not only enhances growth and feed efficiency but also results in leaner carcasses with higher protein and mineral content. Additionally, these adjustments provide economic benefits by lowering feed costs and producing meat with less fat, resulting in a higher-quality product. This research emphasizes the importance of adjusting amino acid ratios based on growth phase to improve both broiler performance and meat yield.

Description of problem

Methionine (Met) is a sulphur amino acid of crucial importance for poultry survival, growth, and reproduction one whose postabsorptive metabolism, unlike that of other indispensable amino acids, goes beyond simply serving as a “building block” for protein synthesis. Even when not abundant in all the proteins, Met is the initiating amino acid in the translation of mRNA into proteins in all known eukaryotic cells (Brosnan et al., 2007). Apart from its structural function, Met plays two other crucial roles in poultry, which to different extents will also reflect the efficiency with which body protein deposition and egg protein synthesis occurs. As a methyl donor, Met is involved in the synthesis of important molecules such as creatine, choline, polyamines, carnitine, melatonin, and epinephrine (Baker, 1991; Brosnan et al., 2007; Chung & Baker, 1992) and in the methylation of DNA, RNA, and histones, regulating, for

example, gene expression. When not remethylated, homocysteine from Met is consumed in irreversible trans-sulfuration reactions with the participation of serine (Ser) to form cysteine (Cys) (Ingenbleek & Kimura, 2013). The latter, in addition to being utilized for maintenance functions and body protein accretion, is also essential for antioxidant defense, taurine synthesis, and feather composition (Hosseini et al., 2012; Jong et al., 2012; Marí et al., 2009). For feathers, Cys is an amino acid of tremendous importance as its concentration in such a tissue might range between 50 and 70 g/kg protein, whereas in the feather-free body, Cys corresponds to approximately 6 to 11 g/kg protein (Gous et al., 1999 & Nogueira et al., 2021).

Because Met is irreversibly converted into Cys, the latter is considered a nonessential amino acid whose requirements are partially fulfilled by Met. Therefore, broiler requirements for both sulphur amino acids, are expressed as Met+Cys, and usually considered together when

[☆] **Primary Audience:** nutritionist, researchers, poultry producers.

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<https://doi.org/10.1016/j.japr.2025.100563>

Received 17 February 2025; Received in revised form 17 May 2025; Accepted 10 June 2025

Available online 11 June 2025

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designing feeding programs (Goulart et al., 2011). Regarding to commercial feeds, as far as broiler amino acid nutrition is concerned, Met has been universally acknowledged as the first limiting essential amino acid in feeds. Because feed ingredients usually do not provide enough Met to fulfill the sulphur amino acid needs of poultry (Rostagno et al., 2017), synthetic Met sources must be provided in feeds to optimize broiler feeding programs. Conventionally, decisions regarding the concentrations of Met + Cys to be provided in feeds have generally been made on economic grounds, considering the price of Met+Cys doses in feeds, the performance responses to such Met + Cys doses, and the price of broiler final live weight. Without accurate knowledge regarding broiler responses to different levels of Met + Cys, and the doses of both amino acids that optimize the responses of economic interest (e.g., body weight, feed conversion, average daily gain), the profit margin and revenue of the intensive broiler rearing industry could be seriously compromised (Tavárez & De Los Santos, 2016; Zhai et al., 2016). To decrease the environmental impacts of poultry meat production, crude protein content of feeds has been systematically reduced (Lemme et al., 2019), which might consequently restrict the supply of protein-bound Cys, changing the relevance of Met to meet broiler requirements for sulfur amino acids.

Literature describes the ideal SID Met+Cys:Lys ratio for broilers ranging between 66 % and 82 % (Chamruspollert et al., 2004; Cobb-vantress, 2018; FEDNA, 2008; Kalinowski et al., 2003; Millicam et al., 2021; Macelline et al., 2022; Neto et al., 2005; Neto et al., 2007; NRC, 1994; Rodrigueiro et al., 2000; Rostagno et al., 2017; Tavernari et al., 2014). This discrepancy among published findings might be attributed to several factors, which include broiler strain, growth phase, and statistical model used for the optimal ratio estimation. Poultry breeding has succeeded in improving broiler growth rates, and it might also affect the dynamics with which the body and feathers grow, the allometric relationships between tissues, and the efficiency with which amino acids such as Met and Cys are partitioned for different functions in modern broiler strains.

Given this background, we considered opportune to investigate broiler growth and carcass responses to increasing SID Met+Cys:Lys ratios in the starter, grower, and finisher phases of the male Cobb500 broiler chicken, furthermore, based on such responses, to estimate the optimal ratios for both amino acids utilizing different regression models.

Materials and methods

Ethics

Three trials were conducted at the Poultry Sector of the Animal Science Department of Universidade Federal de Viçosa (UFV) for each broiler starter, grower and finisher phase. The trials were previously approved by the Ethics Committee of Animal Production of Universidade Federal de Viçosa (CEUAP – UFV n°029/2020), and the animal handling procedures were performed in strict accordance with the committee guidelines.

Experimental diets

Ingredients utilized in feed manufacturing for all rearing phases were acquired from the same batch to avoid differences in nutrient content among experimental feeds. The crude protein and total amino acid contents of corn, soybean meal, and soy concentrate were analyzed by near infrared spectroscopy (Lemme et al., 2020) (NIRs) before feed manufacturing. The NIRs was well validated to be able to predict amino acids. The experimental diets for the starter, grower, and finisher phases were formulated based on the nutritional recommendations described by Rostagno et al. (2017), for high performance male broilers, except for Met and other essential amino acids. Standardized ileal digestible Lys was included at 94 % of the recommended levels, whereas the other essential amino acids were supplied at 110 % of the recommended levels

to ensure that Met was the first limiting amino acid in the experimental diets. For each growth phase, the SID Met+Cys:Lys ratios under study were obtained through the dilution method. The experimental diets administered in each trial were produced using a basal Met+Cys deficient-diet without DL-methionine (DLM) supplementation and a diet supplemented with DLM (MetAMINO, Evonik Operations GmbH, Essen, Germany, min. 99 % DL-methionine) in replacement of starch (high SID Met+Cys) (Table 1). Thereafter, the intermediate SID Met+Cys:Lys ratios were obtained between low- and high-SID Met+Cys diets. The total amino acid contents of the experimental diets were determined by AminoLab (Evonik Operations GmbH), and the SID amino acid content was estimated using the respective SID amino acid coefficient of Rostagno et al. (2017).

Bird husbandry and experimental design

Three trials were conducted with an experimental period of two weeks each with broilers at the starter (1 to 14 days), grower (14 to 28 days), and finisher (28 to 42 days) phases. In all the trials, birds were housed in 0.6 m × 1.5 m × 2.0 m (height × width × length) pens with new wood shavings as litter. Pens were equipped with one hanging feeder and two nipple drinkers which offered ad libitum access to water and feeds throughout the 3 trials.

In the beginning of the trial, all birds were randomly assigned to one of 6 treatments (SID Met+Cys:Lys ratios) with 7 replicates of 20 birds each (Table 2). Each pen was considered the experimental unit. For the starter phase trial, a total of 840 Cobb500 male chickens (average BW: 43.84 ± 0.03 g) were randomly distributed to treatments. The lighting consisted of 12 h of natural light and 12 h of artificial light to ensure adequate feed and water intake by the birds. In the following days, the light conditions were applied according to generic guidelines for each experimental phase. The ambient temperature ranged from 33 to 30°C throughout the entire experimental period.

For the grower phase trial, Cobb500 male chickens were reared in a conventional poultry house from 1 to 14 days of age and fed a commercial starter diet (CP:23.5 %, ME: 2983 kcal/kg of feed). After 14 days of age, 840 chickens were selected, weighed (average BW: 429.50 ± 0.001 g) and randomly distributed to treatments. The environmental temperature recorded throughout the trial ranged between 27 and 23°C.

For the finisher phase trial, Cobb500 male chickens were reared in a conventional poultry house from 1 to 28 days of age and fed commercial starter (CP:23.5 %, ME: 2983 kcal/kg of feed) and grower diets (CP:20.4 %, ME: 3098 kcal/kg of feed). After reaching 28 days of age, 840 chickens were selected, weighed (average BW: 1.54 ± 0.003 kg) and randomly distributed to treatments. The environmental temperature ranged between 23 and 20°C throughout the entire experimental period.

Growth performance

For each trial all birds and feed leftovers were weighed in the beginning and the end of the experimental period, respectively, to obtain the final body weight (FBW), average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR). The ADG was calculated as the difference between the FBW and initial body weight divided by the number of days in the experimental period. The ADFI of a bird was calculated as the difference between the total amount of feed provided and the amount of feed leftovers divided by the number of days in the experimental period. The FCR was calculated from the ratio between ADFI and ADG gain during the entire experimental period, adjusting for mortality when necessary.

Body composition and carcass yield traits

In the beginning of the experimental period, 9 birds were randomly selected at days 1, 14, and 28 of age sacrificed by cervical dislocation to determine the initial body composition of the starter, grower and

Table 1

Feed formulation of basal diet (Low SID Met+Cys) and High SID Met+Cys diet used to obtain intermediate experimental diets from starter (1 to 14 days of age), grower (14 to 28 days of age), and finisher (28 to 42 days of age) broilers.

Ingredients (%)	Starter		Grower		Finisher	
	Low SID Met+Cys	High SID Met+Cys	Low SID Met+Cys	High SID Met+Cys	Low SID Met+Cys	High SID Met+Cys
Corn	53.32	53.32	61.20	61.20	59.61	59.61
Soybean meal (46 %)	32.05	32.05	25.55	25.55	27.56	27.56
Soy Concentrate (62 %)	6.37	6.37	4.00	4.00	0	0
Starch	2.00	1.60	2.00	1.67	2.00	1.68
Soy oil	2.36	2.36	3.14	3.14	6.87	6.87
Salt	0.28	0.28	0.17	0.17	0.56	0.56
Sodium bicarbonate	0.15	0.15	0.49	0.49	0.17	0.17
Dicalcium phosphate	1.84	1.84	1.67	1.67	1.40	1.40
Limestone	0.71	0.71	0.64	0.64	0.64	0.64
DLMet (99.7 %)	0	0.40	0	0.33	0	0.32
Biolys (54.6 %)	0.12	0.12	0.22	0.22	0.26	0.26
L-Threonine	0.13	0.13	0.15	0.15	0.16	0.16
L-Valine	0.10	0.10	0.12	0.12	0.14	0.14
L-Isoleucine	0.04	0.04	0.10	0.10	0.10	0.10
L-Arginine	0	0	0.06	0.06	0.08	0.08
Glycine	0.11	0.11	0.10	0.10	0.11	0.11
Choline chloride 70 %	0.08	0.08	0.08	0.08	0.08	0.08
Mineral premix ¹	0.13	0.13	0.12	0.12	0.09	0.09
Vitamin premix ²	0.13	0.13	0.12	0.12	0.09	0.09
Coccidiostatic ³	0.06	0.06	0.06	0.06	0.06	0.06
Antioxidant ⁴	0.02	0.02	0.02	0.02	0.02	0.02
Composition ⁵						
ME, kcal/kg	2974	2974	3107	3107	3344	3344
Crude protein, %	24.20 (23.10)	24.68 (23.10)	19.70 (19.53)	19.35 (19.53)	18.35 (17.98)	18.79 (17.98)
SID Met + Cys, %	0.70 (0.60)	1.03 (1.00)	0.56 (0.52)	0.92 (0.85)	0.55 (0.48)	0.86 (0.80)
SID Met, %	0.34 (0.31)	0.67 (0.71)	0.27 (0.27)	0.63 (0.59)	0.26 (0.25)	0.56 (0.55)
SID Cys, %	0.37 (0.29)	0.36 (0.29)	0.29 (0.26)	0.29 (0.26)	0.28 (0.24)	0.29 (0.24)
SID Lys, %	1.38 (1.20)	1.34 (1.20)	1.15 (1.03)	1.14 (1.03)	1.07 (0.96)	1.10 (0.96)
SID Thr, %	1.00 (0.86)	1.00 (0.86)	0.83 (0.75)	0.83 (0.75)	0.77 (0.71)	0.80 (0.71)
SID Arg, %	1.63 (1.41)	1.58 (1.41)	1.30 (1.20)	1.29 (1.20)	1.22 (1.14)	1.27 (1.14)
SID Val, %	1.19 (1.06)	1.18 (1.06)	0.98 (0.92)	0.98 (0.92)	0.96 (0.86)	0.98 (0.86)
SID Ile, %	1.06 (0.91)	1.04 (0.91)	0.89 (0.81)	0.86 (0.81)	0.84 (0.76)	0.85 (0.76)
SID Leu, %	1.95 (1.71)	1.92 (1.71)	1.52 (1.48)	1.51 (1.48)	1.50 (1.35)	1.52 (1.35)
SID His, %	0.62 (0.57)	0.60 (0.57)	0.49 (0.46)	0.48 (0.46)	0.46 (0.42)	0.47 (0.42)
SID Phe, %	1.16 (1.01)	1.15 (1.01)	0.90 (0.84)	0.90 (0.84)	0.86 (0.77)	0.88 (0.77)
Na, %	0.16	0.16	0.21	0.21	0.23	0.23
Non-phytate P, %	0.49	0.49	0.42	0.42	0.36	0.36

¹ Composition per kg of product: manganese, 58.36 mg; zinc, 54.21 mg; iron, 41.68 mg; copper, 8.31 mg; selenium, 250 mg and iodine 843 mg.

² Composition per kg of product: vit. A, 9,637,000 UI; vit. D3, 2,409,000 UI; vit. E, 36,100 UI; vit. B1, 2.59 g; vit B2, 6.45 g; vit. B6, 3.61 g; pantothenic acid, 12.95 g; vit K3, 1.93 g; Folic acid, 903 mg; niacin, 39.20 g; vit B12, 15.9 mg; biotin, 89.8 mg.

³ Salinomycin (12 %).

⁴ Hydroxybutyltoluene – BHT.

⁵ The total amino acid contents of the experimental diets were determined by AminoLab (Evonik Operations GmbH), and the SID amino acid content was estimated using the respective SID amino acid coefficient of Rostagno et al. (2017). Inside the parentheses, the estimated nutritional values are presented based on the nutritional composition of the ingredients of Rostagno et al. (2017).

Table 2

Analyzed SID Met levels and SID Met+Cys:Lys ratios of experimental diets from starter (1 to 14 days of age), grower (14 to 28 days of age), and finisher (28 to 42 days of age) broilers used in the studies. Calculated level or ratio are presented inside the parenthesis.

Diet	Starter		Grower		Finisher		Replicates	n
	SID Met, %	SID Met+Cys:Lys, %	SID Met, %	SID Met+Cys:Lys, %	SID Met, %	SID Met+Cys:Lys, %		
1	0.37 (0.35)	53 (53)	0.31 (0.30)	53 (54)	0.31 (0.28)	56 (54)	7	140
2	0.42 (0.39)	56 (57)	0.35 (0.34)	56 (58)	0.34 (0.32)	58 (58)	7	140
3	0.47 (0.44)	61 (61)	0.40 (0.38)	62 (62)	0.40 (0.36)	63 (63)	7	140
4	0.51 (0.51)	66 (67)	0.45 (0.43)	64 (67)	0.44 (0.41)	69 (68)	7	140
5	0.60 (0.61)	74 (75)	0.49 (0.51)	68 (75)	0.49 (0.48)	72 (75)	7	140
6	0.67 (0.71)	77 (83)	0.63 (0.59)	81 (83)	0.56 (0.55)	79 (83)	7	140

finisher phases, respectively. At the end of each experimental period, one bird per experimental unit (n= 42) with the BW closest to the pens average BW was selected, weighed, and sacrificed by cervical dislocation after 12 h of fasting with ad libitum drinking water, to be further used in body composition assessments. The process was similar for starter, grower and finisher trials. After slaughter, sacrificed birds were plucked, and the feather-free body whole carcasses (containing viscera,

blood, feet and head) were stored under –20°C. Carcasses were cut using a band saw (SI-282HD, Skymesen, Santa Catarina, Brazil), ground twice with an industrial grinder (PBM081, Beccaro, São Paulo, Brazil), and homogenized. A sample was collected for lyophilization for chemical analysis at the Laboratory of Animal Nutrition of the Department of Animal Science of the UFV. One-day-old birds were also sacrificed, plucked, and the ground samples were pooled before lyophilization to

obtain three representative samples. The lyophilized samples were ground in a ball mill and used for analysis of dry matter (INCT-CA, G-003/1, 2021), crude protein Kjeldahl N \times 6.25 (INCT-CA, N-001/2, 2021), ether extract (AOCS, Am 5-04, 2009), and ash (INCT-CA, M-001/2, 2021). The average daily depositions of protein, fat, and ash were calculated as the difference between the body composition at the end and at the beginning of each experimental period and then divided by 14 (number of experimental period days) for each starter, grower and finisher trial.

Concerning the grower and finisher phases, beyond the bird selected for body composition, two more birds ($n=84$, each trial) with the BW close to the respective pen average BW were selected, weighed, and sacrificed by cervical dislocation after 12 h of fasting with ad libitum drinking water. Such birds were used to determine the yields of the carcass, such as carcass weight (CW), carcass yield (CY), viscerossomatic index (VI), breast yield with skin, drumstick yield, and thigh yield. For CW measurements, birds were bled, plucked and the hot carcasses with head, feet and viscera were weighed. The hot carcass was then eviscerated. Viscera weight was obtained from the difference between the carcass weight and the eviscerated carcass. The VI was calculated as the weight of the viscera relative to the fasting weight. The CY was calculated as the eviscerated carcass weight as a percentage of the fasting weight. The eviscerated carcass was processed to obtain the weight of the noble cuts (e.g., breast, drumstick and thighs) whose yields were calculated as percentages of the eviscerated carcass.

Statistical analysis

All the statistical analysis procedures were performed using SAS statistical software (SAS Institute, 2003). The raw data were evaluated for outliers per trial period, and significant outliers (those outside the range of the mean \pm 2 times the standard deviation) were excluded from the statistical analysis. Using this procedure, 4.8 % of the dataset was excluded as an outlier. Data from the three trials were analyzed as one-way ANOVA at the 5 % probability level. Data collected from birds fed Met supplemented feeds were subjected to polynomial contrast analysis to evaluate whether there was a linear or quadratic effect of the tested SID Met+Cys:Lys ratios on the responses assessed ($P < 0.05$). The nutritional optimal ratio of SID Met+Cys:Lys for broilers was estimated using the quadratic polynomial (QP), linear broken-line (LBL), quadratic broken-line (QBL) and exponential asymptotic (EA) models, where the growth performance, body composition, and meat yield trait parameters were regressed against the SID Met+Cys:Lys ratios (Cemin et al., 2017). The 95 % asymptotic response was used to estimate the optimal SID Met+Cys:Lys ratio for the EA model. The criterion for choosing the model that best fit the data were that one presented, in this order: the lowest value of the Akaike information criterion (AIC), the highest R^2 or the best estimated value of the SID Met+Cys:Lys optimal ratio.

Results and discussion

Effects of SID Met+Cys:Lys ratios on broiler growth performance

The performance responses of broilers in the starter, grower and finisher phases are shown in Table 3. The FBW and ADG of broilers fed Met-supplemented diets in the starter and grower phases were quadratically affected by Met+Cys:Lys ratios ($P < 0.01$). However, in the finisher phase, ($P < 0.01$) broiler FBW and ADG were linearly increased as the Met+Cys:Lys ratios increased, whereas ADFI was linearly decreased ($P = 0.01$). Regarding FCR, for broilers from starter, grower and finisher phases, a quadratic response ($P < 0.01$) was observed. Regardless of the growth phase, dietary methionine supplementation improved performance outcomes. The literature clearly demonstrates that methionine supplementation enhances growth performance (Chamrusspollert et al., 2004; Millecam et al., 2021;

Table 3

Growth performance of broilers fed graded SID Met+Cys:Lys ratios in starter (1 to 14 days of age), grower (14 to 28 days of age), and finisher (28 to 42 days of age) phases.

SID Met+Cys:Lys (%)	FBW (g)	ADG (g/day)	ADFI (g/day)	FCR (g feed/g weight gain)
<i>Starter</i>				
53	484 \pm 16.4	31.4 \pm 1.17	44.7 \pm 1.35	1.42 \pm 0.08
57	514 \pm 17.3	33.5 \pm 1.23	44.9 \pm 1.40	1.34 \pm 0.03
61	521 \pm 13.0	34.1 \pm 0.93	44.3 \pm 3.25	1.30 \pm 0.10
67	531 \pm 16.8	34.8 \pm 1.20	43.6 \pm 1.80	1.25 \pm 0.04
75	522 \pm 14.2	34.2 \pm 1.01	44.3 \pm 2.69	1.30 \pm 0.07
83	515 \pm 8.69	33.7 \pm 0.61	44.1 \pm 3.48	1.31 \pm 0.10
linear	<0.01	<0.01	0.55	<0.01
quadratic	<0.01	<0.01	0.73	<0.01
<i>Grower</i>				
54	1631 \pm 30.1	85.8 \pm 2.01	130 \pm 2.36	1.52 \pm 0.01
58	1690 \pm 32.9	90.0 \pm 2.19	132 \pm 3.43	1.47 \pm 0.02
62	1699 \pm 65.4	90.7 \pm 4.38	130 \pm 4.68	1.44 \pm 0.03
67	1741 \pm 46.2	93.7 \pm 3.15	132 \pm 5.31	1.41 \pm 0.02
75	1765 \pm 32.5	95.4 \pm 2.19	133 \pm 2.47	1.40 \pm 0.01
83	1740 \pm 26.7	93.6 \pm 1.75	130 \pm 2.65	1.39 \pm 0.01
linear	<0.01	<0.01	0.53	<0.01
quadratic	<0.01	<0.01	0.20	<0.01
<i>Finisher</i>				
54	3267 \pm 76.4	124 \pm 5.22	212 \pm 5.19	1.72 \pm 0.03
58	3324 \pm 44.5	128 \pm 3.43	210 \pm 2.86	1.65 \pm 0.03
63	3320 \pm 66.7	127 \pm 4.63	208 \pm 5.51	1.64 \pm 0.04
68	3363 \pm 49.7	130 \pm 3.46	208 \pm 4.26	1.60 \pm 0.02
75	3352 \pm 52.2	130 \pm 3.90	206 \pm 3.91	1.59 \pm 0.04
83	3362 \pm 95.3	130 \pm 6.78	206 \pm 8.55	1.58 \pm 0.05
linear	<0.01	<0.01	0.01	<0.01
quadratic	0.25	0.25	0.54	<0.01

FBW = final body weight; ADG = average daily weight gain; ADFI = average daily feed intake; FCR = feed conversion ratio.

Values are presented as means \pm standard deviation.

Pokoo-Aikins et al., 2021; Rehman et al., 2019; Wen et al., 2017)

Overall, the results from the current study support published literature on broiler in starter (Çenesiz et al., 2022; Goulart et al., 2011; Macelline et al., 2022; Neto et al., 2005), grower (Agostini et al., 2016; Çenesiz et al., 2022; Goulart et al., 2011; Macelline et al., 2022; Rodrigueiro et al., 2000; Tavernari et al., 2014), and finisher (Çenesiz et al., 2022; Goulart et al., 2011; Neto et al., 2007; Tavernari et al., 2014) phases. Nonetheless, differing from this study, for ADG of grower phase broilers, an ascending linear response was observed in the research of Neto et al. (2005). In addition, in finisher broilers, several authors reported a quadratic response for ADG (Rodrigueiro et al., 2000; Goulart et al., 2011), and Kalinowski et al. (2003) reported linear improvements in FCR. The differences among studies may be related to the length and number of phases investigated in the referred trials, the Met source supplemented in diets, the dietary Met concentration under study, and bird sex and genotype.

SID Met+Cys:Lys ratios alter body composition and broiler meat yields

Methionine supplementation does not only affect the broiler growth performance but also alters their body composition. To the best of our knowledge, there is no published research about the optimal SID Met+Cys:Lys ratios required to optimize body composition traits of broilers (feather-free body, containing head, feet, and viscera) in all growing phases.

The carcass body composition and broiler meat yields results are shown in Table 4. Regarding carcass body composition, in the starter phase, a quadratic response ($P < 0.01$) was observed for average daily protein deposition (ADPD). For the grower phase, an ascending linear response was observed for the ADPD ($P < 0.01$) of birds fed Met-supplemented diets, and a quadratic response ($P < 0.01$) of average daily ash deposition (ADAD) was observed. The average daily fat deposition (ADFD) of birds in the finisher phase decreased linearly ($P < 0.01$).

A graded increase in dietary SID Met+Cys:Lys ratios increased the ADPD in broiler carcasses in the starter and grower phases. Similar results were observed in eviscerated carcasses, however, for broilers in the starter (Neto et al., 2005) and finisher (Rodríguez et al., 2000) phases. Neto et al. (2005) observed a quadratic response for protein deposition in starter broilers. In animals, Met is an essential sulfur amino acid for protein synthesis and influences lipid metabolism via carnitine synthesis and hormone-sensitive lipase activity (Zhan et al., 2006). Most studies have evaluated the influence of Met on the abdominal fat of poultry (Çenesiz et al., 2022; Goulart et al., 2011; Kalinowski et al., 2003; Neto et al., 2007; Wu et al., 2019; Zhai et al., 2016). Nevertheless, this technique results in a greater coefficient of variation due to biases beyond not representing the whole body. Hence, chemical analysis methods for investigating lipid deposition in broiler bodies can generate more reliable results.

In the present study, a reduction in lipid deposition was observed

with increasing ratios of SID Met+Cys:Lys in broilers in the grower (trend, $P = 0.09$) and finisher phases. This finding is relevant for the poultry industry and for consumers seeking meat products with better nutritional quality. The protein deposition to lipid deposition (PD:LD) ratio of grower broilers fed 54 % SID Met+Cys:Lys (105 %) compared to broilers fed 83 % SID Met+Cys:Lys (149 %) increased 1.41-fold, while in finisher broilers, a 1.47-fold increase was observed. In grower phase of broilers, an increase in ADAD was observed as the ratios of SID Met+Cys:Lys increased, which may be related to increased growth performance, with the mineral content being deposited in bone and muscle tissue to a greater extent.

During the grower and finisher phases, the CWs of the broilers fed SID Met+Cys:Lys ratios from the Met-supplemented diets showed a quadratic response ($P < 0.01$). Broilers in the grower phase fed increasing SID Met+Cys:Lys ratios showed an increasing linear response for CY and breast yield ($P < 0.01$), whereas for VI, drumstick and thigh yields, a decreasing linear response was observed ($P < 0.01$). Regarding the finisher phase, a quadratic response ($P < 0.01$) was observed for CY and thigh yield. Broilers fed Met-supplemented diets showed greater CW and CY in the grower and finisher phases. Literature has extensively shown that Met supplementation improves carcass weight and yield (Çenesiz et al., 2022; Millicam et al., 2021; Pokoo-Aikins et al., 2021; Rehman et al., 2019; Rodríguez et al., 2000; Zhai et al., 2016). In fact, the improvements observed herein in growth performance were expected to be reflected in the broiler carcasses.

The yields of noble cuts from the breast, drumstick and thighs were also influenced by the SID Met+Cys:Lys ratios under study in grower broilers. While the breast yield increased in response to the ratios, the drumstick and thigh proportionally decreased, as observed by some researchers (Çenesiz et al., 2022; Millicam et al., 2021; Zhai et al., 2016). In finisher broilers, only thigh yield was influenced by dietary Met supplementation. These responses might be associated with the higher CW, and similar findings were observed in geese (Ashour et al.,

Table 4

Carcass body composition and yields of broilers fed graded SID Met+Cys:Lys ratios in starter (1 to 14 days of age), grower (14 to 28 days of age), and finisher (28 to 42 days of age) phases.

SID Met+Cys:Lys (%)	Carcass body composition			Yield traits					
	ADPD (g/day)	ADFD (g/day)	ADAD (g/day)	CW (g)	CY (%)	VI (%)	Breast (%)	Drumstick (%)	Thigh (%)
<i>Starter</i>									
53	4.99 ± 0.44	2.70 ± 0.31	1.04 ± 0.10						
57	5.40 ± 0.39	2.41 ± 0.15	1.11 ± 0.17						
61	5.61 ± 0.12	3.00 ± 0.42	1.16 ± 0.06						
67	5.48 ± 0.33	2.41 ± 0.18	1.02 ± 0.05						
75	5.51 ± 0.46	2.77 ± 0.67	1.02 ± 0.06						
83	5.14 ± 0.35	2.65 ± 0.45	1.03 ± 0.13						
linear	0.38	0.85	0.27						
quadratic	<0.01	0.95	0.24						
<i>Grower</i>									
54	12.0 ± 1.11	11.4 ± 1.01	2.02 ± 0.22	1472 ± 26.7	80.5 ± 0.78	11.6 ± 0.54	28.3 ± 1.42	14.3 ± 0.90	12.6 ± 0.68
58	13.1 ± 0.21	10.6 ± 1.60	2.01 ± 0.09	1537 ± 53.0	81.8 ± 1.64	12.4 ± 1.21	29.8 ± 1.54	13.0 ± 0.35	11.7 ± 0.54
62	13.8 ± 0.72	11.1 ± 0.49	2.39 ± 0.24	1549 ± 58.0	81.6 ± 1.10	11.1 ± 0.90	30.1 ± 1.73	13.3 ± 0.83	11.9 ± 0.85
67	13.8 ± 1.37	9.76 ± 1.79	2.41 ± 0.29	1552 ± 14.5	81.4 ± 0.98	11.1 ± 0.38	31.4 ± 0.32	13.2 ± 0.77	11.6 ± 0.48
75	14.3 ± 0.34	10.3 ± 1.97	2.28 ± 0.33	1565 ± 34.1	81.9 ± 0.92	10.3 ± 0.51	33.4 ± 1.36	13.1 ± 0.33	11.5 ± 0.22
83	14.8 ± 1.18	9.94 ± 0.68	2.18 ± 0.35	1558 ± 20.7	82.1 ± 0.53	10.5 ± 0.49	33.2 ± 0.92	12.8 ± 0.73	11.3 ± 0.55
linear	<0.01	0.09	0.12	<0.01	0.03	<0.01	<0.01	<0.01	<0.01
quadratic	0.28	0.66	0.02	0.02	0.47	0.92	0.75	0.17	0.25
<i>Finisher</i>									
54	16.2 ± 0.95	22.4 ± 3.14	2.91 ± 0.61	2911 ± 55.6	83.2 ± 0.91	9.24 ± 0.45	33.9 ± 1.15	13.2 ± 0.61	12.0 ± 0.60
58	18.4 ± 0.80	18.8 ± 0.89	2.43 ± 0.13	3005 ± 43.7	84.2 ± 0.51	8.67 ± 0.46	34.9 ± 1.04	13.3 ± 0.37	11.4 ± 0.45
63	17.2 ± 1.32	17.3 ± 3.34	2.82 ± 0.21	3007 ± 69.1	83.5 ± 0.49	9.11 ± 0.39	34.4 ± 0.94	13.0 ± 0.55	11.6 ± 0.26
68	17.0 ± 1.03	18.4 ± 4.11	2.77 ± 0.97	3030 ± 104	84.0 ± 0.74	8.71 ± 0.65	35.0 ± 1.24	13.5 ± 0.45	11.1 ± 0.35
75	17.3 ± 1.53	17.0 ± 1.15	3.18 ± 0.73	3030 ± 68.7	83.8 ± 0.62	8.60 ± 0.37	35.2 ± 1.27	13.3 ± 0.58	11.1 ± 0.50
83	18.5 ± 3.00	17.4 ± 1.91	2.66 ± 0.58	3020 ± 74.2	83.5 ± 0.39	9.01 ± 0.33	34.7 ± 1.03	13.5 ± 0.34	11.4 ± 0.36
linear	0.15	0.02	0.64	<0.01	0.68	0.25	0.22	0.22	<0.01
quadratic	0.91	0.11	0.95	0.02	0.04	0.12	0.37	0.48	0.03

ADPD = average daily protein deposition; ADFD = average daily fat deposition; ADAD = average daily ash deposition; CW = carcass weight; CY = carcass yield; VI = viscerosomatic index.

Values are presented as means ± standard deviation.

2020).

Different models estimate the SID Met+Cys:Lys ratio of broilers

The statistical models used to estimate the SID Met+Cys:Lys broiler optimal ratios include QP, LBL, QBL, and EA (Table 5). The model that fulfilled the following criterion was chosen: lowest value of the AIC, highest R² or the best estimated value of the SID Met+Cys:Lys optimal ratio. The SID Met+Cys:Lys ratio (Fig. 1) that optimized FBW and ADG of starter broilers were estimated using the LBL model that estimated optimum responses at 58 % SID Met+Cys:Lys. Conversely, the SID Met+Cys:Lys ratio that optimized the FBW and ADG of grower broilers were estimated at 76 % by the QP model. The optimal ratio for FCR of broilers from the starter phase was estimated by the QP model as 71 % SID Met+Cys:Lys. The EA model better fit the FCR responses in the grower and finisher phases, estimating ideal SID Met+Cys:Lys ratio as were 81 and 77 %, respectively.

Usually, the ideal SID Met+Cys:Lys ratio to optimize FCR has been shown to be greater than those estimated for ADG (Chamrupollert et al., 2004; Neto et al., 2005; Tavernari et al., 2014).

For broilers during the starter phase (0-14d of age), particularly, there are few references in the literature regarding ideal SID Met+Cys:Lys ratios. However, the recommendations of Cobb-Vantress (2018); FEDNA (2008) and Rostagno et al. (2017) are 74 %, 73 % and 74 %, respectively, for this growth phase. In the last revised edition of the Nutrient Requirements of Poultry, the NRC Poultry Subcommittee describes broiler requirements from 1 to 21d of age for Met and Lys as 0.90 and 1.10 %, respectively, which corresponds to a Met+Cys:Lys ratio of approximately 82 % NRC (1994). The recommendation is higher than the ideal ratio estimated in the current study for ADG. The reasons underlying the differences could be the age of birds (14d vs. 21d age), and the genotype of birds reared at the time of NRC publication year, whose efficiency of nutrient utilization is far different from broiler strains currently reared. Moreover, Baker & Han (1994) found 75 % SID Met+Cys:Lys for birds during the first three weeks posthatching, and Goulart et al. (2011) reported a 71 % SID Met+Cys:Lys ratio for ADG of broilers until 7 days of age, a similar ratio to what we found for FCR. Our findings suggest that modern broiler strains in the starter phase might be more efficient in utilizing Met+Cys for growth. Nonetheless, to confirm such hypothesis, further investigations about the effect of different SID

Met+Cys:Lys ratios on the metabolism of early-stage broilers are necessary.

For grower broilers, the optimal SID Met+Cys:Lys ratio estimated for ADG in the current study is similar to that reported by other authors (Goulart et al., 2011; Macelline et al., 2022) but higher than that reported by others: 66 % (Chamrupollert et al., 2004), 72 % (Neto et al., 2005), and 75 % (Mack et al., 1999; Tavernari et al., 2014). Nonetheless, for the FCR, the estimated SID Met+Cys:Lys ratio in this study is far greater than the values reported in the literature: 69 % (Chamrupollert et al., 2004), 74 % (Neto et al., 2005), and 76 % (FEDNA, 2008; Macelline et al., 2022). This highlights the importance of constantly investigating broiler requirements for proper recommendations. The SID Met+Cys:Lys recommendations for the growth performance of grower broilers from Rostagno et al. (2017) and FEDNA (2008) is 74 %, and that from Cobb-vantress (2018) and NRC (1994) is 77 %, which is close to the optimal SID Met+Cys:Lys ratio estimated for ADG in this study.

For the finisher phase, only the FCR observed results for the optimal SID Met+Cys:Lys was in accordance with the recommendations of Neto et al. (2007) and Tavernari et al. (2014). The estimated values in this study are close to those of the FEDNA (2008) (76 %) and Cobb-vantress (2018) (78 %) but higher than those of the Rostagno et al. (2017) (74 %) and NRC (1994) (72 %) recommendations. In regard to ADG, the stable results observed in finisher birds fed diets with 68 to 83 % SID Met+Cys:Lys ratios indicate that additional Met supplementation could not increase ADG further if the birds had already approached nutritional adequacy, and 54 % of the SID Met+Cys:Lys starting range was not sufficient to promote different stages of growth response for a descriptive response curve for assessment of the optimal level (Baker, 1986; Pacheco et al., 2018).

The optimal SID Met+Cys:Lys ratios for body composition and meat yields are shown in Fig. 2. For the starter phase, the SID Met+Cys:Lys ratio of 69 % for optimal ADPD was estimated by the QP model. This SID Met+Cys:Lys optimal ratio for ADPD in starter broilers was lower than the 74 % reported by Neto et al. (2005). However, the age of the birds used in the referred study ranged from 1 to 21 days, which may explain this discrepancy. For the grower phase, the QP model better fit the ADAD responses, and estimated the ideal SID Met+Cys:Lys ratio as 70 %, while for the CW, the EA estimated the ideal ratio as 63 %. Regarding the finisher phase, the CW was optimized at 61 % SID Met+Cys:Lys ratio by the EA model, and the CY at 58 % SID Met+Cys:Lys by the LBL model.

Table 5

Models used for SID Met+Cys:Lys optimal ratios estimation of growth performance, carcace composition and yields parameters for starter (1 to 14 days of age), grower (14 to 28 days of age), and finisher (28 to 42 days of age) broilers.

Parameter	QP			LBL			QBL			EA		
	Optimal ratio	AIC	R ²	Optimal ratio	AIC	R ²	Optimal ratio	AIC	R ²	Optimal ratio	AIC	R ²
<i>Starter</i>												
FBW	72	336.4	0.49	58	327.1	0.48	61	327.1	0.48	61	327.2	0.47
ADG	71	124.2	0.49	58	121.3	0.47	61	121.3	0.47	61	121.3	0.47
FCR	71	-91.4	0.35	60	-88	0.33	64	-88.1	0.33	60	-87.7	0.32
ADPD	69	35.7	0.25	57	39.7	0.17	58	39.7	0.17	56	39.7	0.17
<i>Grower</i>												
FBW	76	413.9	0.52	69	415.4	0.50	75	414.6	0.51	77	414.9	0.50
ADG	76	198.6	0.56	67	200.4	0.53	75	199.1	0.54	77	199.5	0.54
FCR	76	-190.5	0.81	66	-190	0.81	75	-194.5	0.83	81	-195	0.83
ADAD	70	139.4	0.19	-	-	-	-	-	-	-	-	-
CW	74	391.4	0.36	59	387.5	0.42	61	387.5	0.42	63	387.3	0.43
<i>Finisher</i>												
FCR	77	-141.6	0.62	61	-136.8	0.51	75	-143.5	0.59	77	-144.2	0.59
CW	73	482.2	0.23	59	480.2	0.27	61	480.2	0.27	61	480.1	0.27
CY	-	-	-	58	89.0	0.12	58	89.0	0.12	55	89.1	0.12
Thigh	73	54.3	0.27	59	56.4	0.24	61	56.4	0.24	67	55.8	0.25

QP = Quadratic Polynomial; LBL = Linear Broken-Line; QBL = Quadratic Broken-Line; EA = Exponential Asymptotic; FBW = final body weight; ADG = average daily weight gain; FCR = feed conversion ratio; ADPD = average daily protein deposition; ADAD = average daily ash deposition; CW = carcass weight; CY = carcass yield. AIC = Akaike information criterion.

Bold values represent the model chosen, based on following order criterion: lowest value of the AIC, highest R² or best value of SID Met+Cys:Lys optimal ratio estimated.

If model fit were not accepted ($P > 0.05$), no values are given in the table and “-” is displayed.

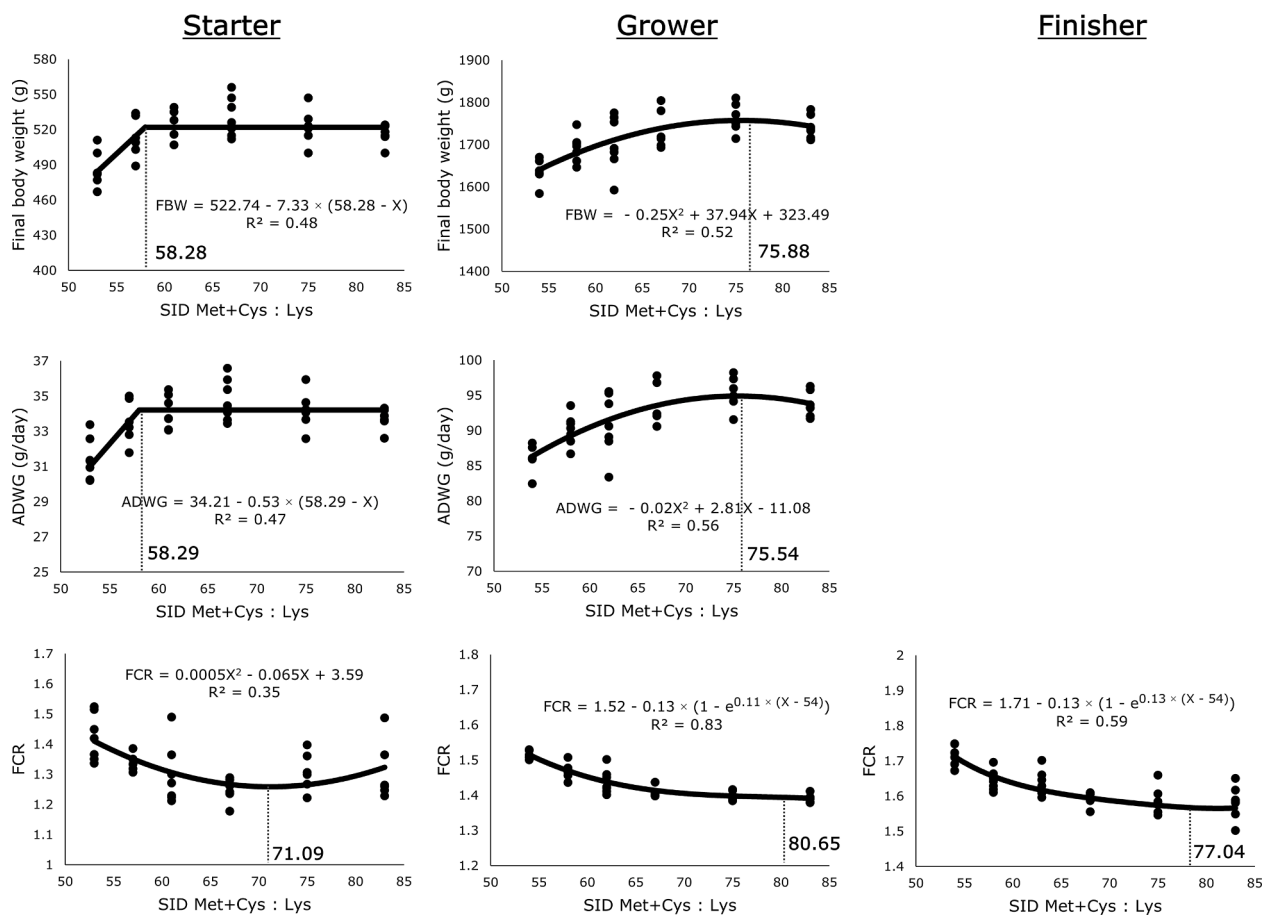


Fig. 1. SID Met+Cys:Lys ratio for growth performance of starter (1 to 14 days of age), grower (14 to 28 days of age), and finisher (28 to 42 days of age) broilers. FBW = final body weight; ADG = average daily weight gain; FCR = feed conversion ratio. Optimal SID Met+Cys:Lys ratios are shown in every graphic by a vertical pointed line.

High yield data fit better in the QP model with 73 % SID Met+Cys:Lys as the ideal ratio. To the best of our knowledge, this is the first study to identify a the ideal SID Met+Cys:Lys ratio for ADAD in broilers. More studies about the effects of SID Met+Cys:Lys ratios on the carcass body composition of broilers are needed since most published literature have massively focused on growth performance and meat yield. The SID Met+Cys:Lys ratio for CW was 63 % and 61 % for grower and finisher broilers, respectively, while for the CY of finisher broilers, the ideal SID Met+Cys:Lys ratio was 58 %, which is lower than the 73 % estimated by [Rodrigueiro et al. \(2000\)](#).

The variations in SID Met+Cys:Lys optimal ratios observed among the works for all variables discussed in this study can be attributed to the statistical model used for estimation. Most studies use the QP model to estimate the optimal nutrient levels for broilers ([Goulart et al., 2011](#); [Macelline et al., 2022](#); [Neto et al., 2005, 2007](#); [Rodrigueiro et al., 2000](#); [Tavernari et al., 2014](#)). However, one single model might not necessarily describe with the same accuracy all the responses to increasing doses of a given nutrient ([Cemin et al., 2017](#)) due to variations in tissue deposition and body composition during growth. In this sense, the results of experiments should dictate the model choice ([Cemin et al., 2017](#); [Pesti et al., 2009](#)). In the current study, we observed that for the responses assessed, the model used to estimate the ideal SID Met+Cys:Lys ratios varies according to the growth phase. Additionally, comparisons with previous research results should consider differences in the conditions under which responses were collected such as strain, sex, age, diet formulation, statistical model, and method (factorial vs. empirical) ([Cemin et al., 2017](#)).

In summary, the ideal Met+Cys:Lys ratios estimated herein suggest a

dynamism in nutrient utilization as birds grow as the optimal ratios increased to a certain extent, and then slightly decreased close to slaughter age. Higher SID Met+Cys:Lys ratios were estimated in the grower phase, which may likely be related to the growth of feathers since the feather growth rate reaches a peak between d 14- and 28 post hatch ([Pacheco et al., 2018](#)). As protein accretion rates increase in feathers, increased amino acid requirements, particularly Cys, are expected during this period ([Pacheco et al., 2018](#)). In the animal body, Met is transformed into Cys by a nonreversible process in the homocysteine cycle. As previously mentioned, the body composition of broilers as birds grow, and Met- supplemented diets with supply of Met+Cys closer to bird requirements elicited lower lipid deposition in carcasses, but higher concentrations of protein and mineral.

Conclusions and applications

1. In conclusion, diets formulated with ideal SID Met+Cys:Lys ratios optimize performance and carcass traits.
2. The SID Met+Cys:Lys ratios for starter broilers were estimated in 58 % for FBW and ADG, 71 % for FCR, and 69 % for ADPD. For grower broilers, the ideal ratios were 76 % for FBW and ADG, 81 % for FCR, 70 % for ADAD, and 63 % for CW. For the finisher phase, the ideal ratios were 77 % for FCR, 61 % for CW, and 58 % for CY. These Met+Cys:Lys ratios were found for Cobb500 males which can be different than other genetic lines.
3. The estimated optimal levels for broiler performance parameters significantly depend on the statistical model used, the analyzed parameter, and the production phase. This reinforces the need to

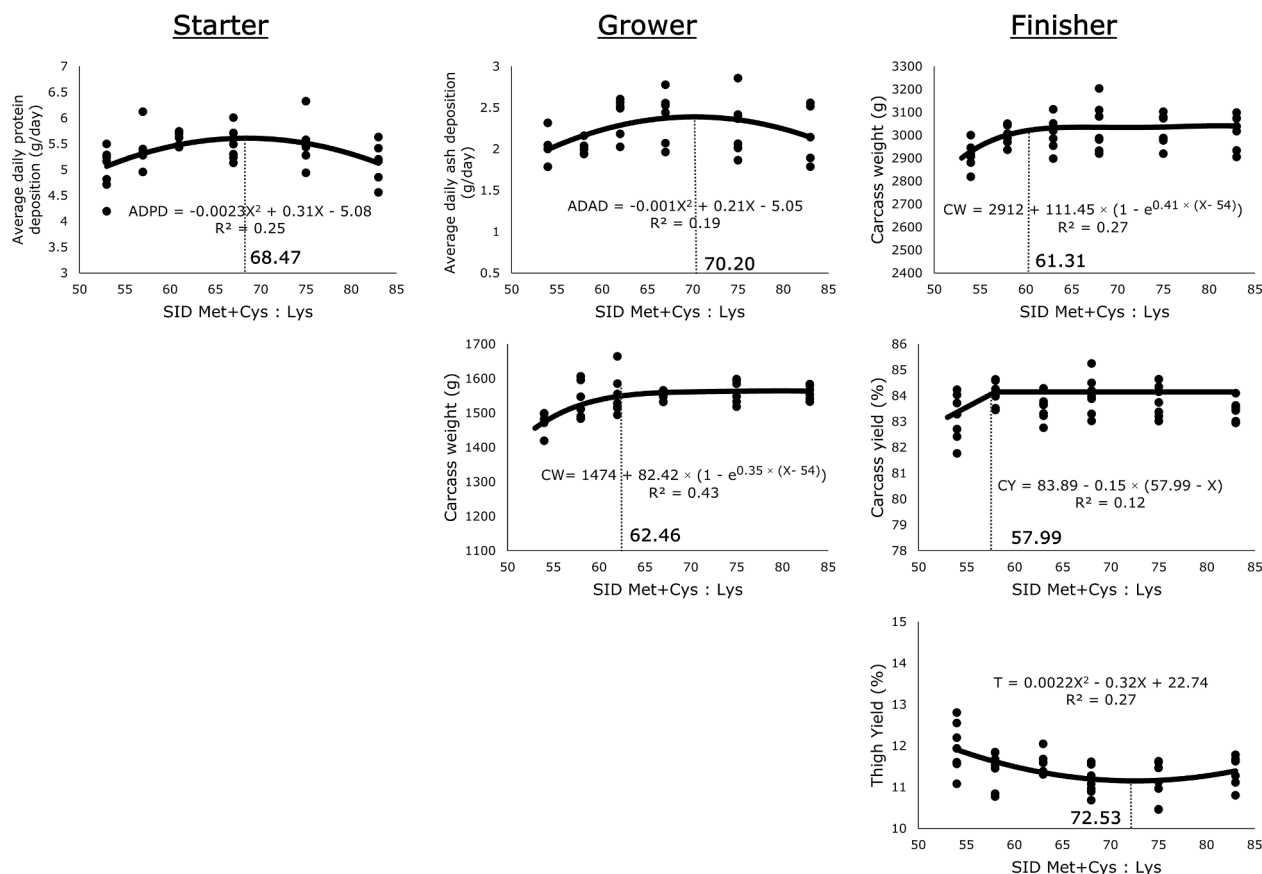


Fig. 2. SID Met+Cys:Lys ratios for carcass body composition and yield of starter (1 to 14 days of age), grower (14 to 28 days of age), and finisher (28 to 42 days of age) broilers. ADPD = average daily protein deposition; ADAD = average daily ash deposition; CW = carcass weight; CY = carcass yield; T = thigh yield. Optimal SID Met+Cys:Lys ratios are shown in every graphic by a vertical pointed line.

consider the specificity of each context when defining nutritional recommendations.

4. Met supplementation produces improved broiler carcass traits, by decreasing fat deposition and increasing protein and mineral deposition.
5. Further research is needed to explore the impact of different SID Met+Cys:Lys ratios on the metabolism and growth of broilers in their early stage of life.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Victor D. Naranjo reports financial support was provided by Evonik Operations GmbH. V. D. N. is an employee of Evonik GmbH. All authors declare that they have no known competing financial interests, and the funding source had no influence on this study design, data analysis and article interpretation.

Acknowledgments

We thank Evonik GmbH for donating the amino acids and financial support. We also thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) [n° 465377/2014-9], Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG) [n° 6.36/2021], Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) [n° 88887.510984/2020-00, n° 88887.844747/2023-0], and Instituto Nacional de Ciência e Tecnologia de Ciência Animal (INCT-CA) [n° 465377/2014-9] for the support.

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