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The optimal concentration of digestible methionine plus cysteine for broiler chicken performance determined by statistical models

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ABSTRACT - The objective of this study was to determine the optimal concentration of digestible methionine plus cystine (Met+Cys) for broiler chickens. We allocated 1,296 male broiler chickens in a completely randomized design with six treatments and nine replicates of 24 broiler chickens each. From 1 to 21 days old, broiler chickens were fed a basal diet. At day 21, the experimental diets were offered, which consisted of a basal diet (with no inclusion of synthetic methionine) and five diets supplemented with increasing concentrations of DL-methionine, which supplied 7.70, 8.29, 8.89, 9.48, 10.07, and 10.67 g/kg Met+Cys in the grower phase (21 to 35 d); and 6.92, 7.41, 7.90, 8.38, 8.87, and 9.36 g/kg Met+Cys in the finisher phase (36 to 42 d). Feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR), methionine intake, efficiency of methionine utilization, carcass yield, breast yield, and abdominal fat percentage were recorded. The quadratic polynomial (QP) regression, segmented, linear response plateau (LRP), and broken-line regression (BL) statistics were used to calculate the optimal Met+Cys concentrations for BWG and FCR. In the grower phase, FI was not affected by the increasing concentrations of Met+Cys, but it was linearly reduced during the finisher period ($P<0.05$). Greater Met+Cys concentrations positively affected the other analyzed variables in every phase ($P<0.05$). During the grower phase, the QP and LRP models were better fit to the BWG data, respectively indicating 10.30 and 9.10 g/kg optimal Met+Cys concentrations, whereas the QP model estimated 10.03 g/kg Met+Cys for optimal FCR. For the finisher phase, LRP was a better fit to BWG and FCR data, respectively estimating 8.20 g/kg optimal Met+Cys concentrations.

Keywords: digestible Met+Cys, optimal concentration, performance, statistical model

1. Introduction

Methionine is an essential amino acid (AA) for poultry and is involved in important functions (Jankowski et al., 2014; Wen et al., 2014), serving as the first limiting AA and a precursor to cysteine. When methionine is properly supplied, cystine (oxidized form of cysteine) is considered a nonessential AA for poultry (Goulart et al., 2011), hence the requirements of both are expressed as methionine plus cystine (Met+Cys). Meeting the precise nutritional requirements of Met+Cys is challenging, so nutritionists can use statistical models to predict optimal levels.

A greater diversity is reported regarding the requirements of Met+Cys for broiler chickens in grower and finisher phases. According to the Cobb 500 nutritional specifications (Cobb-Vantress, 2022), the recommendations of total sulfur amino acids (TSAA) vary in a range of 6.60 to 9.40 g/kg. The values recently updated by Rostagno et al. (2024), suggested for broiler chickens with high performance, the optimal digestible TSAA concentrations (g/kg) of 9.98 (0 to 8 d of age), 9.74 (8 to 17 d), 9.67 (17 to 27 d), 9.14 (27 to 35 d), and 9.14 (35 to 43 d). In comparison with NRC (1994), this range is even more pronounced, whereby the requirements listed are 9.0 g/kg (0 to three weeks of age), 7.2 g/kg (three to five weeks), and 6.2 g/kg (six to eight weeks).

Quadratic polynomial regression (QP) is often used because it is easier to execute and interpret the results (Nunes et al., 2004); it is influenced by the number of tested doses in optimal concentration evaluation, requiring at least three concentrations per dose, and is sensitive to concentration variations, estimating the range between tested doses (Kaps and Lamberson, 2004; Souza et al., 2014). The QP model assumes equal effects on growth performance when a limiting nutrient is added, suggesting that both reduction and gain happen at the same intensity. However, a severe drop in performance is observed when the nutrient level is high enough to be toxic (Siqueira et al., 2009).

The broken line regression (BL) is used when the ideal protein concept is adopted, i.e., with lower AA requirements (Mack et al., 1999; Deponti et al., 2007), thus reducing nitrogen excretion and avoiding the diversion of AA for energy production (Emmert and Baker, 1997). The BL model identifies the optimal nutrient dose as the point with minimum statistical error. The animal response increases until it reaches the breakpoint, beyond which no further gains are observed (Pack et al., 2003). However, past this breakpoint, the animal may have additional performance gains even with larger statistical errors. Thus, the optimal concentration suggested by this model, may not be the one that maximizes performance.

The linear response plateau (LRP) determines the optimal concentrations due to its simple outcome interpretation (Oviedo-Rondón and Waldroup, 2002; Pack et al., 2003). The LRP model implies that when the concentration of limiting nutrients grows, the animal response will reach a maximum point, beyond which the additional dose will not affect the response. This breakpoint is controversial and may lead to incorrect interpretations and underestimations of optimal nutrient levels (Rezende et al., 2007).

The BL and LRP models are discontinuous; after the breakpoint, the BL model forms a straight line, curve, or plateau (Portz et al., 2000), and the LRP model forms a plateau (Sakomura and Rostagno, 2007), so different models can lead to distinguished interpretations and recommendations. Therefore, the objective of this study was to determine concentrations of digestible Met+Cys for optimal performance of grower and finisher broiler chickens through statistical models.

2. Material and methods

This research was approved by the Ethics Committee on the Use of Animals of the Universidade Federal of Paraná, in Curitiba, Paraná, Brazil, under protocol number 072/2019. The experiment was conducted in Curitiba, Paraná, Brazil (25°25'40" S and 49°16'23" W).

2.1. Birds and housing

A total of 1,296 one-day-old male broiler chickens (Cobb 500; Cobb Brazil Ltda, SP, Brazil) were housed in 54 experimental pens of 2.06 m^2 each (24 birds/pen) with wood shavings as litter, equipped with nipple drinkers and tube feeders. Feed and water were offered *ad libitum* throughout the experimental period. A continuous lighting program was set for the first 24 h, and the time (h) of light per day were gradually decreased according to the Cobb Broiler Management Manual (Cobb-Vantress, 2021). The initial temperature was set to 32 °C on day 0 and gradually reduced to 18 °C on day 42. All pens were daily checked for the removal of dead broiler chickens.

2.2. Experimental design and diets

The broiler chickens were distributed in a completely randomized design with six treatments and nine replicates of 24 broiler chickens each. Each pen was considered an experimental unit, to a total of 54 units. From 0 to 21 days old, all broiler chickens received the same basal diet, formulated to be nutritionally adequate for broiler chickens at that age. From day 21 to 42, the experimental diets were offered and divided into grower (21 to 35 d) and finisher phase (36 to 42 d). Diets were based on corn and soybean meal and offered in mash form (Table 1).

The dietary treatments consisted of a basal diet (without the inclusion of synthetic methionine) and five diets supplemented with increasing concentrations of DL-methionine. For grower-phase diets, in each treatment 0.59 g/kg more methionine was added than in the previous treatment, ranging from 0.00 g/kg in treatment 1 to 2.95 g/kg in treatment 6; for the finisher-phase diets, this rise of methionine supplementation was 0.49 g/kg, ranging from 0.00 g/kg in treatment 1 up to 2.45 g/kg in treatment 6.

Table 1 - Calculated nutritional composition of experimental diets

Ingredient (%)	Experimental diet		
	Starter	Grower	Finisher
Corn (7.5% crude protein)	55.98	68.34	71.75
Soybean meal (45% crude protein)	36.40	24.70	21.50
Soybean oil	3.50	3.50	3.20
Dicalcium phosphate	1.70	1.69	1.73
Limestone	0.80	0.66	0.69
Salt	0.30	0.30	0.30
Sodium bicarbonate	0.29	0.23	0.19
DL-Met (99%)[1,2]	0.37	variable	variable
L-Thr (98%)	0.11	0.01	0.08
Choline chloride	0.06	0.07	0.08
Mineral premix[3]	0.05	0.05	0.05
Vitamin premix[4]	0.15	0.15	0.15
L-Lys[5]	0.22	0.30	0.27
Growth promoter[6]	0.01	0.01	0.01
Anticoccidial[7]	0.05	-	-
Inert substance[8,9]	-	variable	variable
Monensin	-	0.03	-
Calculated composition			
Metabolizable energy (kcal/kg)	3,070	3,201	3,222
Crude protein (%)	21.63	17.63	16.12
Ca (%)	0.78	0.75	0.76
Available P (%)	0.42	0.39	0.40
Digestible methionine (%)	0.66	0.52	0.46
Digestible met+cys (%)	0.95	0.77	0.69
Digestible lysine (%)	1.23	1.00	0.89
Digestible threonine (%)	0.81	0.66	0.59

[1] DL-Methionine in the grower phase (g/kg): T1 = 0.00; T2 = 0.59; T3 = 1.18; T4 = 1.77; T5 = 2.36; T6 = 2.95.
[2] DL-Methionine in the finisher phase (g/kg): T1 = 0.00; T2 = 0.49; T3 = 0.98; T4 = 1.47; T5 = 1.96; T6 = 2.45.
[3] Provided per kilogram of the diet: Cu, 0.01 g; Fe, 0.05 g; Mn, 0.08 g; Co, 10 mg; I, 10 mg; Zn, 0.05 g; Se, 0.25 mg.
[4] Provided per kilogram of the diet: vitamin A, 9000 UI; vitamin D3, 2500 UI; vitamin E, 20 UI; vitamin K3, 2.5 mg; vitamin B1, 1.5 mg; vitamin B2, 6 mg; vitamin B6, 3 mg; vitamin B12, 12 mg; pantothenic acid, 0.012 g; niacin, 0.03 g; folic acid, 0.80 mg; biotin, 0.06 mg.
[5] Biolys 70% (Evonik Brazil Ltda, São Paulo, Brazil).
[6] Enradin F80 (MSD Saúde Animal, São Paulo, Brazil).
[7] Nicamix 25% (Phibro Animal Health Corporation, NJ, USA).
[8] Kaolin (Mineração Itapeva Ltda.) inclusion in the grower phase (g/kg): T1 = 3.78; T2 = 3.19; T3 = 2.59; T4 = 2.00; T5 = 1.41; T6 = 0.81.
[9] Kaolin (Mineração Itapeva Ltda.) inclusion in the finisher phase (g/kg): T1 = 3.00; T2 = 2.51; T3 = 2.03; T4 = 1.54; T5 = 1.05; T6 = 0.56.

2.3. Growth performance

At d 21, all broiler chickens were weighted individually for uniformity evaluation and were distributed to each treatment in groups of 24 broiler chickens within the average weight (970 g) to avoid a high variability in the weight of broiler chickens between treatments. Broiler chickens were then weighted by pen at days 21, 35, and 42 to calculate body weight gain (BWG). Weekly feed allowance and feed refusal were weighed to determine feed intake (FI). Feed conversion ratio (FCR) was calculated as the ratio between FI and BWG. Methionine intake (MI) was obtained by dividing FI by the percentage of dietary Met+Cys on each diet. The efficiency of methionine utilization (EM) was determined by the ratio between BWG and MI (Cella et al., 2001). Culled and dead broiler chickens were weighed, and the performance variables were adjusted to their weight.

2.4. Carcass yield and parts yields

At 42 d, two broiler chickens per experimental unit (n = 108) were identified, weighed, and transported to the experimental processing plant. After 12 h of feed withdrawal, the broiler chickens were euthanized by cervical dislocation. The carcasses were plucked, eviscerated, weighed, and cut, after which breast and abdominal fat were weighed. Carcass yield was calculated as a percentage of carcass weight relative to body weight, and part yield was calculated as part weight relative to carcass weight. Yields were expressed as percentages, according to the procedure described by Dahlke et al. (2001).

2.5. Statistical models

To determine optimal concentrations of digestible Met+Cys, the data were fit using the QP and LRP regression models and BL, in which BWG and FCR were considered the dependent variables and the concentration of digestible Met+Cys was the independent variable according to the following mathematical models:

$$\text{QP: } Y = \beta 0 + \beta 1X + \beta 2X^2,$$

in which Y = dependent variable, X = is the Met+Cys dietary concentration, β0 = intercept, β1 = linear coefficient, and β2 = quadratic coefficient. The optimal concentration was obtained by: −β1/(2 × β2).

$$\text{LRP: } Y = \beta 0 + \beta 1^*(X - V), \text{ if } (X \leq V),$$

in which Y = dependent variable, β0 = intercept of the maximum response, β1 = the slope of the straight line before the breakpoint, and (X − V) in which X is the Met+Cys level in the feed and V is the Met+Cys level estimated by the breakpoint. This equation is a Boolean expression (Gries and Schneider, 1993), as in (X ≤ V) = 1 only if X, the Met+Cys dietary concentration, is less than or equal to V, the Met+Cys dietary concentration at the breakpoint of the function. (X ≤ V) = 0 if X is greater than V.

$$\text{BL: } Y = \beta 0 + \beta 1^*X^*(X \leq V) + (\beta 1^*V + \beta 2 * (X - V)) * (X > V),$$

in which, Y = dependent variable, β0 = intercept, β1 = the slope of the line before the breakpoint, and β2 = the slope of the line after the breakpoint. The terms (X ≤ V) and (X > V) are Boolean expressions, as in (X > V) = 1 only if X, the Met+Cys dietary concentration, is greater than V, the Met+Cys dietary concentration at the breakpoint of the function. (X > V) = 0 if X is less than V. Similarly, (X ≤ V) = 1 only if X is less than or equal to V, and (X ≤ V) = 0 if X is greater than V.

The evaluation parameters used for assessing the model that provided the best fit to the data were R^2 (with values closer to 1 meaning a better fit to the data), Akaike's Information Criterion (AIC), and residual sum of squares (RSS). For both AIC and RSS, the model with the lowest values was considered the most adequate (Emiliano et al., 2009; Siqueira et al., 2009).

3. Results

3.1. Growth performance

During the grower phase (21 to 35 d), FI was not influenced by the different concentrations of digestible Met+Cys (P>0.05; Table 2), but an improvement was observed for BWG as Met+Cys concentrations were increased (P<0.05; Table 2), as maximum BWG (1,428 g) was obtained with 10.67 g/kg Met+Cys. A quadratic effect was observed for FCR (P<0.05; Table 2), which was improved up to 1.62 with 10.07 g/kg Met+Cys but increased to 1.64 with 10.67 g/kg Met+Cys. The MI was linearly increased with increasing concentrations of Met+Cys, maximum of 24.89 g with 10.67 g/kg Met+Cys (P<0.05; Table 2), whereas EM showed a quadratic effect, reaching its maximum value (67.61) up to 8.89 g/kg Met+Cys and decreasing afterward with higher Met+Cys concentrations.

In the finisher phase (36 to 42 d), FI was linearly reduced (P<0.05; Table 2) when increasing the Met+Cys dietary concentrations (1,248 g at 9.36 g/kg), whereas BWG showed a quadratic effect (P<0.05; Table 2). An increase in BWG was observed in broiler chickens receiving diets with intermediate Met+Cys concentrations (maximum of 661 g with 8.87 g/kg) but reduced when Met+Cys was increased to 9.36 g/kg. Diets with lower Met+Cys concentrations resulted in worse FCR, which then showed a quadratic effect (P<0.05; Table 2) as FCR improved with intermediate concentrations (best value of 1.90 with 8.87 g/kg) but increased with the highest Met+Cys concentration. Similarly, to the grower phase, MI was linearly increased with increasing Met+Cys concentrations, maximum of 11.73 g with 9.36 g/kg (P<0.05; Table 2), but EM again showed a quadratic effect: the best result (65.22) was obtained with an intermediate Met+Cys concentration (7.90 g/kg) but decreased with higher concentrations.

Table 2 - Growth performance of broiler chickens fed different concentrations of digestible Met+Cys from 21 to 42 d of age

Phase (d)	Variable	Digestible Met+Cys (g/kg)						SEM	P-value
		7.70	8.29	8.89	9.48	10.07	10.67		
21 to 35	FI (g)	2,363	2,329	2,337	2,316	2,307	2,350	20.14	0.368
	BWG (g)[1]	1,215	1,305	1,405	1,411	1,422	1,428	13.84	<0.001
	FCR (g/g)[2]	1.88	1.77	1.67	1.64	1.62	1.64	0.007	<0.001
	MI (g)[3]	18.19	19.31	20.78	21.91	23.23	24.89	0.18	<0.001
	EM (g/g)[4]	66.79	67.58	67.61	64.28	61.23	57.37	0.27	<0.001
		Digestible Met+Cys (g/kg)							
		6.92	7.41	7.90	8.38	8.87	9.36		
36 to 42	FI (g)[5]	1,309	1,274	1,281	1,286	1,252	1,248	11.62	<0.001
	BWG (g)[6]	581	547	660	658	661	654	8.43	<0.001
	FCR (g/g)[7]	2.26	2.33	1.94	1.96	1.90	1.91	0.02	<0.001
	MI (g)[8]	9.06	9.44	10.12	10.77	11.11	11.73	0.09	<0.001
	EM (g/g)[9]	64.13	57.94	65.22	61.10	59.43	55.99	0.57	<0.001

FI - feed intake; BWG - body weight gain; FCR - feed conversion ratio; MI - methionine intake; EM - efficiency of methionine utilization; SEM - standard error of the mean.

[1] Quadratic effect (P = 0.001): $-1261.34 + 5230.91x - 2539.11x^2$; ($R^2$ = 0.72).
[2] Quadratic effect (P = 0.001): $6.3 - 9.33x + 4.65x^2$; (R^2 = 0.95).
[3] Linear effect (P = 0.001): $16.746 + 1.3254x$; (R^2 = 0.99).
[4] Quadratic effect (P = 0.001): $-54.825 + 296.53x - 179.65x^2$; ($R^2$ = 0.98).
[5] Linear effect (P = 0.001): $1311.6 - 10.457x$; (R^2 = 0.75).
[6] Quadratic effect (P = 0.001): $-1155.85 + 4002.06x - 2203.07x^2$; ($R^2$ = 0.52).
[7] Quadratic effect (P = 0.001): $8.28 - 13.65x + 7.29x^2$; (R^2 = 0.70).
[8] Linear effect (P = 0.001): $8.4707 + 0.5431x$; (R^2 = 0.99).
[9] Quadratic effect (P = 0.001): $-28.129 + 244.54x - 164.74x^2$; ($R^2$ = 0.46).

3.2. Carcass yield and parts yields

Carcass yield, breast yield, and abdominal fat percentage demonstrated a quadratic behavior ($P<0.05$; Table 3). Increasing Met+Cys concentrations up to 9.48/8.38 g/kg Met+Cys resulted in the maximum values of carcass (79.77%) and breast (39.71%). The lowest values for abdominal fat percentage were obtained with medium-to-high Met+Cys concentrations (0.018 and 0.017%; T4 and T5, respectively), although it increased with the lowest Met+Cys concentrations (T1 and T2).

Table 3 - Effect of different dietary concentrations of digestible Met+Cys on carcass yield, breast yield, and abdominal fat percentage of 42-day-old broiler chickens

Variable (%)	Digestible Met+Cys (g/kg)						SEM	P-value
	T1	T2	T3	T4	T5	T6		
Carcass yield[1]	77.79	79.17	79.76	79.77	79.57	79.30	0.44	<0.001
Breast yield[2]	34.92	36.47	38.13	39.71	38.47	39.23	0.42	<0.001
Abdominal fat[3]	0.027	0.024	0.021	0.018	0.017	0.020	0.001	<0.001

Digestible Met+Cys concentrations in the grower/finisher phases (considering the weighted average of the grower (66.66%) and finisher (33.34%) periods). T1 = 7.70/6.92; T2 = 8.29/7.41; T3 = 8.89/7.90; T4 = 9.48/8.38; T5 = 10.07/8.87; T6 = 10.67/9.36.
SEM - standard error of the mean.
[1] Quadratic effect ($P = 0.001$): $24.987 + 120.87x - 66.511 x^2$; ($R^2 = 0.96$).
[2] Quadratic effect ($P = 0.001$): $-43.537 + 172.87x - 90.24x^2$; ($R^2 = 0.92$).
[3] Quadratic effect ($P = 0.001$): $0.2143 - 0.4158x + 0.2203x^2$; ($R^2 = 0.94$).

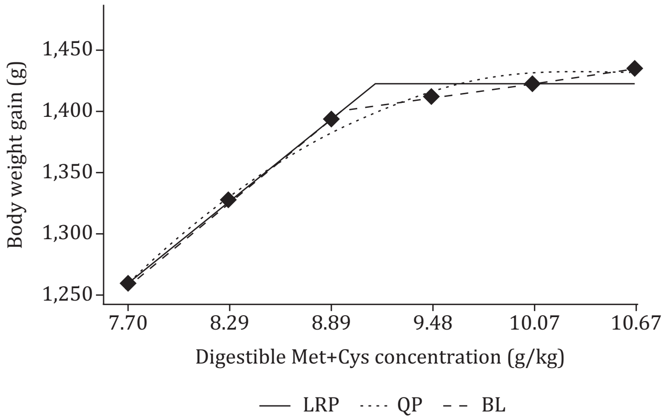
3.3. Optimal Met+Cys concentrations for maximum BWG and best FCR

On the grower phase, the optimal Met+Cys concentrations for BWG were 10.30, 9.00, and 9.10 g/kg using QP, BL, and LRP models, respectively, whereas optimal concentrations for FCR were 10.03, 9.10, and 9.10 g/kg (Table 4). When evaluating the data fitness for BWG and FCR, the evaluated regression models had similar RSS and R^2 values between them, but different AIC: the QP and LRP showed the lowest AIC values for BWG, and the QP model showed the lowest AIC for FCR (Figures 1 and 2; respectively).

Table 4 - Determination of ideal dietary concentrations of digestible Met+Cys for broiler chickens from 21 to 42 days old aiming for optimal body weight gain (BWG) and feed conversion ratio (FCR) through different statistical models

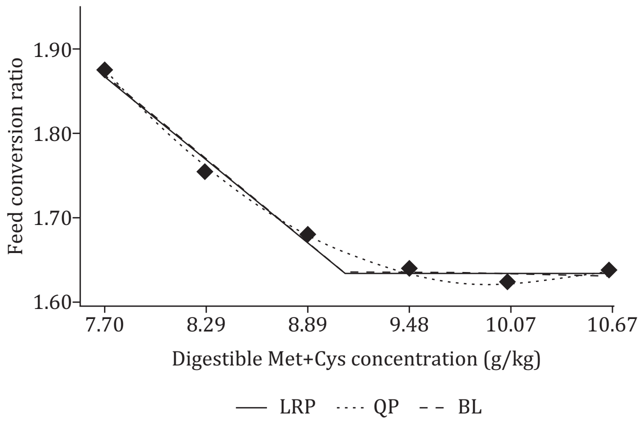
Phase (d)	Variable	Model	Criterion for data fitness			Optimal Met+Cys content (g/kg)
			AIC	R^2	RSS	
21 to 35	BWG	QP	524.91	0.72	39.61	10.30
		BL	525.39	0.72	39.44	9.00
		LRP	524.89	0.72	39.60	9.10
	FCR	QP	−242.24	0.95	0.02	10.03
		BL	−232.19	0.94	0.02	9.10
		LRP	−234.10	0.94	0.02	9.10
36 to 42	BWG	QP	517.46	0.52	36.82	9.08
		BL	514.84	0.56	35.56	8.20
		LRP	512.89	0.56	35.21	8.20
	FCR	QP	−81.11	0.70	0.10	9.36
		BL	−84.57	0.73	0.10	8.10
		LRP	−85.64	0.73	0.10	8.20

QP - quadratic polynomial; BL - broken-line; LRP - linear response plateau; AIC - Akaike Information Criterion; R^2 - coefficient of determination; RSS - residual sum of squares.



LRP - linear response plateau; QP - quadratic polynomial; BL - broken-line regression.
LRP: 1,422.79 + 1,122.22 * X – 0.910, if X ≤ 0.910 (R^2 = 0.72; AIC = 524.89; RSS = 39.60).
QP: −1,261.34 + 5,230.91 * X – 2,539.11 * X^2 (R^2 = 0.72; AIC = 524.91; RSS = 39.61).
BL: 396.12 + (1,122.2) * X * (X ≤ 0.900) + (1,122.22 * 0.900 + (−924.7 * (X – 0.900)) * (X > 0.900) (R^2 = 0.72; AIC = 525.39; RSS = 39.44).

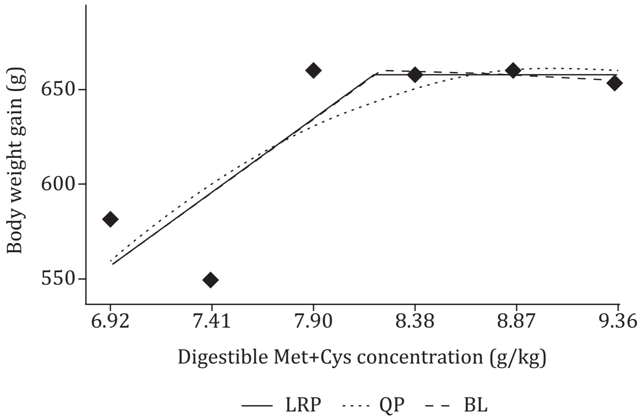
Figure 1 - Behavior of different statistical models to determine the optimal concentrations of digestible Met+Cys in diets of broiler chickens from 21 to 35 days of age for body weight gain.



LRP - linear response plateau; QP - quadratic polynomial; BL - broken-line regression.
LRP: 1.63 − 1.66 * X – 0.910, if X ≤ 0.910 (R^2 = 0.94; AIC = −234.10; RSS = 0.02).
QP: 6.3 − 9.33*X + 4.65 * X^2 (R^2 = 0.95; AIC = −242.24; RSS = 0.02).
BL: 3.15 + (−1.66) * X * (X ≤ 0.910) + (− 1.66 * 0.910 + (1.64 * (X – 0.910)) * (X > 0.910) (R^2 = 0.94; AIC = −232.19; RSS = 0.02).

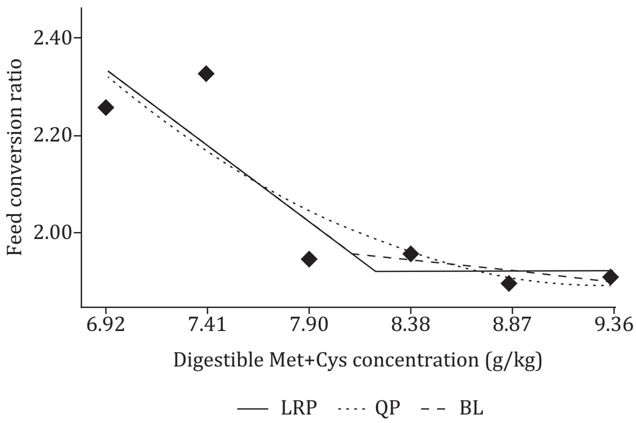
Figure 2 - Behavior of different statistical models to determine the optimal concentrations of digestible Met+Cys in diets of broiler chickens from 21 to 35 days of age for feed conversion ratio.

In the finisher phase, optimal Met+Cys concentrations estimated by QP, BL, and LRP models were, respectively, 9.08, 8.20, and 8.20 g/kg for BWG and 9.36, 8.10, and 8.20 g/kg for FCR. It was observed that the LRP model had the best data fitness by presenting the lowest AIC values for BWG and the lowest AIC for FCR, although the RSS for FCR was similar among models, and the R^2 value was similar between BL and LRP models (Figures 3 and 4; respectively).



LRP - linear response plateau; QP - quadratic polynomial; BL - broken-line regression.
LRP: 657.75 + 804.99 * X – 0.820, if X ≤ 0.820 (R^2 = 0.56; AIC = 512.89; RSS = 35.21).
QP: −1,155.85 + 4,002.06 * X – 2,203.07 * X^2 (R^2 = 0.52; AIC = 517.46; RSS = 36.82).
BL: (−0.53) + (804.99) * X *(X ≤ 0.820) + (804.99 * 0.820 + (−846.26 * (X – 0.820)) * (X > 0.820) (R^2 = 0.56; AIC = 514.84; RSS = 35.65).

Figure 3 - Behavior of different statistical models to determine the optimal concentrations of digestible Met+Cys in diets of broiler chickens from 36 to 42 days of age for body weight gain.



LRP - linear response plateau; QP - quadratic polynomial; BL - broken-line regression.
LRP: 1.92 – 3.19 * X – 0.820, if X ≤ 0.820 (R^2 = 0.73; AIC = −85.64; RSS = 0.10).
QP: 8.28 – 13.65*X + 7.29 * X^2 (R^2 = 0.70; AIC = −81.11; RSS = 0.10).
BL: (4.54) + (−3.19) * X * (X ≤ 0.810) + (– 3.19*0.810) + (2.7 * (X – 0.810)) * (X > 0.810) (R^2 = 0.73; AIC = −84.57; RSS = 0.10).

Figure 4 - Behavior of different statistical models to determine the optimal concentrations of digestible Met+Cys in diets of broiler chickens from 36 to 42 days of age for feed conversion ratio.

4. Discussion

4.1. Grower performance and optimal Met+Cys concentrations

Broiler chickens may have FI influenced by an excess or deficiency of dietary AA (Sigolo et al., 2019; Pokoo-Aikins et al., 2021), although the different digestible Met+Cys concentrations used in the current study did not affect FI. Probably, the difference among the tested Met+Cys concentrations was not enough to cause any increase or reduction in diet intake. However, broiler chickens efficiently responded to higher Met+Cys concentrations (between 10.07 and 10.67 g/kg) with greater BWG and more efficient FCR.

The estimated optimal concentrations of Met+Cys were different according to the regression model utilized in all periods. Overall, the QP model indicated higher concentrations than BL and LRP models, but this difference between models was expected due to the distinct parameters employed in their equations (Pesti et al., 2009). The QP model, for instance, tends to overestimate nutritional values for poultry broiler chickens, as it can be influenced by factors such as the variation between concentrations, the number of concentrations being tested, or the positioning of these concentrations in the response curve (Euclydes and Rostagno, 2001; Souza et al., 2014).

In the study conducted by Nogueira et al. (2022), who investigated the daily requirements of digestible lysine (Lys) for male broilers, the mathematical models best fitted differently in each performance phase. According to the authors, for the starter and grower phases, the ADG fitted better to the LRP model than the QP model. In the starter phase, the authors estimated 0.415 vs. 0.507 g Lys/bird/d using the LRP and QP models, respectively; in the grower phase, they estimated 1.38 vs. 1.73 g Lys/bird/d using the LRP and QP models, respectively. Similar results were observed by Pinheiro et al. (2011), who evaluated optimal dietary Ca concentrations for broiler chickens, which were estimated by the LRP model to be 9.5 g/kg versus 10.7 g/kg by the QP model.

During the grower phase, the evaluation parameters indicated that both QP and LRP models were better fit to the BWG data, with the lowest AIC values, even though they had similar R^2 and RSS; for FCR data, the QP model was deemed the best fit, based on its lowest AIC values. The LRP and QP models estimated, respectively, 9.10 and 10.30 g/kg as optimal Met+Cys concentrations for a maximum BWG of 1,432 g. The QP model was best fit for the FCR data, estimating 10.03 g/kg Met+Cys for an optimum FCR of 1.61. This estimated concentration was slightly higher than the recommendation of Rostagno et al. (2017): 8.32 g/kg Met+Cys for 22-to-33-d-old male broiler chickens of regular performance. Tavernari et al. (2014) also indicated lower Met+Cys concentrations of 7.90 and 7.51 g/kg for optimal BWG and FCR of growing broiler chickens through simple linear and QP regressions, and approximate values were also estimated by Goulart et al. (2011) and Carvalho (2017) for the variables BWG and FCR for broiler chickens in the same age, using QP regression: 7.48 and 7.16 g/kg, respectively.

The synthesis of body protein is one of the many functions of methionine on the metabolism of broiler chickens, and an insufficient supply of methionine can lead to higher FI, reduced BWG, and worse FCR (Carew et al., 2003; Tesseraud et al., 2007), which was observed in the finisher phase of this study. The low difference of 6 g in BWG from 36 to 42 days between 7.90 and 9.36 g/kg Met+Cys suggests that 7.90 g/kg (or the reduction of 1.46 g/kg in digestible Met+Cys) for finisher broiler chickens is sufficient to achieve good BWG, which was also observed in FCR. Rostagno et al. (2017) estimated an approximate Met+Cys requirement of 7.50 g/kg for 34-to-42-d old male broiler chickens of regular performance. The LRP model presented the best data fitness with the lowest AIC result and estimated 8.20 g/kg Met+Cys as the optimal concentration for maximum BWG (658 g) and best FCR (1.92).

Lower nutritional requirements of Met+Cys are typically recommended. Nascimento et al. (2009) showed 7.16 g/kg digestible Met+Cys for Isa Label broiler chickens from 28 to 56 d old for FCR, and the same Met+Cys concentration was estimated by Carvalho (2017) for maximum performance of 22-to-42-d old broiler chickens.

As previously mentioned, the ideal Met+Cys concentrations that optimized BWG and FCR found in this study are a bit higher than previous concentrations reported in the literature. The improvement of broiler chickens' genetic potential for fast growth over the years could explain this higher requirement of Met+Cys. As part of the genetical improvement, broiler chickens need an increased nutritional input—especially greater AA intake—to achieve maximum protein deposition and growth performance.

4.2. Methionine intake and efficiency of methionine utilization

The ingestion of AA and its efficient utilization in diets by broiler chickens are fundamental, and any excess or deficiency in AA concentrations must be avoided (Buteri et al., 2009; Sklan and Plavnik, 2002). Throughout the experimental period, a greater MI was observed not due to greater FI, but to the higher inclusion of synthetic methionine in the diet. The higher concentrations of digestible Met+Cys led to higher MI, as similarly reported by Nascimento et al. (2009) and Haese et al. (2012). When deriving the QP equation, maximum EM was obtained with 8.25 g/kg in the growth phase (67.53 g/g) and 7.42 g/kg in the finisher phase (66.61 g/g).

Methionine is considered the main limiting AA for broiler chickens, and it plays a role in diverse metabolic processes—either related to maintenance requirements or protein synthesis (Jankowski et al., 2014). In this study, it was possible to observe that in all the production phases, lower concentrations of Met+Cys resulted in better EM. A possible explanation is that when diets with a deficit of Met+Cys were given, these AA were more rapidly absorbed and directed to the organism's maintenance demands, and once the maintenance requirement was supplied, the remaining AA were used for body protein deposition. This allows us to explain why diets with reduced Met+Cys also resulted in lower BWG. However, the opposite was also observed, diets with higher Met+Cys concentrations resulted in lower EM, although showing the best results for BWG.

4.3. Carcass yield and parts yields

A consequence of the deficiency of methionine supply to broiler chicken diets is the reduction of body protein deposition/lean tissue, in addition to higher fat deposition (Carew et al., 2003). In the current study, the lower inclusions of digestible Met+Cys resulted in lighter carcasses, whereas carcass yield and breast yield were significantly recovered with increasing Met+Cys—and fat deposition was reduced. When deriving the QP equation, the most adequate Met+Cys concentrations for maximum carcass yield (79.90%), breast yield (39.25%), and lower fat deposition (0.02%) ranged from 9.08 to 9.58 g/kg. Nonetheless, Carvalho (2017) reported a lack of significant effect of digestible Met+Cys concentrations on breast yield and abdominal fat in broiler chickens; carcass yield was influenced, although with an indicated concentration of 7.61 g/kg—lower than the estimated by the current study.

5. Conclusions

The QP and LRP regression models showed the best data fit for the growth phase, respectively indicating 10.30 and 9.10 g/kg as optimal Met+Cys concentrations for maximum BWG, and the QP regression model showed the best data fit for the 10.03 g/kg for best FCR. In addition, for the finisher phase, the LRP model indicated 8.20 g/kg as the optimal Met+Cys concentration for maximum BWG and FCR.

Data availability

The entire dataset supporting the results of this study is available upon request to the corresponding author.

Author contributions

Conceptualization: Maiorka, A. **Data curation:** Goes, E. C. **Formal analysis:** Alvarez, M. V. N. **Investigation:** Marx, F. O. **Supervision:** Félix, A. P. **Validation:** Oliveira, S. G. and Maiorka, A. **Visualization:** Maiorka, A. **Writing – original draft:** Marx, F. O. **Writing – review & editing:** Vieira, V. I.; Félix, A. P. and Oliveira, S. G.

Conflict of interest

The authors declare no conflict of interest.

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