

Article

Responses of Broiler Breeder Hens to Dietary Digestible Lysine, Methionine+Cystine, and Threonine [†]

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Abstract

To evaluate the response of broiler breeder hens submitted to different amino acid intakes of methionine+cystine, lysine, and threonine, and to determine the coefficients for egg output and body weight for maintenance. Three studies were performed using 160 broiler breeder hens housed individually in metabolic cages. A summit diet and a nitrogen-free diet were formulated. The levels ranged from 1.79 to 7.13, 2.49 to 8.3, and 2.04 to 6.79 g/kg of methionine+cystine, lysine, and threonine, respectively. The variables measured were feed intake, amino acid intake, rate of lay, egg weight, and egg output. The broken line model was used to evaluate the responses. It was verified that higher values of the rate of lay, egg weight, and egg output were observed for the higher concentrations of amino acids studied. A significant difference was observed for the variables rate of lay, egg weight, egg output, and body weight ($p < 0.05$) for the three amino acids evaluated. The amount of each amino acid required to produce one gram per egg was estimated at 12.4 mg, 14.5 mg, and 11.2 mg for methionine+cystine, lysine, and threonine, respectively. The values estimated by coefficient b that represent the amino acid for maintenance requirement were methionine+cystine, lysine, and threonine of 30.2, 32.2, and 42.4 mg/kg BW, respectively. The coefficients may be used to design additional models to study requirements nutrition in broiler breeders, allowing a better understanding of how these birds respond to different dietary amino acids.

Keywords: amino acid; egg output; maintenance requirement; rate of lay



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1. Introduction

Broiler breeder hens have unique characteristics that allow nutritionists to establish adequate nutritional plans for these birds [1–6]. When fed less energy than recommended, these birds mobilize their body fat reserves to maintain egg production [1–3,7,8].

Bowmaker and Gous [9] demonstrated that birds fed diets containing 39.7 g of crude protein (CP) per kilogram consumed less protein than the maintenance requirement of 2.282 g CP per kg of metabolic body weight ($BW^{0.75}$) [10].

Bowmaker and Gous [9] showed that birds fed with diets containing 39.7 g CP/kg ingested less protein than the maintenance requirement of 2.282 g CP/ $BW_{kg}^{0.75}$ [10]. As a result, birds produced one egg per week, generating a deficit between protein input and

output. This result indicates that, besides lipid mobilization, these birds also mobilized body protein [1–3].

Researchers use response values to methionine+cystine (Met+Cys), lysine (Lys), and threonine (Thr) intakes obtained with laying hens [11–13] to study these responses in broiler breeder [1,9,14]. This limitation impairs our understanding of how broiler breeders actually respond to these amino acids.

Several mathematical models can be applied to fit the responses to amino acid intake and to provide some important parameters applied in response modeling. These parameters are (a) mg of amino acids per gram of egg, and (b) mg per kg of body weight for maintenance [1–3,11,15,16]. Bowmaker and Gous [9] and Lima et al. [2] showed that broiler breeders at peak production were less efficient in the use of amino acid, when compared to laying hens [11,12] and thus presented a lower laying rate. The authors [2,9] expressed difficulties in estimating the maintenance requirement for broiler breeder hens.

A previous study with Met+Cys [17] done in post-peak production birds showed that age is an important parameter to be considered when evaluating the responses to amino acid intake [18]. Indeed, it has long been known that decreased utilization of amino acids is related to the maintenance of unproductive layers, which increase in number with advanced age [11–13]. However, no study has investigated the response to Met+Cys, Lys, and Thr in broiler breeders older than 60 weeks. It is hypothesized that broiler breeders over 60 weeks of age exhibit altered efficiency in the utilization of Methionine+Cysteine (Met+Cys), Lysine, and Threonine. These changes are expected to enable the estimation of precise coefficients. This study aimed to evaluate the response of post-peak production hens submitted to different intakes of Met+Cys, Lys, and Thr and to determine the coefficients for egg output and body weight maintenance in broiler breeders older than 60 weeks.

2. Materials and Methods

2.1. Animal Ethics and Welfare Committee, Hens and Experimental Design

All experimental procedures were approved by the Animal Ethics and Welfare Committee of UNESP (CEUA) under protocol number 007125-08, with the approval date being the 26 April 2013, in Jaboticabal-SP, Brazil. The trials were conducted at the Poultry Science Laboratory of Poultry Science in 2013, of the São Paulo State University, UNESP, Jaboticabal, São Paulo, Brazil with the objective of measuring the responses of broiler breeder hens to digestible Met+Cys, Lys and Thr with a view to determining the coefficients of response for egg output and body weight maintenance.

Two dose response studies were performed simultaneously (methionine+cystine and lysine trial), then the threonine trial.

A total of 160 broiler breeder hens of Cobb 500[®] genotype were used, being 56 birds for methionine+cystine and lysine trials (at 86 weeks of age) and 48 birds for threonine trial (at 60 weeks of age). The experimental design was a completely randomized design with eight treatments (seven increasing levels of amino acid and a validating diet) and seven replicates for Met+Cys and Lys trials and six replicates for Thr trial. In all trials, the hens were raised in the same poultry house with a negative-pressure system with controlled temperature of 21 °C and individually housed in metabolic cages measuring 50 × 50 × 50 cm equipped with individual feeders and nipple drinkers.

2.2. Bird Management

The birds were provided by a commercial farm situated in the state of Minas Gerais, Brazil. Two weeks before the beginning of the experiment, all birds were fed an adequate diet designed to meet the nutritional requirements of the birds during this period according to the recommendation of [19] at the rate of 150 g/d, and the rate of lay was monitored to

provide a baseline for the experimental period. The experiment lasted ten weeks, the first six weeks being an adaptation period, and the last four weeks for data collection, and there was no mortality during the experimental period. The lighting program adopted during the experiment was 17 h of light with the average daily temperature maintained at 24 °C, and the daily management was performed according to the breeder guidelines [20].

2.3. Experimental Diets

A high-protein summit feed, based on corn and soybean meal, was formulated for each trial containing 7.13 g/kg of digestible Met+Cys, 8.30 g/kg of digestible Lys, and 6.79 g/kg of digestible Thr (Table 1). The contents of each limiting amino acid in the summit diets were calculated to provide 1.2 times the requirement of Met+Cys (4.58 g/kg), Lys (5.27 g/kg), and Thr (4.27 g/kg), respectively, according to the recommendations proposed by Rostagno et al. [19]. The other amino acids were included at a minimum of 1.4 times the requirement with the purpose of ensuring that the test amino acid studied was first limiting in the summit feed.

Table 1. Composition (g/kg) of the summit (high protein) and nitrogen-free basal feeds used in the lysine (Lys), methionine+cysteine (Met+Cys), and threonine (Thr) response trials.

Ingredients (g/kg)	Met+Cys Summit	Lys Summit	Thr Summit	Nitrogen-Free
Soybean meal	276	222	266	
Corn	557	587	555	
Wheat bran (meal)	21.5	94	68.5	
Corn starch				469
Corn gluten meal	38			
Rice husk				119
Sugar				150
Soybean oil	30	12.7	35.0	60
Dicalcium phosphate	8.53	8.06	8.37	14.2
Limestone	62.7	63.8	57.4	59.3
Potassium chloride				12.7
Salt	3.28	3.75	3.73	3.88
DL-Methionine	0.54	2.70	2.42	
L-Lysine HCl			0.34	
L-Threonine	0.29	1.56	0.01	
L-Valine		0.89	0.27	
L-Isoleucine		1.57	0.89	
L-Tryptophan		0.21	0.04	
Choline chloride	0.7	0.70	0.7	0.7
Vitamin premix ^a	1	1.00	1	1
Mineral premix ^b	0.5	0.5	0.5	0.5
BHT ^c	0.1	0.1	0.1	0.1
Inert (Sand)				110
----- Calculated nutrient content -----				
AMEn (MJ/kg)	11.72	11.72	11.72	11.72
Crude protein	206	175	187	2.10
Methionine+Cystine ^d	7.13	8.14	8.20	
Methionine ^d	3.76	5.24	5.14	
Lysine ^d	9.76	8.30	9.64	
Threonine ^d	7.80	7.71	6.79	

Table 1. Cont.

Ingredients (g/kg)	Met+Cys Summit	Lys Summit	Thr Summit	Nitrogen-Free
Tryptophan ^d	2.08	2.02	2.05	
Arginine ^d	12.8	10.9	12.1	
Valine ^d	9.46	8.63	8.74	
Isoleucine ^d	8.46	8.32	8.40	
Leucine ^d	20.1	15.1	16.2	
Histidine ^d	5.43	4.59	4.98	

^a This premix supplied the following per kilogram of diet: Vit A 10,000 IU; Vit D₃ 2700 IU; Vit E 40 IU; Vit K₃ 2.0 mg; Vit B₁ 2.5 mg; Vit B₂ 7 mg; Vit B₃ 35 mg; Vit B₅ 15 mg; Vit B₆ 2.5 mg; Vit B₁₂ 0.020 mg; Folic Acid 0.9 mg; Biotin 0.11 mg; Selenium 0.36 mg. ^b This premix supplied the following per kilogram of diet: Cu 12 mg; Fe 60 mg; I 1.2 mg; Mn 84 mg; Zn 78 mg. ^c Butylated hydroxytoluene. ^d Digestible amino acid composition.

For each trial, a nitrogen-free diet was formulated to meet the same nutritional and energy levels as the high-protein summit diets, except for protein and amino acids. The nitrogen-free diets were used to dilute the high-protein summit diets, in appropriate proportions, to obtain the range of Met+Cys, Lys, and Thr (Table 2) contents required for each dilution series according to [21].

Table 2. Proportion of summit basal used with the corresponding protein-free basal in the methionine+cysteine (Met+Cys), lysine (Lys), and threonine (Thr) trials and the concentrations of limiting amino acid in the resultant feed.

Feed	Met+Cys Limiting			Lys Limiting			Thr Limiting		
	Summit	Dilution	Met+Cys	Summit	Dilution	Lys	Summit	Dilution	Thr
	g/kg	g/kg	g/kg	g/kg	g/kg	g/kg	g/kg	g/kg	g/kg
D1	1000	0	7.13	1000	0	8.30	1000	0	6.79
D2	900	100	6.42	900	100	7.48	800	200	5.43
D3	700	300	4.99	700	300	5.81	700	300	4.75
D4	600	400	4.28	600	400	4.98	600	400	4.07
D5	500	500	3.56	500	500	4.16	500	500	3.39
D6	350	650	2.49	400	600	3.32	400	600	2.72
D7	250	750	1.79	300	700	2.49	300	700	2.04
D8 ^{a,b,c}	250	750	2.49	300	700	3.32	300	700	2.72

^a D8_{Lys} = 0.106 g L-Lysine HCl 780 g/kg added to D7_{Lys}. ^b D8_{Met+Cys} = 0.070 g DL-Methionine 990 g/kg added to D7_{Met+Cys}. ^c D8_{Thr} = 0.069 g L-Threonine 980 g/kg added to D7_{Thr}.

To confirm that the response of the hens was a function of the respective limiting factor, a control diet was included. A small quantity of the respective crystalline amino acid was added (0.106 g of L-Lysine, 0.070 g of DL-Methionine and 0.069 g of L-Threonine) (Table 2) to the diet with the lowest level of the amino acid tested (D7) sufficient to meet the level of the amino acid in the second-lowest level in the dilution series (D6).

2.4. Allocation of Diets and Measurements

The birds were fed 150 g of feed at the same time each morning, and at the end of the week, the leftovers were weighed to quantify the weekly consumption of the feed. The body weight of the birds was measured on the first, sixth, and tenth weeks of the experiment. The rate of lay was recorded daily, and egg weight was measured on three consecutive days each week.

2.5. Modeling of Responses

The individual responses (Y) were divided by the maximum individual response for each amino acid (Y_{max}). The responses for rate of production, egg weight, and egg output were analyzed as a percentage of the maximum response. The variable Y_p represents

the predicted response and varies according to the dependent variable under evaluation, such as rate of lay, egg weight, or other relevant production parameters, according to Equation (1).

$$Y_p = Y/Y_{\max} \quad (1)$$

Obtained for each amino acid and regressed together as a function of the relative intake to the maximum intake. The relative intake to the maximum (I_r) is calculated as 1 minus the relationship between the individual intake (I) by the maximum individual intake ($I_{\max}$), according to Equation (2).

$$I_r = 1 - I/I_{\max} \quad (2)$$

The data for the rate of lay and relative egg weight were adjusted together using the polynomial model with intercept equal to 1, as follows: Equation (3), as described by Morris and Gous [22].

$$Y_p = 1 - A \times I_r^2 - B \times I_r \quad (3)$$

Conversion of dietary amino acids to egg output (a) and body maintenance (b). To determine the coefficients a and b , the intake for egg output (E) and body weight (BW) was used in the equations: Equation (4) and Equation (5), respectively, as described by Bowmaker and Gous [9]

$$a \text{ (mg/g)} = [I - (b \times BW)]/E \quad (4)$$

$$b \text{ (mg/kg)} = [I - (a \times E)]/BW \quad (5)$$

Based on these derivations, a linear model was fitted to the responses in egg output and body weight to amino acid intake, considering Equation (6).

$$I = a \times E + b \times BW \quad (6)$$

Net efficiency for deposition of amino acid in egg—To estimate the efficiency of utilization of the amino acid, the amount of amino acid deposited (D_{aa}) in egg output was plotted as a function of the intake corrected (I_c) for maintenance of Met+Cys, Lys, and Thr. To calculate the deposition, the concentration of the amino acid was multiplied by the egg output. The concentration of the amino acids per unit of egg output was obtained from [23]. The corrected intakes (I_c) were obtained by subtracting the daily intake of the amino acid (iMet+Cys, iLys, and iThr) from their amino acid requirement for maintenance of body weight [24], according to Equations (7), (8), and (9), respectively.

$$I_{c\text{Met+Cys}} = i\text{Met+Cys} - b \times BW \quad (7)$$

$$I_{c\text{Lys}} = i\text{Lys} - b \times BW \quad (8)$$

$$I_{c\text{Thr}} = i\text{Thr} - b \times BW \quad (9)$$

The b values for Met+Cys, Lys, and Thr were calculated as described in the previous section (Equation (5)). The deposition of the amino acids (D_{aa}) in eggs and the corrected intakes (I_c) of the amino acids were used to fit the broken line model with one inclination, according to Equation (10).

$$D_{aa} = L + U \times (R - I_c) \quad (10)$$

where D_{aa} and I_c are the variables of the model and L , U , and R are the model parameters, meaning: maximum response for deposition (R), inclination (U), and intake for maximum deposition (R), respectively.

The efficiency of utilization (k) was obtained by relating the parameters $k = L/R \times 100$ [16].

2.6. Statistical Analysis

The linear and quadratic coefficients of the polynomial model for rate of lay, egg weight, and relative egg output were estimated using the PROC NLIN procedure, and the coefficients a and b of the relationship between intake, egg output, and body weight. The linear and quadratic-plateau models were adjusted according to the procedures described by Robbins et al. [25]. The statistical analyses were performed using SAS software (Statistical Analysis System, version 9.2).

3. Results

The performance of birds fed different diets of Met+Cys, Lys, and Thr is given in Tables 3–5. Means and standard errors refer to data from the period from six to ten weeks of feeding the diet. D8 is the validating diet.

Table 3. Mean and standard deviation ($\mu \pm$ SD) feed intake (FI, g/bird d), limiting amino acid intake—Methionine+Cystine (LAAI, mg/bird d), rate of lay (RL, per 100/hen d), egg weight (EW, g), egg output (EO, g/bird d) and body weight (BW, kg) responses to treatments over the final 4-week.

Diet	Amino Acid in Feed (g/kg)	FI	LAAI	RL	EW	EO	BW
D1	7.13	133.2	949.5	57.9	72.4	38.3	4.7
D2	6.42	138.9	891.7	62.8	69.9	44.1	5.0
D3	4.99	136.1	679.0	61.3	72.1	44.2	4.5
D4	4.28	137.1	586.6	60.2	69.3	41.7	4.3
D5	3.56	137.9	490.8	53.1	69.8	41.8	4.7
D6	2.49	134.6	335.2	45.2	66.2	31.1	4.1
D7	1.79	121.2	217.0	32.7	66.5	21.7	4.2
D8 ^a	2.49 ^a	124.0	308.4	45.2	62.8	29.1	4.0
Average		134.0	581.6	53.3	69.5	37.7	4.5
SEM ^b		1.3	37.2	2.2	0.6	1.6	0.1
<i>p</i> -value							
<i>F</i> -test ANOVA		0.0009	<0.0001	0.0001	0.0305	<0.0001	0.0016
Linear effect		0.0102	<0.0001	<0.0001	0.0013	<0.0001	0.0004
Quadratic effect		0.0006	0.7143	0.0036	0.5104	<0.0001	0.9319

^a validating diet. ^b standard error of men.

Table 4. Mean and standard deviation ($\mu \pm$ SD) feed intake (FI, g/bird d), limiting amino acid intake—Lysine (LAAI, mg/bird d), rate of lay (RL, per 100/hen d), egg weight (EW, g), egg output (EO, g/bird d) and body weight (BW, kg) responses to treatments over the final 4-week.

Diet	Amino Acid in Feed (g/kg)	FI	LAAI	RL	EW	EO	BW
D1	8.30	129.7	950.9	63.1	70.9	42.7	4.5
D2	7.48	126.0	831.3	56.8	73.0	41.5	4.5
D3	5.81	128.9	661.0	56.3	71.6	40.3	4.4
D4	4.98	131.6	579.1	59.2	70.9	39.3	4.5
D5	4.16	133.1	488.4	54.1	70.8	38.3	4.5
D6	3.32	131.2	384.3	50.5	67.3	33.8	4.3
D7	2.49	127.4	280.3	35.7	66.7	24.0	4.1
D8 ^a	3.32 ^a	122	356	47.0	63.0	31.1	3.8

Table 4. Cont.

Diet	Amino Acid in Feed (g/kg)	FI	LAAI	RL	EW	EO	BW
Average		129.7	602.3	53.4	70.2	37.1	4.4
SEM ^b		0.6	33.3	1.6	0.6	1.2	0.040
<i>p</i> -value							
<i>F</i> -test ANOVA		0.0044	<0.0001	<0.0001	0.0138	<0.0001	0.0253
Linear effect		0.2697	<0.0001	<0.0001	0.0009	<0.0001	0.0019
Quadratic effect		0.0331	<0.0001	0.0129	0.0678	0.0108	0.0876

^a validating diet. ^b standard error of men.

Table 5. Mean and standard deviation ($\mu \pm$ SD) feed intake (FI, g/bird d), limiting amino acid intake—Threonine (LAAI, mg/bird d), rate of lay (RL, per 100/hen d), egg weight (EW, g), egg output (EO, g/bird d) and body weight (BW, kg) responses to treatments over the final 4-week.

Diet	Amino Acid in Feed (g/kg)	FI	LAAI	RL	EW	EO	BW
D1	6.79	149.2	1163.7	63.4	72.6	45.7	4.7
D2	5.43	147.2	1034.9	64.3	70.7	44.1	4.5
D3	4.75	147.4	804.6	72.3	73.7	48.3	4.4
D4	4.07	147.2	689.1	67.0	71.4	47.4	4.7
D5	3.39	148.7	579.8	55.4	69.7	41.7	4.5
D6	2.72	136.8	372.2	56.4	68.2	37.9	4.0
D7	2.04	135.3	265.2	46.7	68.3	34.4	3.9
D8 ^a	2.72 ^a	143.9	391.3	59.3	63.8	37.6	3.9
Average		144.3	695.3	60.2	70.9	42.5	4.4
SEM ^b		1.7	50.9	2.2	0.7	1.4	0.08
<i>p</i> -value							
<i>F</i> -test ANOVA		0.0818	<0.0001	0.0226	0.0419	0.0241	0.0350
Linear effect		0.0082	<0.0001	0.0040	0.0140	0.0040	0.0027
Quadratic effect		0.1510	0.0304	0.0378	0.1283	0.0602	0.2266

^a validating diet. ^b standard error of men.

Feed intake was affected by different diets in all three trials (Tables 3–5). The Met+Cys (Table 3) and Lys (Table 4) trials had similar patterns of feed intake among the eight diets, whereas the Thr trial had a different pattern, with generally higher intake levels (Table 5). All birds received 150 g of feed per day. The maximum feed intake on an individual basis for Met+Cys, Lys, and Thr was 142, 136, and 150 g/bird d, respectively.

As shown in Tables 3–5, the maximum rate of lay was 62.8, 63.1, and 66.1 per 100 hen d, observed in the diets with higher levels of each amino acid (Met+Cys, Lys, and Thr, respectively). With lower levels of the amino acids in the diet, the rate of lay was reduced to 56%, 57% and 86% of the maximum rate of lay for the Met+Cys, Lys, and Thr trials, respectively. Diets with higher levels of amino acids showed lower variation in average rate of lay, even though egg weight variation did not differ between treatments. Reductions in egg weight were similar to those in the rate of lay. Although egg weight decreased, the reduction was 20%, 6% and 3% of the maximum egg weight recorded for the Met+Cys, Lys, and Thr trials, respectively.

Thus, egg weight was less affected than the rate of lay. The variation in egg output was lower than that observed for egg weight and rate of lay. The rate of lay and egg weight was expressed as a proportion of the maximum response obtained for each amino acid and plotted as a function of the maximal feed intake.

To obtain the best response, which is equal to 1 when $X_r = 0$, we considered $X_r = 1 - (I/I_{\max})$, where I is the amino acid intake and $I_{\max}$ is the amino acid intake that provided maximum individual response. The data for Met+Cys, Lys, and Thr were analyzed together, and the rate of lay and egg weight showed coefficients of variation of 24% and 14%, respectively, relative to the maximum value. Using the quadratic-plateau model, the following equations were applied:

Rate of lay: $Y_p = 0.81 \pm 0.02 - 1.48 \pm 0.42 \times (0.30 \pm 0.07 - X_r)^2$; if $X_r > 0.30$, then we consider $(0.30 - X_r)^2 = 0$.

Egg weight: $Y_p = 0.90 \pm 0.01 - 2.60 \pm 0.47 \times (0.55 \pm 0.03 - X_r)^2$; if $X_r > 0.55$, then we consider $(0.55 - X_r)^2 = 0$.

Observed and predicted values obtained for the rate of lay and egg weight are presented in Figures 1–3. The standard error associated with the break point was 23% for the rate of lay and 5% for egg weight.

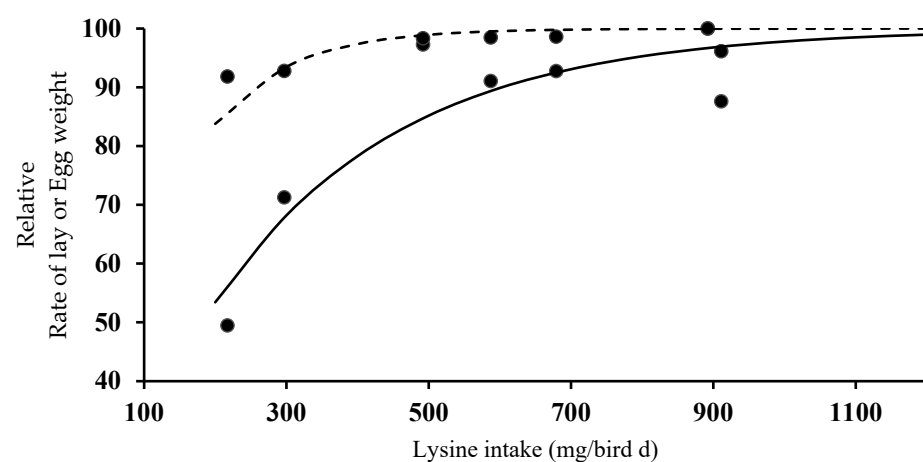


Figure 1. The relationships between Lysine and relative rate of lay (—) and egg weight (-----), where these are expressed as a proportion of the maximum rate of lay and maximum egg weight, respectively. $p < 0.05$.

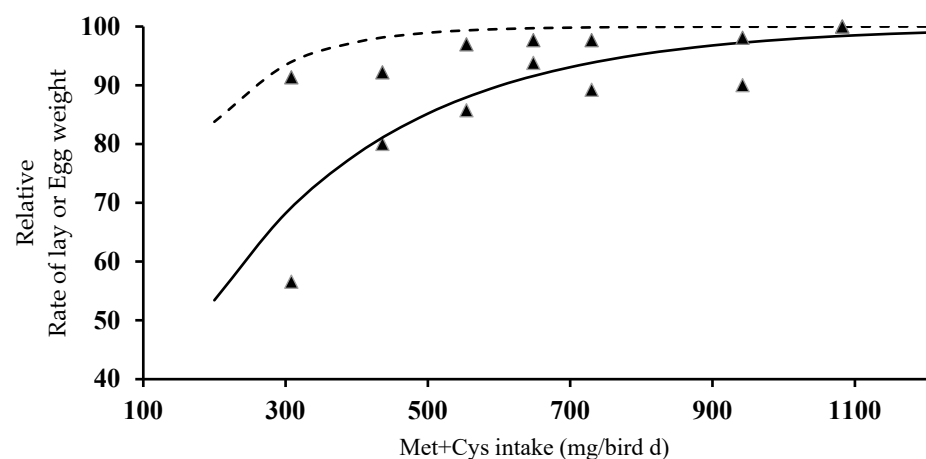


Figure 2. The relationships between Methionine+cystine intake and relative rate of lay (—) and egg weight (-----), where these are expressed as a proportion of the maximum rate of lay and maximum egg weight, respectively. $p < 0.05$.

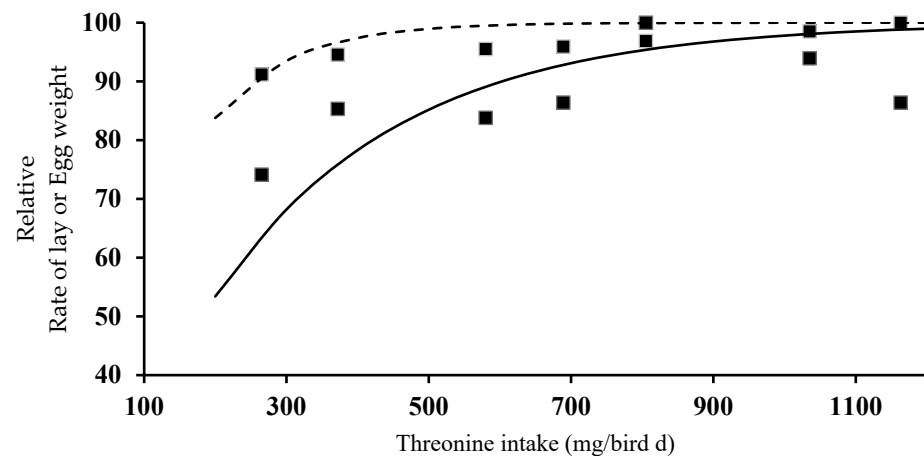


Figure 3. The relationships between threonine intake and relative rate of lay (—) and egg weight (-----), where these are expressed as a proportion of the maximum rate of lay and maximum egg weight, respectively. $p < 0.05$.

A regression model in which intake of Met+Cys, Lys, and Thr (mg/day) was considered as a function of egg output (E, g) and body weight (BW, kg) was used to determine the parameters a and b as follows:

$$i\text{Met+Cys} = 12.4 \pm 4.1 \times E + 30.2 \pm 1.1 \times \text{BW}$$

$$i\text{Lys} = 14.5 \pm 3.1 \times E + 32.2 \pm 0.9 \times \text{BW}$$

$$i\text{Thr} = 11.2 \pm 6.3 \times E + 42.4 \pm 1.2 \times \text{BW}$$

The variation coefficient for egg output was 33%, 21% and 56% for Met+Cys, Lys, and Thr, respectively. The ratio between the a coefficient of Met+Cys and Thr to that of Lys showed that the optimum ratio for egg output was 86% for Met+Cys ($100 \times 12.4/14.5$) and 77% for Thr ($100 \times 11.2/14.5$).

The variation coefficient for body maintenance was 4%, 3% and 3% for Met+Cys, Lys, and Thr, respectively. The ratio of the b coefficient for the amino acids resulted in values of optimum ratio for maintenance of 94% ($100 \times 30.2/32.2$) for Met+Cys and 132% ($100 \times 42.4/32.2$) for Thr, respectively.

The relationship between deposition of an amino acid in egg output (mg/bird d) and amino acid intake (mg/bird d) was fitted by the broken line model, according to the following equations:

$\text{DMet+Cys} = 312.6 \pm 12.5 - 0.43 \pm 0.07 \times (475.6 \pm 47.9 - \text{IcMet+Cys})$; if $\text{IcMet+Cys} > 475.6$ then we considered $(475.6 - \text{IcMet+Cys}) = 0$.

$\text{DLys} = 364.4 \pm 9.3 - 0.35 \pm 0.06 \times (546.8 \pm 45.9 - \text{IcLys})$; if $\text{IcLys} > 546.8$ then we considered $(546.8 - \text{IcLys}) = 0$.

$\text{DThr} = 254.6 \pm 12.4 - 0.18 \pm 0.08 \times (464.8 \pm 133.3 - \text{IcThr})$; if $\text{IcThr} > 464.8$ then we considered $(464.8 - \text{IcThr}) = 0$.

The errors associated with each parameter were higher for the slope (U) of the model, being 16%, 17% and 44% for Met+Cys, Lys, and Thr, respectively.

The maximum deposition predicted for the amino acid (L) by the model was higher for Lys, followed by Met+Cys and Thr. The intake for maximum deposition (R) was higher for Lys, followed by Thr and Met+Cys (547, 476 and 465 mg/bird day, respectively), resulting in a greater utilization efficiency (k) for Met+Cys, followed by Lys and Thr, with values of 0.67 mg/mg ($k = 364.4/546.8$), 0.66 mg/mg ($k = 312.6/475.6$) and 0.55 mg/mg ($k = 255/465$), respectively.

The ratio between the estimated intake of maximum deposition of Met+Cys and Thr to that of Lys indicated that the ideal ratio of amino acid deposition in the egg was 87% and 85% for Met+Cys and Thr, respectively.

4. Discussion

This study evaluated the response of post-peak production broiler breeders subjected to different intake levels of the amino acids Met+Cys, Lys, and Thr, and estimated the coefficients for egg output and body weight maintenance in hens older than 60 weeks. The results show the importance of evaluating the responses of broiler breeder hens at 60 weeks and older to amino acid intake. The methods used in this study to determine the response coefficients (*a* and *b*) might be used as a model in other studies.

Comparison of the validating diet D8 to D7 showed that the amino acid tested was limited in each trial, which was confirmed by the egg output response. This positive response was due to an increased rate of lay, because no response was observed in egg weight (Table 3). This finding agrees with that observed in other studies [1–3]. The variables feed intake and body weight were unchanged between D7 and D8.

D6 diets had the same concentration of the limiting amino acid as the D8 diets, but they had protein levels 35% higher than those of D7 and D8. Likewise, D6 had higher protein intake than D7 and D8, and showed improved responses for rate of lay, egg weight, egg output, and recovery in body weight (Table 3). This improvement was due to increased feed intake, corroborating the theory of regulation of intake and indicating an increase in voluntary intake when the concentration of a given nutrient in a balanced diet is lower [1–3,16].

As observed in the studies of Bowmaker and Gous [9], the birds consumed less than the provided 150 g/day. The maximum reduction in feed intake for Met+Cys, Lys, and Thr was 13%, 8% and 10%, respectively (Table 3). This reduction was less than that found by Bowmaker and Gous [9] of 33% for methionine and 37% for lysine.

The rate of lay in the present study differs from those in [9]. In the present study, the birds produced 32.7 (Met+Cys), 35.7 (Lys), and 49 (Thr) eggs in the last four weeks of the trial. Bowmaker and Gous [9] reported that in an equivalent period, the birds in their study produced 3.8 (lysine) and 2.4 (methionine) eggs, feeding diets with 39.7 g (lysine trial) and 40.5 g (methionine test) of CP/kg, which provided less protein intake than the requirement of 2.282 g CP/BWkg^{0.75} [10] to maintain the rate of lay. This low protein intake resulted in a mobilization of approximately −33 g (Met+Cys) and −45 g (Lys) of body protein. A higher deficit was observed in the present study: −92 g (Met+Cys) and −85 g (Lys) of body protein that were mobilized for maintenance and rate of lay.

An alternative to ensure that weight gain is zero is to use the partition coefficients obtained by multiple regression analysis, considering amino acid intake as a function of either egg output or body weight. The coefficient *b*, representing the amino acid maintenance requirement, had values for Met+Cys, Lys, and Thr of 30.2 ± 1.1 , 32.2 ± 0.9 , and 42.4 ± 1.2 mg/kg BW, respectively, which agree with other studies [26–28].

Heavier birds in the Met+Cys (5.59 kg), Lys (4.92 kg), and Thr (5.33 kg) trials used for maintenance the following values: 130 mg, 154 mg, and 224 mg, respectively. The amount of each amino acid required to produce one gram per egg was estimated at 12.4 mg, 14.5 mg, and 11.2 mg for Met+Cys, Lys, and Thr, respectively. Due to the lack of this information for these amino acids in the literature, these values were recalculated using the following partition for egg output: $a = [I - (b \times BW)]/E$, with the coefficient *b* fixed as the value determined in this study and the values for *I*, *BW* and *E* obtained from the literature [9,17]. As a result, the value of *a* found for Lys was similar to those reported by [9,23,29] and lower than the calculated value ($a = 43 \pm 11$ mg/g) described in [30].

Considering that methionine represents 52% of Met+Cys, the calculated value in [9], which was 12.9 ± 3.2 mg/g Met+Cys, and in [31], which was 13.0 ± 3.4 mg/g Met+Cys, are similar to the values found in this study but lower than the value calculated by [17], which was 21.1 ± 3.8 mg/g Met+Cys.

Due to the scarcity of information on Thr for broiler breeder hens, data from laying hens were used. The values for the coefficient a found for laying hens range from 7.4 to 9.4 mg/g, resulting in an average of 8.5 ± 0.7 mg of Thr per gram of egg [27,32–34], which is 33% lower than that observed in this study.

The ratios between the a coefficients of Met+Cys and Lys and of Thr and Lys were calculated to obtain the optimal ratio for egg output of 86 and 77% for Met+Cys/Lys and Thr/Lys, respectively. The same calculation was made using the estimated levels to maximize amino acid deposition in the egg, resulting in similar values of the optimum ratio: 87% for Met+Cys/Lys and 85% for Thr/Lys. These findings agree with those in other recommendations of [35].

Utilization efficiencies of Met+Cys, Lys, and Thr were similar to those determined for broiler breeder hens by [9,29,36]. For laying hens, the values of utilization efficiency are greater than those in this study (around 80%) [1]. One explanation for this difference can be attributed to the greater number of eggs produced per week (6–7 eggs) by laying hens, while broiler breeder hens produce two to four eggs per week. Bowmaker and Gous [9] demonstrated increases in utilization efficiency of 31% for methionine and of 21% for lysine when considering only birds with a rate of lay greater than 50%.

The relationships between amino acid intake and both rate of lay and egg weight, expressed as percentages of the maximum response, are shown in Figures 1–3. Decreased amino acid intake has a greater effect on the rate of lay than on egg weight. The two variables should not be affected when the reduction in amino acid intake is less than 18%. As was previously observed by [22], the present study showed that many individuals attain the maximum egg weight value on diets with a low protein concentration. The minimum value of egg weight in this study was 80% of the highest values observed, and the largest reduction in amino acid intake did not change egg weight. Because the bird uses the limiting amino acid efficiently, an egg above the minimum weight can be laid. This observation agrees with findings in laying hens [22] and in broiler breeder hens [9].

5. Conclusions

The results support the hypothesis by demonstrating that broiler breeders over 60 weeks of age exhibit distinct amino acid utilization patterns. The estimated amino acid requirements for the production of one gram of egg mass were 12.4 mg for Met+Cys, 14.5 mg for Lys, and 11.2 mg for Thr. Additionally, the maintenance requirements, expressed per kilogram of body weight, were 30.2 mg for Met+Cys, 32.2 mg for Lys, and 42.4 mg for Thr. These results contribute to a more accurate understanding of amino acid utilization and support the formulation of diets that meet the physiological demands of aged breeders, thereby improving the efficiency of amino acid use for both egg production and body weight maintenance.

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Abbreviations

The following abbreviations are used in this manuscript:

Daa	Amino acid deposited
B	Body maintenance
BW	Body weight
A	Conversion of dietary amino acids to egg output
Ic	Corrected intake
iLys	Daily intake of Lysine
iMet+Cys	Daily intake of methionine+cystine
iThr	Daily intake of Threonine
DMet+Cys	Deposition methionine+cystine
DLys	Deposition of lysine
DThr	Deposition of threonine
K	Efficiency of utilization
E	Egg output
EO	Egg output
EW	Egg weight
FI	Feed intake
U	Inclination/slope
I	Individual amino acid intake
Y	Individual responses
R	Intake for maximum deposition
LAAI	Limiting amino acid intake
Lys	Lysine
L	Maximum deposition predicted for the amino acid
I _{max}	Maximum individual intake
Y _{max}	Maximum individual response for each amino acid
R	Maximum response for deposition
Met+Cys	Methionine+cystine
RL	Rate of lay
Ir	Relative intake to the maximum
Thr	Threonine

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