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Dynamic Modeling for Prediction of Amino Acid Requirements in Broiler Diets

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Abstract: Accurate prediction of amino acid requirements in fast-growing broilers is crucial for cost-effective diet formulation and reducing nitrogen excretion to mitigate environmental impact. This study developed a dynamic model to predict standardized ileal digestible amino acid requirements throughout broiler growth using a factorial approach and the comparative slaughter technique, considering maintenance, growth, and gender factors. The model was based on an experiment were designed using 480 15-day-old Arbor Acres chickens randomly assigned to 10 groups. A linear equation was derived using established growth and protein deposition curves to calculate maintenance and growth coefficients. Models for five essential amino acids under different amino-acid-to-protein ratios were created ($R^2 > 0.70$). The model effectively estimated daily amino acid needs and specific time intervals. Comparisons with NRC (1994), BTPS (2011), and Arbor Acres manual (2018) showed higher predicted requirements for lysine, methionine, valine, and threonine than Arbor Acres (2018) and BTPS (2011), significantly exceeding NRC (1994). Arginine predictions aligned with BTPS in early stages, but were slightly lower in later stages. This supports the further development of dynamic amino acid models.

Keywords: protein nutrition; factorial model; maintenance; growth; poultry

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1. Introduction

Over the past few years, extensive studies have demonstrated significant advancements in precision livestock farming. Developing a dynamic and optimized amino acid (AA) supply is advantageous for enhancing feeding efficiency, reducing nitrogen (N) footprint, and minimizing the environmental impact of livestock production [1]. For decades, genetic selective breeding programs have been implemented to promote rapid growth in broilers, particularly in white-feathered broilers [2]. Amino acids are integral and potentially constraining in the feeding strategies for broiler chickens, significantly impacting their growth and overall health. Consequently, the optimal AA requirement recommendations in earlier standards, such as those provided by the NRC (1994) [3], may not be entirely suitable for modern broiler strains. Feeding strategies and nutrient compositions need to align with advancements in broiler genetic selection.

Over the past several decades, factorial models have been increasingly employed to estimate the AA requirements of broiler chickens by partitioning nutrient needs into maintenance and growth components [4,5]. In 1978, a study utilized the factorial method to calculate the AA requirements for broilers by splitting between maintenance and growth [4]. Subsequently, growth models and direct approaches were employed to estimate the maintenance and deposition requirements of nine amino acids (AAs) for Ross 308 broiler chickens [5]. Traditionally, the nitrogen equilibrium method was commonly used to estimate AA maintenance requirements [6,7]. However, the limitations of this method have become increasingly evident, as it fails to consider the potential adverse effects of low-nitrogen diets

on various aspects of body metabolism [8,9]. Similarly, in most related studies, whether using the dose–response or linear regression method, the determination of a single AA requirement typically involves controlling the concentrations of the AA under investigation in varying gradients while maintaining other AAs at levels corresponding to an ideal protein profile [10,11]. Diets in which a single essential AA is restricted as the primary dietary nutrient or provided in excess are known to inhibit feed intake and impede weight gain across various species [12]. Therefore, it is essential that AA requirement models be constructed based on an equilibrium condition, though few studies have adequately addressed this aspect. Moreover, growth-modeling techniques can be designed to predict daily nutrient requirements and allow for practical adjustments [13]. Consequently, an AA requirement model should be dynamic and adaptable. Despite this need, few dynamic AA requirement models have been developed for broilers to date.

The objectives of this study were to develop a dynamic AA requirement model capable of estimating daily AA needs and to validate the model's precision by comparing its outputs with other authoritative recommendations. We speculate that the developed model is more capable of estimating the AA requirements of broilers than previous authoritative guidelines.

2. Materials and Methods

2.1. Birds and Housing

The experiment was conducted at the experimental facility of the Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, and was approved by the Institute's Ethics Committee for Animal Welfare and Ethics (permit number: IAS 2022-154).

A total of 480 one-day-old Arbor Acres chickens from a commercial hatchery were initially reared in single-layer cages and fed a commercial diet until the commencement of the experiment. At 15 days of age, 240 male and 240 female birds with similar initial body weights were randomly selected from the flock and evenly assigned to 10 treatment groups based on gender and five dietary treatments. The birds were then transferred to single-layer cages (0.82 m × 0.70 m × 0.06 m) within climate-controlled chambers (Kooland, China), where environmental parameters were precisely regulated. The ambient temperature was set at 23 °C, the humidity was 60%, and the light program was kept at a 24 L:0 D photoperiod (L: light; D: dark). There were 6 replicates with 8 chickens in each treatment; the experimental unit was one chicken. The experiment lasted 14 days.

2.2. Diets and Feeding

In accordance with varying protein and amino acid (AA) ratios, the experimental diets were formulated into five groups (A, B, C, D, and E). Diet A was designed based on the Brazilian Tables for Poultry (2011). The protein levels in diets B and D were reduced by 1.69% and 3.38%, respectively, compared to diet A, with proportional decreases in AA levels. Conversely, the protein levels in diets C and E were also reduced by 1.69% and 3.38%, respectively, compared to diet A, but their AA levels were maintained equivalent to those in diet A. Detailed compositions of the diets at different life stages are provided in Tables 1 and 2. The AA content of the diets was determined, and the standardized ileal digestibility was based on data from the Chinese Feed Database (31st edition, 2020). To eliminate potential variability due to differing feed intake levels, the same feed intake amount was maintained across the five diet groups. The feed intake was controlled at 95% of the normal intake level during the experiment. If any feed remained at the end of a day, the intake for the following day was reduced by 2%. To ensure precise control of feed intake during the experiment, the feeding regimen was designed to restrict daily feed intake to 95% of the normal intake level. The detailed procedure was as follows: each morning, the planned amount of feed was weighed and provided to the experimental animals. Any uneaten feed was collected and weighed at a fixed time each day to record the remaining amount. If leftover feed was observed for that day, it was considered an

indication that the animals' actual feed requirement was lower than the planned intake. Consequently, the feed allowance for the following day was reduced by 2% based on the previous day's actual feed intake. This adjustment was repeated daily to ensure that the animals' feed intake remained stable and aligned with the experimental goal of maintaining 95% of normal intake.

Table 1. Composition and nutrient levels of 5 treatment diets for broilers (15–21 d).

Items	A	B	C	D	E
Diet components (%)					
Corn	53.84	48.99	48.99	44.15	44.15
Soybean meal	36.5	33.22	33.22	29.93	29.93
Corn gluten meal	0	8.7	8.45	17.37	16.25
Soybean oil	5.4	4.91	5	4.43	4.86
Limestone	0.85	0.84	0.84	0.84	0.84
Dicalcium phosphate	1.80	1.85	1.85	1.90	1.90
Salt	0.30	0.30	0.30	0.30	0.30
Methionine	0.23	0.21	0.26	0.19	0.28
Lysine	0.29	0.26	0.40	0.24	0.51
Threonine	0.17	0.15	0.23	0.14	0.29
Valine	0.05	0.05	0.12	0.04	0.20
Premix [1]	0.50	0.50	0.50	0.50	0.50
Determined component [2]					
MEn (Kcal/kg)	3054	3050	3060	3055	3050
CP	21.77	19.81	20.08	17.85	18.39
Calcium	0.85	0.85	0.85	0.85	0.85
Phosphorus	0.41	0.41	0.41	0.41	0.41
Lysine	1.22	1.11	1.22	1.00	1.21
Methionine	0.50	0.45	0.49	0.41	0.51
Methionine + Cystine	0.79	0.72	0.77	0.65	0.74
Threonine	0.82	0.75	0.80	0.67	0.81
Tryptophan	0.22	0.20	0.21	0.18	0.19
Arginine	1.34	1.21	1.22	1.11	1.10
Histidine	0.49	0.44	0.43	0.40	0.41
Isoleucine	0.77	0.71	0.70	0.63	0.64
Leucine	1.56	1.41	1.42	1.28	1.29
Cystine	0.30	0.27	0.28	0.24	0.25
Phenylalanine	0.92	0.82	0.83	0.76	0.75
Tyrosine	0.61	0.57	0.56	0.50	0.50
Valine	0.89	0.82	0.89	0.73	0.88

Abbreviations: A, B, C, D, E = 5 different protein concentration groups; MEn = N-corrected metabolizable energy; CP = crude protein. Diet A was formulated based on the Brazilian Tables for Poultry (2011). Diets B and D had protein levels reduced by 1.69% and 3.38%, respectively, with proportional decreases in amino acids (AA), while diets C and E had the same protein reductions with B and D, respectively, but maintained AA levels equivalent to diet A. [1] Premix provided per kilogram of diet: Vitamin A 10,000 IU; Vitamin D3, 4500 IU; Vitamin E, 65 mg; Vitamin K3, 3.0 mg; Vitamin B1, 2.5 mg; Vitamin B2, 6.5 mg; Vitamin B6, 3.2 mg; Vitamin B12, 17 μg; calcium pantothenate, 18 mg; niacin, 60 mg; folic acid, 1.9 mg; biotin, 0.18 mg; choline chloride, 1020 mg; Cu, 16 mg; Fe, 20 mg; Zn, 110 mg; Mn, 120 mg; Se, 0.3 mg; I, 1.25 mg. [2] All components were determined beforehand and the AAs were expressed as the standardized ileal digestible concentrations.

Table 2. Composition and nutrient levels of the 5 treatment diets for broilers (21–28 d).

Items	A	B	C	D	E
Diet components (%)					
Corn	59.30	53.96	53.96	48.63	48.63
Soybean meal	31	28.21	28.21	25.42	25.42
Corn gluten meal	0	8.85	8.32	17.26	16.00
Soybean oil	5.50	5.01	5.20	4.68	5.15
Limestone	0.99	1.00	1.00	1.00	1.00
Dicalcium phosphate	1.44	1.44	1.44	1.48	1.48
Salt	0.20	0.20	0.20	0.20	0.20

Table 2. *Cont.*

Items	A	B	C	D	E
Methionine	0.20	0.18	0.22	0.16	0.25
Lysine	0.33	0.30	0.43	0.27	0.53
Threonine	0.12	0.11	0.18	0.09	0.25
Valine	0.11	0.10	0.18	0.09	0.25
Premix [1]	0.50	0.50	0.50	0.50	0.50
Determined component [2]					
MEn (Kcal/kg)	3110	3100	3115	3090	3095
CP	19.88	18.09	18.34	16.34	16.81
Calcium	0.80	0.80	0.80	0.80	0.80
Phosphorus	0.33	0.33	0.33	0.33	0.33
Lysine	1.12	1.03	1.13	0.93	1.11
Methionine	0.45	0.41	0.46	0.37	0.45
Methionine + Cystine	0.79	0.66	0.71	0.59	0.68
Threonine	0.71	0.64	0.70	0.58	0.70
Tryptophan	0.19	0.18	0.18	0.16	0.16
Arginine	1.18	1.07	1.08	0.98	0.97
Histidine	0.44	0.41	0.40	0.36	0.37
Isoleucine	0.68	0.61	0.62	0.56	0.55
Leucine	1.45	1.31	1.32	1.18	1.19
Cystine	0.28	0.27	0.26	0.23	0.24
Phenylalanine	0.83	0.74	0.73	0.68	0.69
Tyrosine	0.56	0.50	0.51	0.47	0.46
Valine	0.88	0.79	0.87	0.71	0.86

Abbreviations: A, B, C, D, E = 5 different protein concentration groups; MEn = N-corrected metabolizable energy; CP = crude protein. Diet A was formulated based on the Brazilian Tables for Poultry (2011). Diets B and D had protein levels reduced by 1.69% and 3.38%, respectively, with proportional decreases in amino acids (AA), while diets C and E had the same protein reductions with B and D, respectively, but maintained AA levels equivalent to diet A. [1] Premix provided per kilogram of diet: Vitamin A, 9000 IU; Vitamin D3, 4000 IU; Vitamin E, 55 mg; Vitamin K3, 2.2 mg; Vitamin B1, 2.2 mg; Vitamin B2, 5.4 mg; Vitamin B6, 2.2 mg; Vitamin B12, 11 µg; calcium pantothenate, 15 mg; niacin, 45 mg; folic acid, 1.6 mg; biotin, 0.15 mg; choline chloride, 950 mg; Cu, 16 mg; Fe, 20 mg; Zn, 110 mg; Mn, 120 mg; Se, 0.3 mg; I, 1.25 mg. [2] All components were determined beforehand and the AAs were expressed as the standardized ileal digestible concentrations.

2.3. Body Composition Analysis

2.3.1. Isolation of Feather and Carcass

Two birds were randomly selected and weighed from each replicate. They were then euthanized in a carbon dioxide chamber following a 12 h fasting period at 8:00 a.m. on days 14, 21, and 28 of the experiment. The entire birds were immersed in water at 70–80 °C to facilitate feather removal using a defeathering machine. The carcasses were weighed and rapidly cryopreserved to prevent body fluid loss.

2.3.2. Carcass Samples Processing

This process was conducted according to the preprocessing of the Chinese standard [14]. The carcass samples were initially ground into a paste using a grinder, followed by further grinding with a smaller grinder. A 200 g portion of the homogenized sample was collected using the quartering method and placed in a tin box. The samples were sterilized at 105 °C for 15 min and then dried in an oven at 65 °C for 72 h. The weight difference was recorded, and the dried samples were ground into a fine powder for subsequent analysis.

2.3.3. Feather Sample Processing

This process was conducted according to the preprocessing of the Chinese standard [14]. Feather samples from each replicate were collected and placed in tin boxes. These samples were dried in an oven at 65 °C for 72 h and then ground into a powder for further analysis.

2.3.4. Determination of Amino Acid Content

The protein content in both carcass and feather samples was determined using an automatic Kjeldahl nitrogen analyzer, following the Chinese standard [14]. The amino acid content was assessed with an automatic amino acid analyzer, adhering to the procedure outlined in the Chinese standard [15]. The process involved oxidation of carcass and feather samples using peroxyformic acid, followed by the addition of sodium metabisulfite and 6 mol/L hydrochloric acid. The samples were hydrolyzed in a constant-temperature drying oven at 110 °C for 24 h, adjusted to a pH of 2.2, and analyzed using a Hitachi S-8900 automatic amino acid analyzer (Hitachi Ltd., Tokyo, Japan).

2.4. Calculation and Statistical Analysis

The modeling approach utilized in this study is based on the factorial equation, which partitions amino acid (AA) requirements into maintenance and growth components as follows: $AA\ intake = M \times BW^{0.666} + D \times AA\ growth$, where "BW" denotes body weight. The equation is linearized by dividing all terms by $BW^{0.666}$, resulting in a relationship between AA intake and deposition. In this form, "M" represents the maintenance requirement (intercept), while "D" indicates the utilization efficiency of a single AA (slope). Growth and protein deposition curves developed in a previous study were used for calculations [16]. The growth curves are:

Male:

$$BWt = 6025.6 \times e^{-4.7847 \times e^{-0.0481 \times t}}$$

Female:

$$BWt = 5086.6 \times e^{-4.6929 \times e^{-0.0503 \times t}}$$

The protein deposition curves for the carcasses and feathers are:

Male:

$$\text{carcass}: \ CPt = 963.3 \times e^{-5.623 \times e^{-0.059 \times t}}$$

$$\text{feather}: \ FPt = 173 \times e^{-5.371 \times e^{-0.043 \times t}}$$

Female:

$$\text{carcass}: \ CPt = 770 \times e^{-5.395 \times e^{-0.061 \times t}}$$

$$\text{feather}: \ FPt = 107 \times e^{-5.172 \times e^{-0.059 \times t}}$$

where CP and FP represent carcass and feather protein deposition, respectively, and "t" denotes age (days). AA growth is calculated as $AA\ growth = PRC \times AAc + PRF \times AAf$, where "PRC" and "PRF" are carcass and feather protein depositions, and "AAc" and "AAf" are the proportions of specific AAs in carcass and feather proteins, respectively. These values are based on our prior findings [16].

Statistical analyses were performed using SPSS software (version 23.0, SPSS Inc., Chicago, IL, USA). Data are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was conducted to evaluate differences in feed intake, weight gain, and AA content between male and female groups. The Shapiro–Wilk test was used for normality checks, and Levene's test confirmed the homogeneity of variances. Tukey's multiple comparison test was applied for post hoc analysis. The sample size for each group was 24. p-values are reported in the results section, and all tests were two-tailed. Pearson's correlation coefficient was calculated to assess the linear relationship between feed intake and growth, and regression analysis was conducted to determine "M" and "D" values from the five diet treatments. A dynamic AA requirement model, with age as the independent variable, was developed, and its predicted values were compared with the recommendations from NRC (1994), BTPS (2011), and the Arbor Acres Feeding Manual (2018) [17].

3. Results

3.1. Growth Performance

Table 3 shows the growth performance of birds fed five different diets under consistent feed intake conditions. The data represent average values recorded during the 15–21 d and 22–28 d growth periods. The results showed no statistically significant difference ($p > 0.05$) in growth performance between group A and group C for either male or female birds, indicating similar growth outcomes between these groups. In contrast, birds in group A and group C grew significantly faster than those in group B and group D ($p < 0.05$), highlighting the superior effectiveness of these diets in promoting growth. Further analysis revealed that diets with the same dilution ratio but different essential amino acid (EAA) levels (e.g., groups B vs. C and groups D vs. E) yielded distinct growth results. Specifically, supplementing diets with EAAs significantly enhanced growth performance, even when the overall dietary protein and amino acid concentrations were low. These findings emphasize the critical role of EAA supplementation in supporting growth under conditions of reduced protein content, as demonstrated by the performance differences across the dietary groups.

Table 3. Growth performance between 5 diet treatments of different genders in broilers (15–21 days) [1].

Age (wk)	FI (g)		Treatments	Gender	
	Male	Female		Male	Female
3	530	518	A	440.20 ± 5.38 [a]	430.18 ± 4.69 [a]
			B	400.02 ± 4.39 [b]	391.41 ± 4.33 [b]
			C	439.98 ± 4.85 [a]	428.20 ± 4.68 [a]
			D	360.03 ± 3.20 [c]	351.84 ± 4.87 [c]
			E	418.60 ± 4.21 [b]	410.50 ± 4.28 [a]
4	783	742	A	533.02 ± 5.83 [a]	505.19 ± 5.66 [a]
			B	485.50 ± 5.91 [b]	460.66 ± 5.20 [b]
			C	531.03 ± 6.25 [a]	503.24 ± 6.13 [a]
			D	437.60 ± 6.30 [c]	414.07 ± 5.21 [c]
			E	510.05 ± 6.11 [b]	485.5 ± 5.12 [b]

Abbreviations: wk = week; FI = feed intake; A, B, C, D, E = 5 different protein concentration treatments. Mean ± SE of performance data. [a,b,c] Values within a column with different superscripts differ significantly at $p < 0.05$.

3.2. Protein Content

Table 4 shows the protein content in carcass and feather samples across different diet groups, stratified by gender and age. The results indicated no significant differences ($p > 0.05$) in the protein contents of carcasses and feathers among the diet groups. Consequently, the mean values for protein content were calculated and used to represent the amino acid (AA) content in carcass and feather samples for each group. These averages were used for further comparisons and analysis.

Table 4. Protein content of carcasses and feathers in broilers of different genders and ages (15–28 d).

Gender	Age (d)	Item			
		CP in Carcass (%)	Carcass Weight/Body Weight (%)	CP in Feather (%)	Feather Weight/Body Weight (%)
Male	15	18.75 ± 0.01	98.10 ± 0.24	0.83 ± 0.01	2.06 ± 0.23
	21	18.98 ± 0.01	97.50 ± 0.31	0.85 ± 0.02	2.49 ± 0.19
	28	19.80 ± 0.02	97.48 ± 0.18	0.90 ± 0.01	2.57 ± 0.15
Female	15	15.51 ± 0.01	97.08 ± 0.41	0.86 ± 0.01	2.85 ± 0.12
	21	18.27 ± 0.02	96.94 ± 0.33	0.86 ± 0.02	3.01 ± 0.23
	28	18.20 ± 0.01	96.90 ± 0.35	0.92 ± 0.01	2.97 ± 0.21

Abbreviations: d = day; CP = crude protein. Mean ± SE of protein content data. No differences were detected among the effects tested at $p > 0.05$.

3.3. Amino Acid Compositions

The AA compositions of both carcasses and feathers are presented in Table 5. Our findings indicate that age (15; 21; 28 days old), gender, and diet (different protein concentration) exert a minimal influence, which can be considered negligible, on the AA composition, leading to the AA compositions being expressed as mean values.

Table 5. Amino acid compositions of carcasses and feathers in broilers within 15–28 days of age (proportion of protein).

Amino Acid	Carcass (%)	Feather (%)
Cysteine	0.80 ± 0.01	5.90 ± 0.04
Methionine	2.53 ± 0.04	0.45 ± 0.08
Aspartic acid	8.30 ± 0.07	6.49 ± 0.06
Threonine	3.98 ± 0.06	4.48 ± 0.02
Serine	3.75 ± 0.03	10.39 ± 0.02
Glutamic acid	13.15 ± 0.12	10.53 ± 0.13
Glycine	7.10 ± 0.06	6.76 ± 0.22
Alanine	6.18 ± 0.05	4.15 ± 0.05
Valine	5.38 ± 0.06	6.54 ± 0.03
Isoleucine	4.20 ± 0.07	4.42 ± 0.06
Leucine	7.12 ± 0.03	7.76 ± 0.04
Tyrosine	2.05 ± 0.07	1.60 ± 0.05
Phenylalanine	3.75 ± 0.07	4.59 ± 0.07
Lysine	6.85 ± 0.04	2.13 ± 0.05
Histidine	2.34 ± 0.02	0.79 ± 0.03
Arginine	6.90 ± 0.05	6.88 ± 0.09
Proline	4.38 ± 0.08	8.44 ± 0.07

3.4. Amino Acid Requirements Models

The parameters (M and D) values for the models of lysine, methionine, valine, threonine, and arginine are shown in Table 6. The values are presented as the male and female average. The M represents the requirement for maintenance per metabolic body weight. The highest need was for valine at 195 mg/$BW^{0.666}$, followed by threonine, arginine, and lysine, and the lowest one was methionine at 77.5 mg/$BW^{0.666}$. The D represents the utilization efficiency of AA. The highest efficiency of use was 88.11% for valine, followed by arginine, lysine, and threonine, and the lowest efficiency of use was 68.9%. The daily AA requirements were computed utilizing the above model and the models presented below:

Table 6. Parameters of the equation for broilers of different genders [1].

Gender	AA	Parameter		R^2
		M	D	
Male	Lysine	0.082	1.240	0.93
	Methionine	0.067	1.496	0.85
	Valine	0.190	1.170	0.81
	Threonine	0.099	1.279	0.88
	Arginine	0.049	1.201	0.73
Female	Lysine	0.090	1.230	0.92
	Methionine	0.088	1.408	0.82
	Valine	0.200	1.135	0.80
	Threonine	0.180	1.220	0.84
	Arginine	0.060	1.220	0.75

[1] The equation: $\frac{amino\ acid\ intake}{BW^{0.666}} = M \pm \frac{D \times amino\ acid\ growth}{BW^{0.666}}$.

Male:

$$Lysine\ intake = 0.082 \times BW^{0.666} + 1.23 \times AA\ growth$$

$$Methionine\ intake = 0.067 \times BW^{0.666} + 1.496 \times AA\ growth$$

$$Valine\ intake = 0.082 \times BW^{0.666} + 1.17 \times AA\ growth$$

$$Threonine\ intake = 0.099 \times BW^{0.666} + 1.279 \times AA\ growth$$

$$Arginine\ intake = 0.049 \times BW^{0.666} + 1.201 \times AA\ growth$$

Female:

$$Lysine\ intake = 0.09 \times BW^{0.666} + 1.23 \times AA\ growth$$

$$Methionine\ intake = 0.088 \times BW^{0.666} + 1.408 \times AA\ growth$$

$$Valine\ intake = 0.2 \times BW^{0.666} + 1.135 \times AA\ growth$$

$$Threonine\ intake = 0.18 \times BW^{0.666} + 1.22 \times AA\ growth$$

$$Arginine\ intake = 0.23 \times BW^{0.666} + 1.034 \times AA\ growth$$

The specific daily predicted values are shown in Table 7 (with lysine as an illustrative example). Lastly, for validation, model predictions were compared with recommended values from NRC (1994), BTPS (2011), and the AA Feeding Manual (2018), and the results are detailed in Table 8.

Table 7. Amino acid requirement prediction analysis for 42 days in broilers of different genders.

Age (d)	Feed Intake (g)	Male		Female	
		Requirement (g/kg)	Content in Diet	Requirement (g/kg)	Content in Diet
1	13.90	0.212	0.015	0.212	0.015
2	15.70	0.250	0.015	0.250	0.017
3	17.29	0.292	0.015	0.292	0.014
4	23.70	0.338	0.014	0.337	0.012
5	30.02	0.387	0.012	0.386	0.012
6	36.23	0.440	0.011	0.439	0.011
7	42.35	0.496	0.012	0.495	0.011
8	48.37	0.560	0.011	0.629	0.012
9	54.30	0.621	0.012	0.698	0.012
10	60.12	0.685	0.012	0.769	0.012
11	65.85	0.750	0.012	0.842	0.012
12	71.48	0.816	0.012	0.916	0.012
13	77.01	0.883	0.012	0.991	0.012
14	82.44	0.949	0.012	1.066	0.012
15	87.78	1.015	0.012	1.111	0.012
16	93.01	1.184	0.014	1.182	0.012
17	98.15	1.253	0.013	1.251	0.012
18	103.19	1.320	0.013	1.317	0.012
19	108.14	1.384	0.013	1.381	0.012
20	112.98	1.445	0.013	1.442	0.012
21	117.73	1.502	0.013	1.499	0.012
22	122.38	1.595	0.012	1.592	0.012
23	126.93	1.645	0.012	1.642	0.012
24	131.38	1.691	0.011	1.688	0.012
25	135.74	1.732	0.013	1.730	0.012
26	139.99	1.769	0.013	1.767	0.012
27	144.15	1.801	0.013	1.799	0.012
28	148.21	1.828	0.012	1.826	0.012
29	152.18	1.899	0.012	1.897	0.012
30	156.04	1.917	0.012	1.915	0.012
31	159.81	1.930	0.012	1.929	0.011
32	163.48	1.939	0.012	1.938	0.011
33	167.05	1.944	0.012	1.943	0.011
34	170.52	1.944	0.011	1.944	0.011

Table 7. *Cont.*

Age (d)	Feed Intake (g)	Male		Female	
		Requirement (g/kg)	Content in Diet	Requirement (g/kg)	Content in Diet
35	173.90	1.941	0.011	1.941	0.010
36	177.17	1.934	0.011	1.935	0.010
37	180.35	1.923	0.010	1.925	0.010
38	183.43	1.909	0.010	1.911	0.010
39	186.42	1.892	0.010	1.895	0.009
40	189.30	1.873	0.010	1.876	0.009
41	192.09	1.851	0.010	1.854	0.009
42	194.78	1.825	0.010	1.829	0.009

Table 8. Comparison of the predicted values of amino acid requirements with standard manuals.

AA	Gender	Male						Female					
	Age (wk)	1	2	3	4	5	6	1	2	3	4	5	6
Lysine	Estimate [1]	1.37	1.30	1.31	1.23	1.17	1.02	1.40	13.30	13.30	1.24	1.10	0.95
	NRC [2]		1.10			1.00			1.10			1.00	
	BTPS [3]	1.32	1.22		1.13		1.06	1.34	1.2		1.06		0.93
	Arbor Acres manual [4]	1.28	1.15		1.02		0.96	1.28	1.15		1.02		0.96
Methionine	Estimate	0.60	0.59	0.55	0.51	0.49	0.43	0.57	0.47	0.44	0.44	0.42	0.40
	NRC		0.50			0.38			0.50			0.38	
	BTPS	0.52	0.48		0.45		0.41	0.52	0.47		0.43		0.37
	Arbor Acres manual	0.51	0.47		0.43		0.4	0.51	0.47		0.43		0.4
Valine	Estimate	1.26	1.13	1.07	1.01	0.98	0.87	1.24	0.99	0.95	0.97	0.92	0.80
	NRC		0.90			0.83			0.90			0.83	
	BTPS	1.02	0.94		0.88		0.79	1.03	0.92		0.82		0.72
	Arbor Acres manual	0.96	0.87		0.78		0.73	0.96	0.87		0.78		0.73
Threonine	Estimate	0.97	0.86	0.86	0.82	0.78	0.69	0.98	0.83	0.83	0.85	0.79	0.70
	NRC		0.80			0.74			0.80			0.74	
	BTPS	0.86	0.79		0.74		0.69	0.87	0.78		0.69		0.60
	Arbor Acres manual	0.86	0.77		0.68		0.64	0.86	0.77		0.68		0.64
Arginine	Estimate	1.39	1.27	1.29	1.17	1.08	0.93	1.46	1.20	1.19	1.18	1.17	0.91
	NRC		1.25			1.10			1.25			1.10	
	BTPS	1.43	1.32		1.22		1.14	1.44	1.30		1.14		1.00
	Arbor Acres manual	1.37	1.23		1.09		1.03	1.37	1.23		1.09		1.03

Abbreviations: wk = week; AA= amino acid; NRC = National Research Council; BTPS = Brazilian tables for poultry and swine. [1] The prediction value by using this model for broilers of different genders within 6 weeks. [2] This recommendation provides 1 value within every 3 weeks. [3] This recommendation provides 1 value in weeks 1 and 6, but provides 2 values during weeks 2 to 5. [4] This recommendation provides 1 value in week 1 and 6, but provides 2 values during weeks 2 to 5.

4. Discussion

The factorial model exhibited increasing advantages in estimating livestock AA requirements. It provided a simplified yet systematic framework, and the maintenance requirement and growth requirement of AA were analyzed separately. The main task in the present approach is to figure out the functional relationship between the AA requirement, BW, and growth. However, that relationship is not linear. Therefore, we divided by the $BW^{0.666}$ to ensure the efficiency of the calculation. The slope of this equation can be seen as the AA utilization efficiency, whereas the intercept is the AA requirement for maintenance. Numerous studies have been conducted on the maintenance requirements of various amino acids in broiler chickens. In this study, the maintenance requirements were higher than those of a previous study [5]. This might be attributed to differences in the genetic strain

and environmental conditions used in the respective research, as well as advancements in broiler breeding that have resulted in increased metabolic demands. The maintenance requirements for threonine and lysine in this study fall to 140 mg/$BW^{0.666}$ and 86 mg/$BW^{0.666}$, respectively, which is within a reasonable range when compared with prior research [18,19]. These results highlight the importance of continuously updating nutritional models to reflect the evolution of broiler genotypes and the ever-changing environmental factors faced by modern poultry farming. The maintenance requirement for valine observed in this study was relatively higher than 46.5 mg/$BW^{0.666}$ and 111 mg/$BW^{0.666}$ in previous research [19,20]. One possible explanation for this is the variation in the modeling approach and the composition of the diet provided. Different methodologies can yield variations in the reported requirements, as well as variations in protein synthesis and the metabolic pathways associated with specific amino acids. Additionally, while our study adhered to standardized procedures and used a controlled environment, subtle differences in diet composition, such as the presence of non-essential amino acids or the level of digestible protein, could impact maintenance requirements [21]. This underlines the necessity for more comprehensive studies to assess how slight adjustments in diet composition can influence amino acid requirements across different broiler breeds. The choice of the mathematical model for calculating AA requirements can also impact the profiles of AA requirements [21,22]. In this study, the highest utilization efficiency was observed for valine, while the lowest was for methionine. This is noteworthy as all amino acids demonstrated similar utilization efficiencies, yet these were significantly higher than those in a previous study [5]. The increased efficiency may reflect the progress in feed composition and the genetic selection of broilers over the past two decades. The lower efficiency of methionine can be attributed to its partial conversion into cysteine, a known biochemical pathway that impacts its overall utilization in protein synthesis [23]. Understanding these interactions can help refine feed formulations that optimize the cost and effectiveness of amino acid supplementation.

The AA pattern was not largely influenced by diet, gender, or environmental factors, according to Baker and Han (1994) [24]. They suggested representing the AA needs of poultry as an optimal proportion relative to lysine. Our study corroborated this, showing that the AA composition pattern in carcasses and feathers was consistent across different diets and genders. This indicates that modern broilers, when fed balanced diets, may have the ability to maintain a stable AA profile irrespective of moderate changes in environmental conditions or dietary composition. Such findings emphasize the robustness of AA metabolism in well-adapted poultry breeds and point toward the importance of ensuring a balanced AA intake over focusing on individual amino acid variability. Studies have shown that, when calculating AA requirements, it is more accurate to relate them to protein rather than body weight [15]. Thus, we determined the protein and AA content for calculating the AA growth based on particular amino acids' proportions in the carcasses and feathers. The different performances between A, C and B, D were evident due to the lower AA concentrations in groups B and D. Protein synthesis requires an adequate supply of AAs, and a lack of any amino acid may limit protein synthesis [25]. Our results indicated that the protein proportions in carcasses and feathers were unaffected by diet, likely due to the balanced AA content in our experimental diets. This further highlights that, when all essential amino acids are present in sufficient amounts, protein deposition remains stable. It underscores the critical need for a comprehensive approach to formulating diets that consider interactions among different amino acids to maintain optimal growth and protein synthesis.

Five types of AA requirements were determined using this model. These amino acids are crucial in practical production as they support essential biochemical functions [26]. The primary amino acids analyzed, such as methionine, lysine, and threonine, are often the limiting factors in poultry diets [27,28]. The findings of this study, when compared with established standards such as NRC (1994), BTPS (2011) [29], and the Arbor Acres manual (2018), revealed that our predicted values were generally higher, indicating that

broiler chickens bred under modern conditions might have greater nutritional demands. This aligns with evidence that broiler strains have been selectively bred for rapid growth, which increases their metabolic and nutritional requirements [30]. Such updated models can help refine industry standards to better support current and future broiler populations. When comparing the AA requirements of males and females, it was evident that during the first week, females required higher amounts of lysine, arginine, and threonine than males. However, as the weeks progressed, males' requirements surpassed those of females, consistent with the BTPS (2011) data. The requirements for methionine and valine were slightly higher for males throughout the experimental period, aligning with previous findings [31]. These gender-specific differences underline the necessity for tailored feeding programs that can optimize growth and nutrient efficiency for both male and female broilers. By addressing these nuanced needs, producers can enhance overall flock performance and reduce feed costs associated with imprecise nutrient delivery.

In conclusion, the model's applicability for practical feeding programs highlights its potential as a valuable tool for optimizing nutrient delivery and improving sustainability in broiler production. The insights from this research could guide future investigations into precision nutrition and support the advancement of feeding strategies that respond to ongoing changes in poultry genetics and industry practices.

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