105 (2026) 106449

Contents lists available at ScienceDirect

Poultry Science

journal homepage: www.elsevier.com/locate/psj

Full-Length Article

Interactive effects of dietary total sulfur amino acids and crude protein on growth performance in broiler chickens: Meta-analysis and experimental validation

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ARTICLE INFO

Scientific section: Metabolism and Nutrition

Keywords:
Broiler chicken
Crude protein
Growth performance
Meta-analysis
Total sulfur amino acid

ABSTRACT

This study conducted the meta-analysis of interactive effects of dietary total sulfur amino acids (TSAA) and crude protein (CP) on average daily feed intake (ADFI), average daily gain (ADG), and feed conversion ratio (FCR) in broiler chickens, aiming to estimate phase-dependent optimal digestible TSAA levels across dietary CP levels. Moreover, the optimal digestible TSAA levels with different CP levels, which were identified in the meta-analysis, were further validated using a broiler feeding trial. In the meta-analysis, a total of 37 studies published between 2007 and 2024 were included. An initial full quadratic model with CP and digestible TSAA showed limited significance of model coefficients with little biological interpretation across growth phases. Therefore, a simplified quadratic model was employed, incorporating the product of digestible TSAA and CP (TSAA × CP) as a single interactive term with quadratic effects. The results indicated significant coefficients ($P < 0.05$) for ADG and FCR in the starter phase, ADFI and FCR in the grower phase, FCR in the finisher phase, and ADG and FCR in the overall growth phase. Nonlinear interactions for FCR were consistent across all phases. Model performance evaluations also indicated that FCR was the most reliable variable for predicted responses, exhibiting the least prediction errors and between-study heterogeneity. Model simulations across fixed CP levels from 16.0% to 24.0% revealed that optimal digestible TSAA levels for ADFI, ADG, and FCR increased as dietary CP decreased, with an increment of approximately 0.02 to 0.07 percentage points in digestible TSAA for each 0.5-percentage point reduction in dietary CP. Experimental validation trial further supported these predictions, demonstrating that diets formulated with the model-derived optimal digestible TSAA levels decreased FCR ($P < 0.05$) compared with diets following their commercial recommendations, particularly under reducing CP levels. In conclusion, this meta-analysis suggested that optimal digestible TSAA levels required to maximize growth performance in broiler chickens consistently increased with reducing CP levels in diets across all growth phases.

Introduction

Dietary protein is an essential nutrient for the supply of essential and non-essential amino acids (**AA**) in animals. Moreover, maintaining the balance of AA composition in addition to dietary protein is critical for achieving optimal performance and health in animals (Wu, 2014). In poultry, total sulfur AA (**TSAA**), comprising methionine (**Met**) and cysteine (**Cys**), are of particular interest to nutritionists because TSAA are typically considered the first limiting AA in corn-soybean meal--based diets with significant impacts on performance and product quality in poultry (Lemme, 2003). It has been suggested that appropriate TSAA levels should be adjusted according to crude protein (**CP**) levels in diets because dietary CP intake is closely associated with Cys intake, thereby affecting Met metabolism involved in Cys synthesis (Pacheco et al., 2018; Wilfred et al., 2024).

Low-CP diets currently receive an increasing attention in the broiler industry as a nutritional strategy to mitigate environmental issues resulting from excessive nitrogen excretion (Chalova et al., 2016). This low-CP diet is generally formulated by reducing inclusion of intact protein ingredients with additional synthetic AA supplementations (Liu et al., 2021). However, reducing CP levels by more than 3% below its recommendations has been demonstrated to impair growth performance and meat quality in broiler chickens, despite adequate supplementation of essential AA in diets (Bregendahl et al., 2002; van Harn et al., 2019; de

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https://doi.org/10.1016/j.psj.2026.106449
Received 24 November 2025; Accepted 13 January 2026
Available online 14 January 2026
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Rauglaudre et al., 2023). These negative outcomes of feeding low-CP diets may be partly attributed to the possible changes in the optimal AA balance in low-CP diets. A meta-analysis reported that broiler performance may be influenced by the interaction between dietary CP and lysine levels, with lower CP levels requiring a higher lysine:CP ratio in broiler diets (Faridi et al., 2015). Therefore, it is necessary to clarify the relationship between digestible TSAA and CP levels in the diet to optimize broiler performance, especially under feeding low-CP diets. However, the available information on the interactive effects between digestible TSAA and CP levels is still limited.

A meta-analysis in animal nutrition quantitatively integrates independent studies to estimate overall effects with a greater precision and assess heterogeneity, thereby allowing for identification of generalizable nutrient-response relationships (Faridi et al., 2015; Gurevitch et al., 2018). For dietary TSAA or Met in broiler chickens, most meta-analyses have addressed their impacts on broiler performance, including dose-dependent responses and comparisons of Met sources, but have not accounted for dietary CP levels as a modifying factor (Sauer et al., 2008; Uddin et al., 2022). To our knowledge, no comprehensive meta-analysis of available data regarding interactive effects of digestible TSAA and CP in diets on broiler performance has been conducted. Moreover, nutritional models developed from the meta-analysis may be required for further validation under a practical feeding condition to improve their applicability; however, such validation is rarely conducted in poultry nutrition.

Therefore, the current meta-analysis aimed to investigate the interactive effects of digestible TSAA and CP in broiler diets, with the specific objective of identifying optimal digestible TSAA levels at different CP levels to maximize growth performance in broiler chickens across growth phases. Furthermore, an experimental validation trial was conducted to evaluate the predictive accuracy of our model-derived optimal digestible TSAA levels under varying CP levels in broiler diets.

Materials and methods

Inclusion and exclusion criteria

The process of literature search, selection, and evaluation of published articles for the present meta-analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (**PRISMA**) guidelines (Fig. 1; Moher et al., 2009). A comprehensive search was conducted among multiple scientific databases, including Scopus, PubMed, and Web of Science, to identify studies investigating the effect of digestible TSAA levels at varying CP levels in diets on growth performance in broiler chickens. The keywords of 'broiler chickens', 'sulfur amino acids', 'methionine', 'low protein', and 'protein level' were primarily used in this search. Dietary treatments included in the meta-analysis were selected from the initial pool based on predefined eligibility criteria, with a focus on studies published between 2005 and 2024. The publication period was selected to reflect modern commercial broiler genotypes and contemporary feeding practices, while also improving the comparability of nutrient specification and reporting

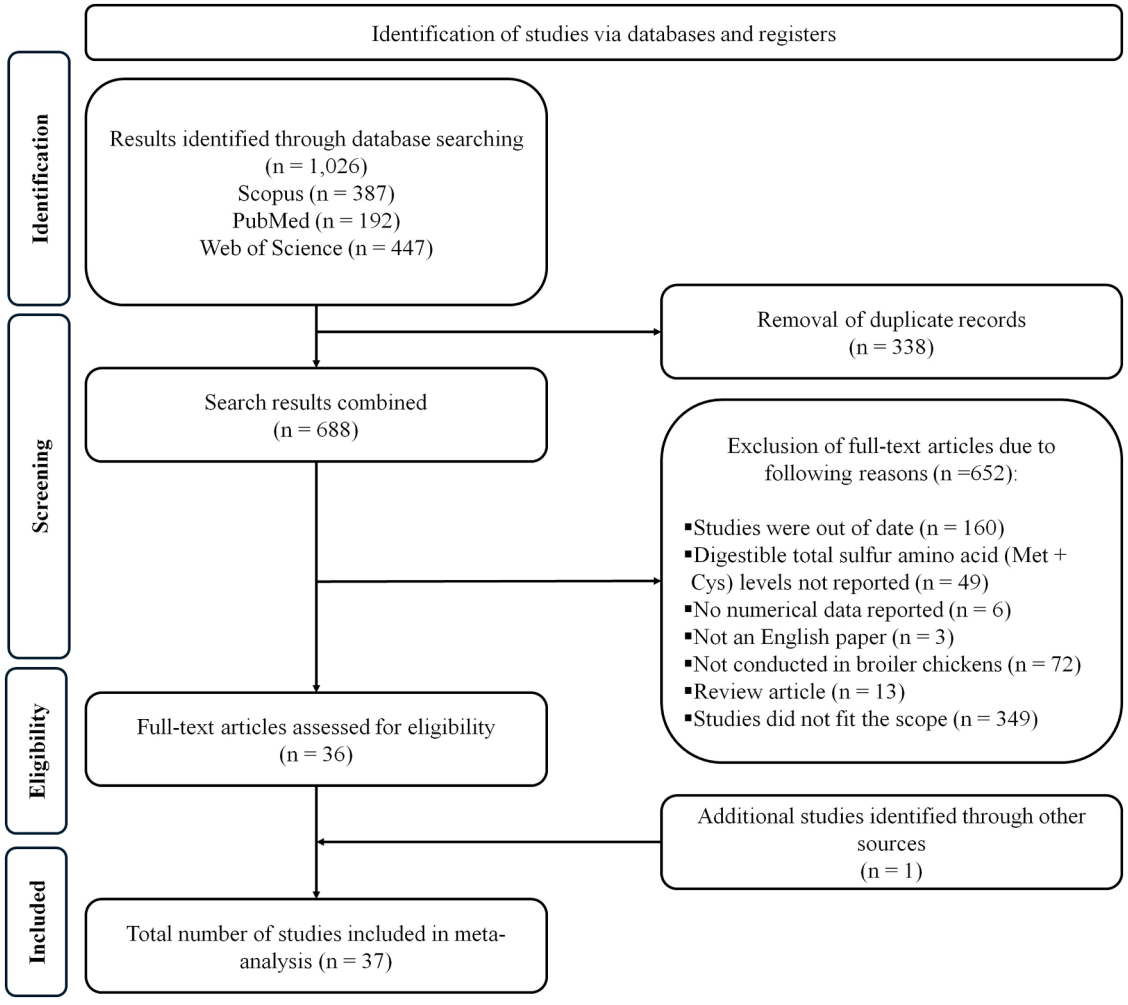


Fig. 1. Selection and evaluation process of published articles following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

among studies. The selection criteria included: (1) each study had to include treatment groups with varying levels of dietary TSAA or CP; (2) dietary concentrations of digestible TSAA or CP had to be clearly specified; (3) no additional AA, other than TSAA (Met or Cys), were supplemented beyond AA requirements met by the control group; (4) the specific experimental period had to be clearly defined; (5) sufficient information describing the experimental factors needed to be provided or made available upon request from the authors; (6) each study was required to report the numerical data regarding growth performance in broiler chickens. In total, 37 articles that satisfied these inclusion criteria were finally selected for the current meta-analysis. The studies were conducted across 12 countries and were published between 2007 and 2024.

Data extraction

Based on the inclusion and exclusion criteria, the factors considered in selected studies included the author, publication year, broiler breeds, number of replicates per treatment, study country, experimental period, dietary CP levels, and digestible TSAA levels (Supplementary Table S1). The experimental periods defined as starter, grower, and finisher phases, while varied substantially among selected studies, and therefore, a midpoint-based classification rule was applied to ensure consistent categorization across datasets. Each dataset was assigned to starter (less than 10 d of age), grower (10–21 d of age), and finisher phase (greater than 21 d of age) based on the midpoint age. These thresholds were selected to reflect typical chronological distinctions among growth phases in the commercial broiler production system, thereby enabling the objective classification of heterogeneous periods in selected studies. All studies supplemented the L- or DL-form of Met or Cys to adjust digestible TSAA levels in treatment diets. When the purity of supplemental TSAA was not specified, it was assumed to be 99.0%. If the studies reported CP levels only for the control diet without specifying those of treatment diets supplemented with Met or Cys, the CP levels of treatment diets were calculated by adding CP contributions from the supplemental TSAA, adopting 58.67% CP for Met or 72.26% CP for Cys, multiplied by the purity of the supplement and its supplemental levels in the diet (Rostagno et al., 2024). Furthermore, average daily feed intake (**ADFI**), average daily gain (**ADG**), and feed conversion ratio (**FCR**) as the main growth performance measurements for broiler chickens were selected as the primary response variables in the meta-analysis because they are the common measurements in broiler studies and the major interests in the broiler industry (Faridi et al., 2015; Zouaoui et al., 2024). Therefore, the data for growth performance were collected for all treatments in selected studies. A summary of the extracted data is presented in Table 1, including the mean, standard deviation, minimum, maximum, number of articles, number of treatments (n), experimental phase, digestible TSAA levels, dietary CP levels, ADFI, ADG, and FCR.

Statistical model for meta-analysis

A meta-analysis was conducted using the MIXED procedure in SAS 9.4 (SAS Institute Inc., Cary, NC, USA) to evaluate the interactive effects of digestible TSAA and CP levels in diets on ADFI, ADG, and FCR in broiler chickens. Initially, a full quadratic regression model was tested, incorporating linear, quadratic, and interaction terms for digestible TSAA and CP, to explore their combined impacts on growth performance in broiler chickens (Supplementary Table S2; Faridi et al., 2015). However, most coefficients in the full quadratic model were either non-significant or inconsistent across growth phases, indicating that this full quadratic model did not provide biologically meaningful predictions of broiler performance based on varying levels of digestible TSAA and CP in diets. As a consequence, a simplified quadratic model was proposed in which only the interactive variable of digestible TSAA and CP, and its quadratic term were included as predictors to better reflect their interactive relationship (Quinn et al., 2013). In this simplified model,

Table 1
Descriptive statistics of datasets by growth phases in the current meta-analysis.

Items[2]	Descriptive statistics[1]					
	Mean	SD	Min	Max	No. of articles	n
Starter phase						
Day of start Exp.	1	0.5	0	1	11	57
Day of finish Exp.	10	2.9	7	14	11	57
CP, %	21.6	2.95	7.4	26.5	11	57
Digestible TSAA, %	0.82	0.217	0.23	1.60	11	57
ADFI, g/bird/d	31.2	22.13	10.2	93.3	11	57
ADG, g/bird/d	22.5	12.77	7.7	61.3	11	57
FCR, g/g	1.34	0.310	1.00	2.8	11	57
Grower phase						
Day of start Exp.	6	5.4	0	15	26	124
Day of finish Exp.	23	4.8	14	35	26	124
CP, %	20.9	1.79	16.5	26.2	26	124
Digestible TSAA, %	0.81	0.139	0.11	1.30	26	124
ADFI, g/bird/d	69.6	28.49	26.0	155.0	26	124
ADG, g/bird/d	46.5	19.90	15.0	99.8	26	124
FCR, g/g	1.53	0.209	1.17	2.32	26	124
Finisher phase						
Day of start Exp.	24	6.2	14	39	22	84
Day of finish Exp.	40	7.4	26	60	22	84
CP, %	18.9	1.29	15.3	21.0	22	84
Digestible TSAA, %	0.75	0.142	0.52	1.47	22	84
ADFI, g/bird/d	143.7	33.92	49.3	193.1	22	84
ADG, g/bird/d	78.7	22.32	27.0	115.6	22	84
FCR, g/g	1.90	0.335	1.37	2.98	22	84

[1] SD, standard deviation; Min, minimum; Max, maximum; n, number of treatments.
[2] Exp, experiment; CP, crude protein, TSAA, total sulfur amino acids; ADFI, average daily feed intake, ADG, average daily gain; FCR, feed conversion ratio.

the interactive variable (TSAA × CP) was calculated by multiplying digestible TSAA levels by corresponding CP levels in each treatment diet. It appeared that this model integrated the combined effects of two dietary variables, thereby improving stability and interpretability of the model. The simplified quadratic model was expressed as follows:

$$Y_{ij} = \beta_0 + \beta_1 TSAA_{ij} \times CP_{ij} + \beta_2 \left[TSAA_{ij} \times CP_{ij}\right]^2 + u_j + \epsilon_{ij}$$

In this quadratic equation, Y_{ij} represents the response variable (i.e., ADFI, ADG, and FCR) for observation i within study (experiment) j. The model considered "study" as a random effect (random intercept) and predictor variables related to dietary treatments were specified as fixed effects (St-Pierre, 2001). The random effect (u_j) was assumed to follow a normal distribution with a mean of 0 and a SD of τ, representing the variability between studies. The residual error (ϵ_{ij}) captured the unexplainable variability within studies and was assumed to follow a normal distribution with a mean of 0 and a SD of σ, representing the residual variability. The number of replicates in each study was used as a weighting factor in the WEIGHT statement of the model (Irawan et al., 2022). Resulting quadratic regression models for growth phases are presented in Table 2.

Model evaluation

Goodness-of-fit statistics were estimated for each growth phase and response variable to evaluate the proposed quadratic models (Table 3). Model performance evaluation was conducted based on the root mean square prediction error (**RMSPE**), RMSPE expressed as a percentage of the mean observed value (**RMSPE%**), and the concordance correlation coefficient (**CCC**), which have been widely used to evaluate predictive goodness-of-fit of regression-based models in poultry studies (Leishman et al., 2021; Richardson et al., 2025). In addition, I^2 statistics were estimated to quantify the proportion of total variability attributable to between-study heterogeneity, calculated as the between-study variance divided by the sum of between-study and within-study (residual) variances (Higgins and Thompson, 2002).

Table 2
Parameter estimates for the regression models regarding the interactive effects of digestible total sulfur amino acids (TSAA) and crude protein (CP) on growth performance in broiler chickens across growth phases[1].

		Growth performance								
		ADFI (g/bird/d)			ADG (g/bird/d)			FCR (g/g)		
Phase	Model parameter	Coefficient	SE	*P*-value	Coefficient	SE	*P*-value	Coefficient	SE	*P*-value
Starter	Intercept	−1.71	5.048	0.742	37.4	6.55	<0.001	2.51	0.126	<0.001
(n = 57)	TSAA × CP	2.01	0.270	<0.001	−0.840	0.2927	0.006	−0.104	0.0114	<0.001
	(TSAA × CP)^2	−0.036	0.0068	<0.001	0.022	0.0074	0.005	0.002	0.0003	<0.001
Grower	Intercept	40.1	12.37	0.003	10.5	10.33	0.321	2.12	0.154	<0.001
(n = 124)	TSAA × CP	3.22	1.338	0.018	3.56	1.166	0.003	−0.054	0.0181	0.004
	(TSAA × CP)^2	−0.080	0.0402	0.049	−0.081	0.0350	0.022	0.001	0.0005	0.050
Finisher	Intercept	78.7	36.02	0.042	−13.8	24.50	0.581	3.36	0.330	<0.001
(n = 84)	TSAA × CP	8.08	4.711	0.091	10.9	3.22	0.001	−0.166	0.0432	<0.001
	(TSAA × CP)^2	−0.237	0.1544	0.131	−0.297	0.1055	0.007	0.004	0.0014	0.004
Overall	Intercept	82.1	9.29	<0.001	23.7	6.01	<0.001	2.57	0.086	<0.001
(n = 265)	TSAA × CP	0.495	0.6777	0.466	2.74	0.509	<0.001	−0.089	0.0085	<0.001
	(TSAA × CP)^2	−0.007	0.0186	0.722	−0.054	0.0140	<0.001	0.002	0.0002	<0.001

[1] ADFI, average daily feed intake; ADG, average daily gain, FCR, feed conversion ratio; n, number of treatments; TSAA × CP, product of digestible TSAA and CP in diets.

Table 3
Goodness-of-fit statistics for the regression models regarding the interactive effects of digestible total sulfur amino acids and crude protein on growth performance in broiler chickens across growth phases[1].

	Model fit statistics[2]			
Items[3]	RMSPE	RMSPE%	CCC	I^2
Starter phase				
ADFI	21.5	69.7	0.07	83.8
ADG	15.5	69.5	−0.25	76.2
FCR	0.185	13.8	0.76	18.3
Grower phase				
ADFI	28.1	39.4	0.01	73.9
ADG	20.0	41.7	0.02	62.2
FCR	0.189	12.5	0.18	34.3
Finisher phase				
ADFI	32.6	21.9	0.09	37.3
ADG	19.3	23.4	0.29	29.7
FCR	0.255	13.6	0.32	33.0
Overall phase				
ADFI	53.8	63.7	−0.01	82.2
ADG	30.3	58.3	−0.05	71.0
FCR	0.276	17.5	0.41	42.9

[1] All goodness-of-fit statistics were calculated using weighted observations based on the number of replicates in each study.
[2] RMSPE, root mean square prediction error; RMSPE% represents RMSPE expressed as a percentage of the mean observed value; CCC, concordance correlation coefficient, which evaluates both precision and accuracy between observed and predicted values; I^2, Higgins' inconsistency statistic, which indicates between-study heterogeneity.
[3] ADFI, average daily feed intake; ADG, average daily gain; FCR, feed conversion ratio.

Visualization of performance response

To illustrate the interactive effects of digestible TSAA and CP levels in diets, performance response curves were generated using quadratic regression models. For each CP level of 16%, 18%, 20%, 22%, and 24%, CP values were substituted into the quadratic regression equations, and then the relationships between digestible TSAA and each performance measurement (i.e., ADFI, ADG, and FCR) were plotted. These visualizations illustrated phase-specific responses in broiler performance to increasing digestible TSAA levels at specific CP levels in diets.

Estimation of optimal digestible TSAA levels at specific CP levels

The quadratic regression equations were used to estimate the optimal digestible TSAA level at each CP level. Firstly, dietary CP levels were fixed at incremental values ranging from 16% to 24% at 0.5% intervals, and these predefined CP values were substituted into the corresponding quadratic model to reparameterize the regression equations in terms of digestible TSAA levels (Supplementary Table S3). Based on this model-based reparameterization, CP-specific quadratic equations were derived to describe ADFI, ADG, and FCR as a function of digestible TSAA levels, from which the optimal TSAA levels were estimated, without subdividing the original dataset or refitting the model. The first derivative of each equation was then solved to determine optimal digestible TSAA levels that maximized ADFI and ADG, while minimizing FCR, depending on the response variable (Faridi et al., 2015). In addition, the relationship between dietary CP levels and the corresponding optimal digestible TSAA levels was also visualized by plotting CP levels on the x-axis and optimal digestible TSAA levels on the y-axis for the starter, grower, finisher, and overall phases. All plots were generated using MATLAB® (The MathWorks, Inc., Natick, MA; version R2025a).

Experimental validation of meta-analysis

A feeding trial was conducted to evaluate whether the optimal digestible TSAA levels derived from the current meta-analysis models could have an impact on FCR in broiler chickens fed diets with varying CP levels. All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Chung-Ang University (Approval No. 202501020115). A total of 420 one-d-old Ross 308 broiler chicks (34.0 ± 0.02 g) were randomly allotted to 6 dietary treatments in 3 × 2 factorial arrangements with 7 replicates per treatment (1:1 male to female ratio). The treatment diets were formulated to contain three CP levels for each phase: the recommended CP level based on Ross 308 nutrition specifications (Aviagen, 2022) and two CP levels reduced by 1.5 and 3.0 percentage points, thereby generating stepwise low-CP and very low-CP diets for each phase (Table 4). At each CP level, two digestible TSAA levels were formulated according to either the recommended level (Aviagen, 2022) or optimal TSAA levels derived from our proposed model to minimize FCR (Supplementary Table S3). Energy and all other nutrients met or exceeded Ross 308 nutrition specifications for each phase (Aviagen, 2022). Diet formulations are detailed in Supplementary Table S4–S6. Birds were raised under standard management and environmental conditions (Aviagen, 2022) for 40 d of the feeding trial. Growth performance, including ADG, ADFI, and FCR, in broiler chickens was determined at 10, 24, and 40 d of age.

All data were analyzed using the PROC MIXED procedure of SAS 9.4. The replicate was considered an experimental unit. The LSMEANS procedure was used to calculate treatment means and the PDIFF option

Table 4
Calculated energy and nutrient content of treatment diets in starter, grower, and finisher phases for the experimental validation trial (as-fed basis)[1].

	Crude protein (CP) and digestible total sulfur amino acids (TSAA) levels					
CP level[2]	Recommend CP		Low-CP		Very low-CP	
TSAA level[3]	Recommend	Optimum	Recommend	Optimum	Recommend	Optimum
Starter (1–10 d)						
AME_n, kcal/kg	2,975	2,975	2,975	2,975	2,975	2,975
CP, %	23.0	23.0	21.5	21.5	20.0	20.0
Digestible TSAA, %	1.00	1.15	1.00	1.23	1.00	1.32
Digestible lysine, %	1.32	1.32	1.32	1.32	1.32	1.32
Grower (11–24 d)						
AME_n, kcal/kg	3,050	3,050	3,050	3,050	3,050	3,050
CP, %	21.5	21.5	20.0	20.0	18.5	18.5
Digestible TSAA, %	0.92	1.17	0.92	1.25	0.92	1.35
Digestible lysine, %	1.18	1.18	1.18	1.18	1.18	1.18
Finisher (25–40 d)						
AME_n, kcal/kg	3,100	3,100	3,100	3,100	3,100	3,100
CP, %	19.5	19.5	18.0	18.0	16.5	16.5
Digestible TSAA, %	0.86	0.99	0.86	1.08	0.86	1.18
Digestible lysine, %	1.08	1.08	1.08	1.08	1.08	1.08

[1] Values for energy, crude protein (CP), and digestible amino acid levels were calculated from Aviagen (2022).
[2] Three CP levels were applied at each growth phase: (1) Recommended CP levels from the Ross 308 nutrition specifications (Aviagen, 2022), (2) Low-CP level formulated with 1.5% CP reduction, and (3) Very low-CP level formulated with 3.0% CP reduction.
[3] Two digestible total sulfur amino acids (TSAA) levels were applied at each CP level: (1) Recommend: recommended digestible TSAA levels from the Ross 308 nutrition specifications (Aviagen, 2022) and (2) Optimum: optimal digestible TSAA at each CP level, which was predicted from the regression model in the current meta-analysis.

of SAS was used to separate the treatment means. The significance for statistical analysis was set at $P < 0.05$.

Results

Literature retrieval and data extraction

A total of 1,026 studies were initially identified through a search of public databases, with 338 duplicates being removed (Fig. 1). The remaining 688 articles were screened using the exclusion criteria, and 36 studies were selected for full-text assessment. Following full-text screening, one additional study was identified through the manual reference searching, resulting in a total of 37 studies that met the inclusion criteria, and therefore, were included in the final meta-analysis.

A summary of the datasets used in this meta-analysis, categorized by growth phases in broiler chickens, is presented in Table 1. For the starter phase, 11 studies were included with experimental ages ranging from 1 to 10 d. The average CP and digestible TSAA levels in diets were 21.6% and 0.82%, respectively. Average values for ADFI, ADG, and FCR were 31.2 g, 22.5 g, and 1.34, respectively. For the grower phase (6 to 23 d), 26 studies were subject to the meta-analysis. The average CP and digestible TSAA levels in diets were 20.9% and 0.81%, respectively. Average values for ADFI, ADG, and FCR were 69.6 g, 46.5 g, and 1.53, respectively. In the finisher phase (24 to 40 d), 22 studies were included. The diets had an average CP level of 18.9% and a digestible TSAA level of 0.75%. Average values for ADFI, ADG, and FCR were 143.7 g, 78.7 g, and 1.90, respectively.

Quadratic regression model

Quadratic regression analyses using the simplified model were conducted for growth performance across the starter, grower, finisher, and overall phases (Table 2). In the starter phase, all coefficients, including the intercept, linear, and quadratic terms of the interactive variable, were significant ($P < 0.01$) for ADG and FCR, indicating a strong nonlinear relationship. The model estimated RMSPE% and CCC values of 69.5 and −0.25 for ADG, and 13.8 and 0.76 for FCR, respectively (Table 3). In the grower phase, the quadratic model displayed all significant coefficients ($P < 0.05$) for ADFI (RMSPE% = 39.4 and CCC = 0.01) and FCR (RMSPE% = 12.5 and CCC = 0.18). In the finisher phase, the model for FCR showed all significant coefficients ($P < 0.01$), with RMSPE% and CCC values of 13.6 and 0.32. In the overall phase, all coefficients were significant ($P < 0.01$) for both ADG (RMSPE% = 58.3 and CCC = −0.05) and FCR (RMSPE% = 17.5 and CCC = 0.41). These findings indicate that the interactive effect of digestible TSAA and CP levels on FCR was consistent across growth phases, whereas its impacts on ADG and ADFI appeared to be more phase-dependent.

Between-study heterogeneity for performance measurements was assessed using the I^2 statistic (Table 3). In the starter phase, heterogeneity was high for ADFI (83.8%) and ADG (76.2%), but low for FCR (18.3%). In the grower phase, heterogeneity remained high for ADFI (73.9%) and ADG (62.2%), and moderate for FCR (34.3%). In the finisher phase, I^2 values indicated low to moderate heterogeneity for ADFI (37.3%), ADG (29.7%), and FCR (33.0%). In the overall phase, heterogeneity was considerable for ADFI (82.2%) and ADG (71.0%), but moderate for FCR (42.9%). These findings also showed substantial variability in ADFI and ADG responses among studies, whereas FCR response was relatively consistent.

Estimation of optimal digestible TSAA levels

The quadratic regression models were used to simulate the effects of varying levels of digestible TSAA at 5 fixed levels of CP in diets (i.e., 16%, 18%, 20%, 22%, and 24%) for each growth phase (Fig. 2). These model-based predictions demonstrated that digestible TSAA levels for maximizing growth performance, as indicated by the vertex in quadratic regressions for ADFI, ADG, and FCR in each growth phase, were progressively increased with reducing CP levels from 24% to 16% in diets.

Optimal digestible TSAA levels for each growth phase were also estimated by solving quadratic regression equations with inclusion of specific CP values in the equation at 0.5% intervals from 16% to 24%. The regenerated quadratic models with digestible TSAA levels as the sole independent variable were solved to identify optimal digestible TSAA levels that maximize ADFI and ADG or minimize FCR in broiler chickens (Fig. 3 and Supplementary Table S3). As a result, the calculated optimal levels of digestible TSAA increased by approximately 0.02 to 0.07 percentage points for each 0.5 percentage point reduction in dietary CP level within the range of 16% to 24%. An inverse relationship for ADFI, ADG, and FCR was consistently observed in all growth phases, indicating that lower CP levels in diets required higher digestible TSAA

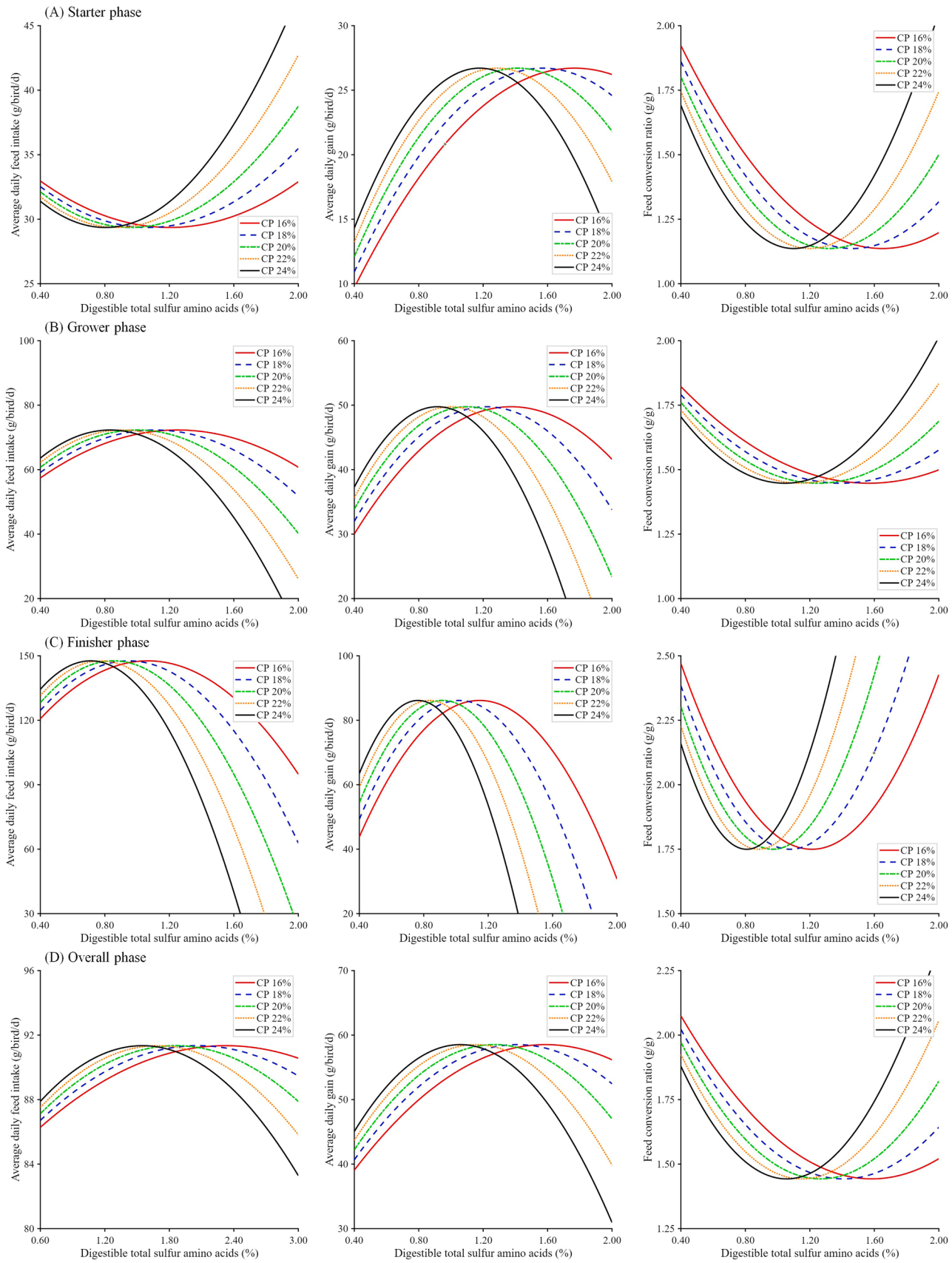


Fig. 2. Growth performance responses of broiler chickens to varying levels of digestible total sulfur amino acids (TSAA) at different crude protein (CP) levels in diets: (A) starter phase, (B) grower phase, (C) finisher phase, and (D) overall phase. Line styles and colors indicate dietary CP levels as follows: solid red = 16% CP, dashed blue = 18% CP, dot-dashed green = 20% CP, dotted orange = 22% CP, and solid black = 24% CP.

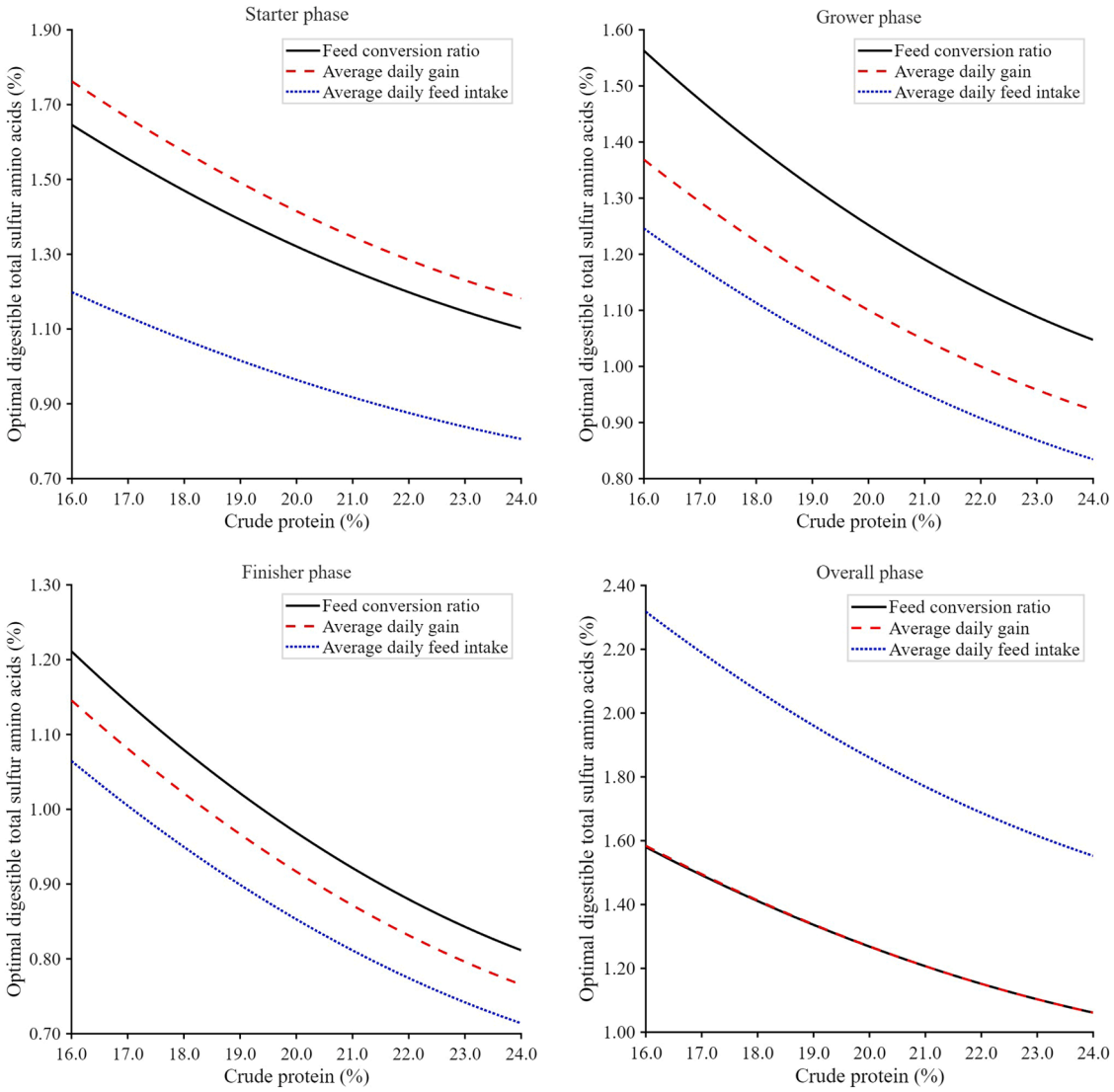


Fig. 3. Relationship between dietary crude protein (CP) and optimal digestible total sulfur amino acids (TSAA) levels for maximizing broiler performance, including feed conversion ratio (black-solid line), average daily gain (red-dashed line), and average daily feed intake (blue-dotted line), across growth phases.

levels to achieve the maximal broiler performance. This relationship was also illustrated in Fig. 3, indicating a clear upward trend in optimal digestible TSAA levels for broiler performance across growth phases as dietary CP levels decreased.

Experimental validation of meta-analysis

In a validation feeding trial, the interaction between digestible TSAA and CP levels in broiler diets for FCR for all growth phases was not significant for all growth phases (Fig. 4). However, despite no significant interaction, birds fed diets containing the model-derived optimal digestible TSAA levels exhibited lower ($P < 0.05$) FCR across all growth phases compared with those fed diets formulated according to commercially recommended digestible TSAA levels. In addition, this consistent decrease in FCR by feeding diets containing model-derived optimal digestible TSAA levels demonstrated that dietary TSAA response was directionally consistent across dietary CP levels, although the magnitude of reduction in FCR varied across dietary CP levels. Detailed results for all growth phases are provided in Supplementary Tables S7–S10.

Discussion

The current meta-analysis investigated the interactive effects of digestible TSAA and CP in broiler diets, specifically aiming to determine optimal digestible TSAA levels at varying CP levels to maximize growth performance in broiler chickens. In our preliminary regression analysis, a full quadratic model, including linear and quadratic terms for both digestible TSAA and CP, as well as their interaction, was evaluated. However, most coefficients in this initial model were not significant and exhibited irregular trends throughout growth phases, indicating that inclusion of TSAA and CP as separate independent terms in the model did not appropriately represent their combined effects on broiler performance. Therefore, a simplified quadratic model was developed in this study, emphasizing the interaction between digestible TSAA and CP, represented by their cross product (i.e., TSAA × CP) in diets. This approach was expected to reflect the biologically flexible interaction with dependence of the optimal TSAA levels on dietary CP (Nukreaw et al., 2011; Jariyahatthakij et al., 2018). Most previous studies typically expressed dietary AA and CP interactions based on a fixed AA:CP ratio, operating under the assumption that their relationship is strictly proportional. However, this approach may have a limitation to describe dynamic interactions between dietary AA and varying CP levels because

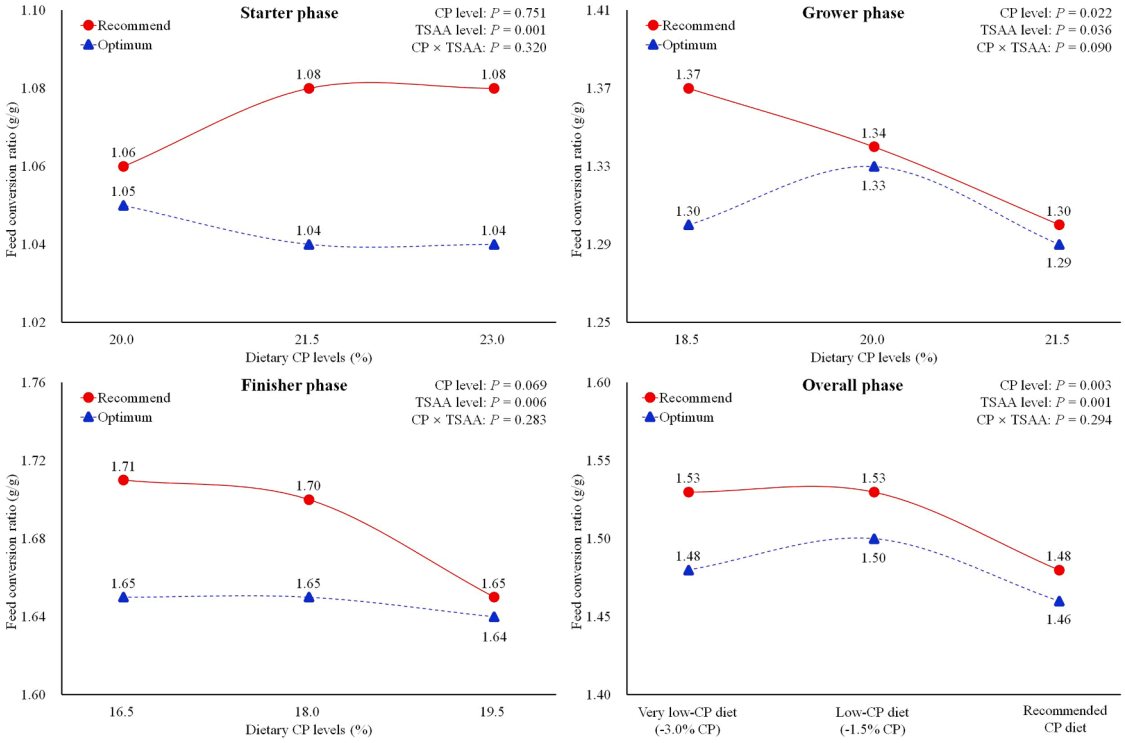


Fig. 4. Interactive effects of dietary crude protein (CP) and digestible total sulfur amino acids (TSAA) levels on feed conversion ratio (FCR) of broiler chickens in starter, grower, finisher, and overall phases in the experimental validation trial. Red circles (Recommend) represent diets formulated with the recommended TSAA levels from Ross 308 nutrition specifications (Aviagen, 2022), whereas blue triangles (Optimum) represent diets formulated with optimal digestible TSAA levels at each CP level predicted from the regression model in the current meta-analysis.

a fixed AA:CP ratio does not fully account for elevated AA recommendation that is often required in low-CP diets (Macelline et al., 2020; Saleh et al., 2021). On the other hand, the simplified quadratic model using the product of digestible TSAA and CP as a predictor in this meta-analysis may better capture the dynamic association of these 2 dietary variables, thereby more accurately reflecting their integrated impacts on broiler performance.

In the meta-analysis, FCR was consistently affected by the interactive variable of digestible TSAA and CP across all growth phases. This consistent significance was accompanied by the results of model performance evaluation for FCR with the least RMSPE and RMSPE%, while the highest CCC among performance measurements. Moreover, the least I^2 statistics for FCR also demonstrated a high degree of agreement among studies. Therefore, it is suggested that FCR is more reliable variable for predicted responses to the interactive effects of digestible TSAA and CP compared with ADFI and ADG. The FCR is an integrated measurement of ADG and ADFI, thereby offering a reliable measure of how broiler chickens efficiently convert dietary energy and nutrients into body growth (Jie et al., 2024). Furthermore, FCR is considered a better indicator of integrated efficiency of nutrient utilization with high sensitivity to imbalances in AA supplies as compared to individual ADG or ADFI (Liu et al., 2021, 2024). This characteristic may be particularly important in feeding low-CP diets, where AA balance is critical for optimizing protein deposition and minimizing excess energy expenditure (Musigwa et al., 2024).

The responses of ADFI and ADG were more phase-specific in this study. For ADFI models, all coefficients were significant only in the grower phase, which also showed high I^2 statistics, suggesting that responsiveness of ADFI to varying levels of dietary TSAA and CP was less consistent than that of FCR. In the starter phase, the immature gastrointestinal tract and rapid post-hatch development in broiler chicks limit voluntary feed intake (Kinstler et al., 2024), and therefore, growth depends largely on the efficiency of dietary AA utilization at a given CP level. Accordingly, all coefficients for the interactive variable of digestible TSAA and CP were significant in the ADG model of the starter phase. In contrast, the intercept coefficient in the ADFI model of the starter phase was not significant, which may imply heterogeneous baseline ADFI and the high sensitivity of ADFI to environmental and management factors in young chicks (Gu et al., 2008; Wasti et al., 2020). In the grower phase, the expansion of intestinal capacity and intensification of feather keratin synthesis may enhance the metabolic demand for TSAA, enabling broiler chickens to adjust their ADFI according to nutrient density (Selle et al., 2023), which agrees with our observation of all significant coefficients for the ADFI model in the grower phase. For ADG models, all coefficients were significant in the starter and overall phases, whereas only intercept coefficients were not significant in the grower and finisher phases, indicating variations in the baseline performance among studies but a maintained sensitivity to the interaction. These phases also exhibited higher I^2 statistics, reflecting a greater between-study variability, which is likely attributed to differences in genetics, housing systems, environment, and management practices (Siegert et al., 2015). In the finisher phase, both ADG and ADFI models were not significant, but FCR remained significant. The reason may be related to the high between-study variability in ADFI and ADG, possibly due to differences in target market weight and grow-out duration across various countries, limited ability of heavy chickens to adjust their ADFI according to dietary nutrient density, and environmental stressors that suppress ADFI and ADG (Schmidt, 2008; Nikbakhtzade et al., 2024).

The development of low-CP diets for broiler chickens recently attracted an increasing attention because excessive nitrogen excretion is a major environmental problem in broiler production (Chalova et al., 2016). However, reducing CP levels in diets has been documented to impair growth performance and meat quality in broiler chickens (Bregendahl et al., 2002; de Rauglaudre et al., 2023), whereas AA fortification in low-CP diets with supplemental synthetic AA is suggested to restore broiler performance and product quality (van Harn et al.,

2019; Woyengo et al., 2023). Nonetheless, the current recommended levels of essential AA in diet formulations for broiler chickens are established primarily based on normal-CP diets, which raises questions about their suitability for low-CP diet formulations.

Therefore, the current meta-analysis developed prediction models to estimate optimal digestible TSAA levels across a range of dietary CP levels, specifically from 16% to 24% in increments of 0.5%. The results clearly demonstrated that optimal digestible TSAA levels for broiler performance across growth phases consistently increased as dietary CP levels decreased. These findings are consistent with previous studies reporting that additional TSAA supplementation over its recommendation levels in low-CP diets improved growth performance in broiler chickens (Nukreaw and Bunchasak, 2015; Jariyahatthakij et al., 2018). Similarly, Macelline et al. (2020) and Saleh et al. (2021) also reported improved growth performance in broiler chickens by additional supplementation of various essential AA, including TSAA, in low-CP diets. Therefore, it may be suggested that AA supplementation in low-CP diets should be adjusted dynamically rather than maintained at a fixed AA:CP ratio in diets (Lemme, 2003; Kidd et al., 2021; Oliveira et al., 2022).

The results of the current validation feeding trial further supported the predictive accuracy of the regression models developed in this study. Although no significant interaction between digestible TSAA and CP levels was observed, birds fed diets containing the model-derived optimal digestible TSAA levels consistently showed lower FCR across all growth phases, regardless of dietary CP levels, compared with birds fed diets formulated according to commercial digestible TSAA recommendations. Interestingly, the extent of reduction in FCR by model-derived optimal digestible TSAA levels was considerable particularly at very low-CP diets in grower, finisher, and overall phases. Collectively, these results may indicate that our developed model successfully captured the dynamic interaction between digestible TSAA and CP levels, providing biologically meaningful predictions of digestible TSAA recommendations across different CP levels.

The observed progressive increase in optimal digestible TSAA levels with reducing CP levels may result from several physiological adaptations of broiler chickens to low-CP diets. For instance, feeding low-CP diets often restricts the supply of methyl-donating nutrients, such as choline and betaine, due to reduced inclusion of protein ingredients in diets (Bunchasak, 2009). Consequently, dietary TSAA may be increasingly utilized for methylation metabolisms, including DNA methylation, creatine synthesis, and other transmethylation reactions (Nutautaitė et al., 2020; Ren et al., 2020; Liu et al., 2022), which may reduce the availability of TSAA for growth-related protein synthesis (Ueland et al., 2005). Moreover, considering the potential methylation contributions of dietary Gly and Ser, it has been suggested that feeding low-CP diets may induce Gly-equivalent deficiencies, which may further enhance dietary TSAA requirement to compensate for the metabolic demands of Gly and Ser (Aguihe et al., 2022). Furthermore, Cys derived from Met in the body plays a role in maintaining cellular redox balance through glutathione metabolism (Lugata et al., 2022). In low-CP diets, dietary Cys supply from protein ingredients may be limited, resulting in enhanced conversion of Met to Cys (Pacheco et al., 2018). Feeding low-CP diets may also enhance oxidative stress in poultry by increasing AA oxidation (Son et al., 2024), which may increase the requirement of glutathione synthesized from Cys. Therefore, this increasing synthesis of Cys from Met may also elevate the overall TSAA requirements to sustain antioxidant capacity in the body (Santana et al., 2021; de Sousa et al., 2022). These physiological changes by feeding low-CP diets to broiler chickens may explain our findings that optimal digestible TSAA levels for broiler performance consistently increased as dietary CP levels decreased. Furthermore, it can also be inferred that our interactive models are highly effective in predicting optimal digestible TSAA levels across different CP levels, especially in low-CP diets for broiler chickens.

Conclusion

The current meta-analysis developed regression models regarding interactive effects of digestible TSAA and CP to determine optimal digestible TSAA at varying CP levels for maximizing growth performance in broiler chickens. Optimal digestible TSAA levels increased consistently as dietary CP levels decreased across growth phases, with an approximate increase of 0.02 to 0.07 percentage points in digestible TSAA for every 0.5 percentage point reduction in dietary CP within the range of 16.0 to 24.0% CP. This prediction was further supported by the validation feeding trial showing decreased FCR in broiler chickens fed diets formulated with the model-derived optimal digestible TSAA levels compared with those fed diets following their commercial recommendations. These findings may suggest that recommended levels of digestible TSAA need to be increased in low-CP diet formulations for all growth phases of broiler chickens.

CRediT authorship contribution statement

Hyun Woo Kim: Writing – review & editing, Writing – original draft, Visualization, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Dong Yong Kil:** Writing – review & editing, Writing – original draft, Validation, Supervision, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Disclosures

The authors declare no conflict of interest for the data presented in this experiment.

Acknowledgments

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (No. RS-2023-00249457). We also thank the BT research facility center, Chung-Ang University.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2026.106449.

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