104 (2025) 105031

Contents lists available at ScienceDirect

Poultry Science

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

Full-Length Article

Effects of maternal dietary threonine concentrations on the productive performance, amino acid profile in plasma and eggs of laying duck breeders, and performance of ducklings one-week post-hatching

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ARTICLE INFO

Keywords:
Production
Reproduction
Egg quality
Hatchability
Intestinal architecture

ABSTRACT

This study's aim was to assess how l- threonine (Thr) affected duck breeders' reproductive and productive performance by examining how it affected the amino acid composition of their eggs and plasma and how well ducklings performed a week after hatching. A total of 648 Longyan duck breeders (23 wk) were randomly allotted to six groups with six replicates of 18 ducks. Ducks were fed a basal control diet deficient in Thr and dietary treatments consisted of the basal diet supplemented l-Thr at 0.0 %, 0.07 %, 0.14 %, 0.21 %, 0.28 %, and 0.35 %, constituting total Thr content of 0.41 %, 0.45 %, 0.51 %, 0.60 %, 0.66 %, and 0.72 %, respectively. At 43 wk of age, the addition of l-Thr at a concentration of 0.28 % had superior ($P < 0.05$) values ($P < 0.05$) for egg production, egg weight, egg weight, egg mass, feed conversion ratio, yolk weight and its ratio to total egg weigh compared to the control diet, which yielded the lowest values. Over the entire experimental period, feed intake was not affected by dietary treatments. Plasma tyrosine elevated ($P = 0.03$ and quadratic trend = 0.02) at 0.28 % l-Thr. In addition, plasma citrulline increased linearly ($P = 0.06$, linear trend $P = 0.003$) at 0.28 % l-Thr as compared to the control diet. The contents of aspartic acid, Thr, tyrosine, and proline in egg yolk exhibited a linear increase ($P < 0.05$) corresponding to increased l- Thr levels. In addition, the contents of serine and cysteine were significantly enhanced at 0.35 % l-Thr, whereas the content of glycine experienced a decrease ($P < 0.05$) as l- Thr levels increased. The amino acid profile in the egg albumen demonstrated a reduction at 0.28 % and 0.35 % l-Thr, whereas feeding l-Thr at 0.21 % led to an increase in proline contents ($P = 0.03$, quadratic trend $P = 0.002$). The addition of l-Thr at a concentration of 0.28 % recorded the heaviest body weight of ducklings after hatching, while the control or 0.07 % Thr group recorded the lowest values. These findings suggest that l-Thr supplementation at 0.28 % (constituting total Thr content of 0.66 %) is an effective nutritional strategy to optimize the performance of duck breeders and the quality of their offspring, providing valuable insights for dietary formulations in poultry production and emphasizing the importance of balanced amino acid nutrition for maximizing breeder performance and offspring quality.

Introduction

It is well acknowledged that the eggs, which serve as the entire nutrition reservoir for embryo development, differ in a variety of qualities among poultry species, ranging from the size to the nutritional composition, which are crucial for hatching eggs. Duck eggs have a greater methionine concentration than other poultry eggs (Nurliyani et al., 2023). In addition, the comparative data of nutritional properties

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https://doi.org/10.1016/j.psj.2025.105031
Received 18 November 2024; Accepted 13 March 2025
Available online 17 March 2025

of egg albumen for poultry showed that duck eggs and goose eggs have relatively higher essential amino acids (EAA) / total amino acids (TAA) ratios (Sun et al., 2019) than other poultry eggs. In the fertilized eggs, the process of embryonic development is contingent on many factors, including the nutritional composition of the eggs. Eggs contain an abundance of protein (amino acid) and various other essential nutrients requisite for embryonic growth (Réhault-Godbert et al., 2019). It has been established that the composition of amino acids in fertilized eggs may be modified through dietary amino acids (Reda et al., 2020; Lee et al., 2023). In this context, the nutritional profile of fertilized eggs could be enhanced with amino acids via maternal dietary supplementation.

Optimal nutritional practices constitute the most effective strategy to mitigate the adverse impacts of environmental stress factors while enhancing both productivity and reproductive success in avian species (Rehman et al., 2019; Alagawany et al., 2024). The addition of amino acids within poultry feed is one of the pillars of nutrition. Since birds cannot synthesis Thr, it is a necessary amino acid. Its catabolism produces a range of intermediaries that are involved in the metabolism of proteins (Zhuang et al., 2024). After lysine and methionine, Thr is the third limiting essential amino acid in corn-soybean based diets. Thr contains both beta and alpha carbons in its structure, and has four isomers (L, D, l-allo and d-allo). Thr comprises 11.7 % nitrogen and is essential for the body's protein synthesis and maintenance (Kidd and Kerr, 1996). In addition to being involved in protein synthesis, Thr is also involved in the synthesis of collagen and elastin and is utilized in several basic metabolic processes (Ospina-Rojas et al., 2014). Thr is a significant biomolecule that affects energy metabolism and protein synthesis (Chen et al., 2018). Thr, existing in its L isomeric form, is supplemented into diets to adequately fulfill the amino acid nutritional prerequisites of highly productive breeds (Ashrafi et al., 2011; Azzam et, al.,2011; Azzam et al., 2014; Xie et al., 2014; Azzam et, al.,2017; Fouad et al., 2017; Azzam et al., 2019; Neto et al., 2020; Gül et, al.,2024). Recently, Azzam et al. (2023) demonstrated that l- Thr at 0.07 % increased egg production as compared to a basal control diet deficient in Thr (65.83 % vs. 55.45 %) without improving FCR. Consequently, Thr should be given with diet to poultry, including laying ducks, to ensure optimal production outcomes.

Our hypotheses suggested that the supplementation of increased levels of l-Thr could potentially affect the productivity and reproductive performance of duck breeders. To our knowledge, there are no existing studies that delineate the effects of increased concentrations of l-Thr on the productive and reproductive performance, amino acid composition in the eggs and plasma of duck breeders, as well as the performance of ducklings one-week post-hatching. This study's goal was to assess how l-Thr affected duck breeders' reproductive and productive performance by examining how it affected the amino acid composition of their eggs and plasma and how well ducklings performed a week after hatching.

Materials and methods

Animal welfare statements

The Animal Care Committee affiliated with the Guangdong Academy of Agricultural Sciences (Guangdong, People's Republic of China) granted approval for the research undertaking (2022006).

Experimental design

A total of 648 Longyan laying duck breeders (23 wk.) were randomly allotted to six groups with six replicates of 18 ducks. Each duck was placed individually per a cage (45 cm length × 30 cm width × 50 cm height). Ducks were fed a basal control diet deficient in Thr, whereas treatments 2 to 6 consisted of the basal diet supplemented with l- Thr (98.5 %, 98.5 %, Evonik Industries AG, Essen, Germany) at varying concentrations, specifically 0.07 %, 0.14 %, 0.21 %, 0.28 %, and 0.35 %, constituting total Thr content of 0.41 %, 0.45 %, 0.51 %, 0.60 %, 0.66 %, and 0.72 %, respectively, as determined by the analytical assessments of the experimental diets (Table 1). Due to the deficiency of Thr in peanut meal, we utilized it as the source of protein to formulate the experimental diets. The crude protein and amino acid levels within the feed were subjected to analysis in accordance with the methodology delineated by Azzam et al. (2011), employing the HITACHIL-8900 apparatus (Hitachi, Ltd., Tokyo, Japan) and the analyzed nutrients are presented in Table 1 and Table 2. The total duration of the trial was 20 wk.

Productive performance and hatching

The ducks were fed the experimental diets for a duration of seven days for the purpose of acclimatization prior to the undertaking of any empirical measurements. The numbers of total intact eggs, feed intake, and egg weight were recorded daily on a per replicate basis. Egg mass and feed conversion ratio were calculated on a per replicate basis. At the end of feeding trial, three eggs per replicate, representative of the average egg weight, were utilized for assessment albumen, yolk, shell weights. The eggshell was detached from the albumen and yolk, subjected to washing in order to eliminate residual albumen, subsequently dried at a temperature of 65°C for a duration of 4 h, and thereafter weighed. The proportion of eggshell (%) was calculated as (eggshell weight/egg weight) × 100. The shell strength was evaluated using an Egg Force Reader (EFR-01, Orka, Ramat HaSharon, Israel) and was determined as the mean thickness at the blunt end, sharp end, and midpoint of the eggs. During the last 3wk, at 40 weeks of age, all female ducks was artificially inseminated twice a week using 100 μL of diluted fresh semen. Three hundred eggs (egg weight > 65 g) per treatment (50 eggs / replicate) were weighed individually, and incubated under standard conditions for hatching using an incubator (Dezhou Jingxiang Technology Co, Dezhou, China). On the 7th day after the start of incubation, unfertilized eggs were recorded and eliminated. The quantity of healthy ducklings, free from deformities, was evaluated, and the weights

Table 1
Ingredients and nutrient composition of the basal control diet (%).

Ingredients	%
Yellow corn	32.50
Wheat	30.00
Peanut meal	13.00
Wheat bran	12.10
Limestone	8.40
DL-Methionine	0.25
L-Lysine	0.50
L-Tryptophan	0.05
L-Isoleucine	0.15
L-Valine	0.15
L-Threonine	0.00
Carrier	0.35
Dicalcium phosphate	1.20
Salt	0.35
Premix[1]	1.00
Total	100
Calculated nutrients, %	
Crude protein	15.90 (15.40)
Lysine	0.89 (1.11)
Methionine	0.42 (0.32)
Threonine	0.39 (0.41)
Calcium	3.50
Total phosphorus	0.58
ME, kcal/100 g	250

[1] Provided per kilogram of diet: Vitamins: A, 12 500 IU; D3, 4 125 IU; E, 15 IU; K, 2 mg; B1 1 mg; B2 8.5 mg; calcium pantothenate 50 mg; niacin 32.5 mg; pyridoxine 8 mg; biotin 2 mg; folic acid 5 mg; VB12 5 mg. Minerals: Zn 90 mg; I 0.5 mg; Fe 60 mg; Cu 8 mg; Se 0.2 mg; Co 0.26 mg; choline chloride 500 mg. * Values in parentheses are the analyzed contents.

Table 2
Analyzed levels of amino acid in the experimental diets[1].

AAs, %	L-Thr, %					
	T1	T2	T3	T4	T5	T6
	0.0	0.07	0.14	0.21	0.28	0.35
Threonine	0.41	0.45	0.51	0.60	0.66	0.72
Alanine	0.69	0.62	0.64	0.65	0.64	0.65
Serine	0.67	0.63	0.63	0.62	0.61	0.64
Leucine	1.06	1.02	1.01	1.01	1.02	1.05
Aspartic acid	1.43	1.35	1.30	1.31	1.26	1.35
Isoleucine	0.64	0.60	0.62	0.60	0.60	0.63
Glycine	0.78	0.74	0.75	0.76	0.73	0.78
Arginine	1.39	1.35	1.33	1.34	1.31	1.42
Histidine	0.35	0.33	0.33	0.32	0.32	0.35
Valine	0.81	0.76	0.76	0.76	0.74	0.76
Proline	0.90	0.90	0.90	0.87	0.90	0.94
Phenylalanine	0.72	0.70	0.70	0.68	0.69	0.73
Methionine	0.32	0.30	0.32	0.30	0.30	0.30
Glutamic acid	3.32	3.30	3.31	3.27	3.31	3.48
Lysine	1.11	0.99	1.00	0.96	0.93	0.98
Tyrosine	0.52	0.50	0.50	0.51	0.51	0.53

[1] Values are the results of a chemical analysis conducted in duplicate.

of the hatchlings on a per replicate basis were recorded at the conclusion of the hatching trial on day 28.

Amino acid profile in plasma and eggs

At the end of the experiment, two birds were selected from each replicate and blood samples were collected from the axillary vein using 5 ml vacutainer tubes after an overnight fast for 8 h in heparinized vacutainer tubes, centrifuged at 2,000 × g for 15 min at 4°C and plasma was stored at −20°C until analysis. Plasma was deproteinized by mixing equal volumes of plasma and trichloroacetic acid (7.5 % w/v) in a 1.5-ml microcentrifuge tube. The samples were vortexed (30 s) and centrifuged for 15 min at 15000 × g. The supernatant was collected in screw-top cryovials and stored at 4°C. Plasma amino acids were quantified using an automatic amino acid analyzer (HITACHI l-8900, Hitachi, Ltd., Tokyo, Japan) equipped with a ninhydrin reagent and lithium buffer system. The yolk and albumen samples were dried to constant weight and then defatted and hydrolyzed. An amino acid analyzer (HITACHI l-8900, Hitachi, Ltd., Tokyo, Japan) was used according to the AOAC method 994.12 (Latimer, 2016). Briefly, 100 mg of each sample plus 800 μl of 0.1 MHCl were mixed and the oxygen was expelled bypassing nitrogen into the ampoule. Then, 800 μl of hexane was added and mixed by vertexing for 1 min, and centrifuged (22,000×g for 5 min), and the lower layer was collected. After acid hydrolysis with 6 NHCl for 20 h at 110°C, the content of amino acids was determined, except for methionine and cysteine, which were measured by acid oxidation before hydrolysis with 6 NHCl (Cho et al., 2020). The absolute concentration was measured from the peaks of the sample and standard by comparing the peak area obtained for each amino acid with the peak area of the amino acid standards.

Intestinal histology

The jejunal and ileal tissue samples, each measuring approximately 1 cm in length, were excised from the midpoint and immediately washed with ice-cold saline, then the samples were submerged in 10 % neutral formalin. The specimens were then embedded in paraffin, from which twenty cross-sections, each with a thickness of 2 μm, were obtained from each tissue sample. The sections were stained following the hematoxylin and eosin protocol and the stained tissues were examined using a light microscope (Nikon microscope, Nikon Corp, Tokyo, Japan). The villus height and crypt depth were measured as described by Azzam et al. (2020).

Performance of ducklings one-week post-hatching

A total of 468 ducklings were allocated into identical treatment groups post-hatching, with each treatment comprising six replicates of 13 ducklings each. All ducklings were administered a basal control diet (Table 3). Feed intake and body weight were assessed on the seventh day of age and daily mortality records were documented. Feed intake relative to body weight gain was utilized to calculate FCR.

Statistical analysis

All data were analyzed using the SPSS16.0 statistical software package (SPSS, Chicago, IL, USA). The replicate was considered an experimental unit. A one-way ANOVA was used to compare the differences among dietary groups. Tukey's HSD, as a post hoc test, was used to compare the differences between the least square means. Orthogonal polynomials for linear and quadratic effects were adopted and the differences between means were considered to be significant at $P < 0.05$.

Results

Productive performance and egg quality

Effects of l- Thr on productive performance data are illustrated in Fig. 1 and 2, respectively. The findings revealed that the duck breeders fed diets enriched with l- Thr at a concentration of 0.28 % exhibited a markedly enhanced laying performance ($P < 0.05$) in comparison to the control diet. No notable differences ($P > 0.05$) in feed intake were observed among dietary groups. The results showed that, when compared with the control diet, L Thr at 0.28 % increased yolk weight and its ratio to total egg weigh ($P < 0.05$). Feeding a diet containing 0.07 %, 0.21 %, 0.28 %, and 0.35 % l-Thr increased ($P < 0.05$) eggshell weight, with a concomitant increase in its proportion relative to the total weight of the egg at 0.35 % l-Thr. In addition, feeding a diet containing 0.21 % l-Thr improved the shell strength ($P < 0.05$) compared with the

Table 3
Ingredients and nutrient composition of the basal control diet of ducklings age from hatchling to 7 d of age.

Ingredient	%
Yellow corn, %	61.90
Soybean meal, %	27.8
Wheat bran, %	6.40
Limestone, %	0.77
Di-calcium phosphate, %	1.60
Salt, %	0.30
L- Lysine, %	0.05
DL- Methionine, %	0.18
Premix[1], %	1.00
Total, %	100
Calculated composition (as fed basis)	
ME, kcal/kg	2800
Crude protein, %	19.00
Calcium, %	0.85
Total phosphorus, %	0.66
Non-phytate phosphorus, %	0.40
Lysine, %	1.05
Methionine, %	0.45
Methionine + cysteine, %	0.76
Threonine, %	0.70
Tryptophan, %	0.22
Arginine, %	1.25

[1] The premix provided the following per kilogram of diet: vitamin A 5,500 IU, vitamin D3 400 IU, vitamin E 10 IU, vitamin K 2.0 mg, vitamin B1 3.0 mg, vitamin B2 4.6 mg, vitamin B6 2.2 mg, vitamin B12 0.02 mg, choline 500 mg, D-calcium pantothenate 7.4 mg, folic acid 1.0 mg, biotin 0.08 mg, Fe 80 mg, Cu 10 mg, Mn 39 mg, Zn 52 mg, I 0.26 mg, and Se 0.15 mg.

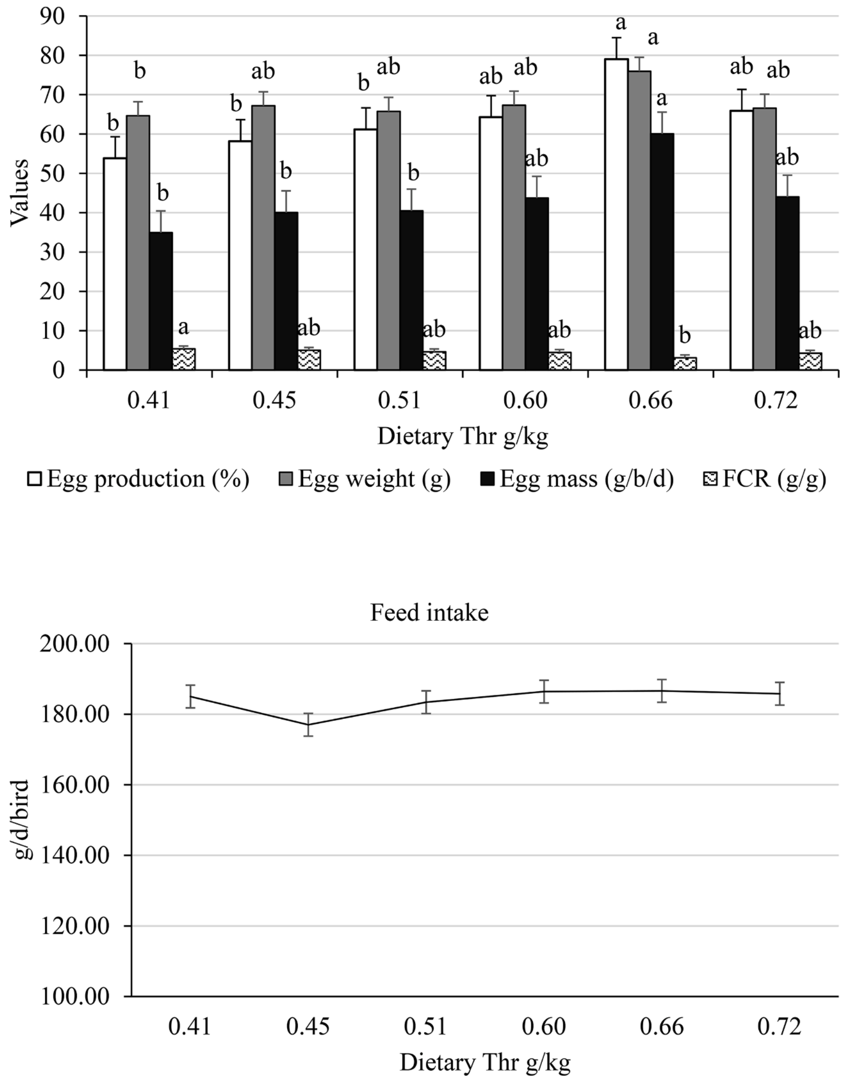

Fig. 1. Effects of the dietary threonine levels on laying performance in duck breeders. Data ± SEM and means on each bar with no common letter differ significantly at $P < 0.05$.

control diet.

Amino acid profile in plasma and eggs

Effects of l- Thr on amino acid profile in plasma, yolk and albumin are illustrated in Tables 4, 5, and 6, respectively. Plasma tyrosine elevated ($P = 0.03$ and quadratic trend $= 0.02$) at 0.28 % l-Thr. In addition, plasma citrulline increased linearly ($P = 0.06$, linear trend $P = 0.003$) at 0.28 % l-Thr as compared to the control diet. The contents of aspartic acid, Thr, tyrosine, and proline in egg yolk exhibited a linear increase ($P < 0.05$) corresponding to increased l- Thr levels. In addition, the contents of serine and cysteine were significantly enhanced at 0.35 % l-Thr, whereas the content of glycine experienced a decrease ($P < 0.05$) as l- Thr levels increased. The amino acid profile in the egg albumen demonstrated a reduction at 0.28 % and 0.35 % l-Thr, whereas feeding l-Thr at 0.21 % led to an increase in proline contents ($P = 0.03$, quadratic trend $P = 0.002$).

Structural integrity of the intestinal architecture

Effects of l- Thr on intestinal structure are illustrated in Table 7. The highest ileum villus width was observed in ducks fed 0.21 and 0.28 % l-Thr diet ($P < 0.05$). The jejunal villus height-to-crypt depth ratio exhibited the highest value (Linear trend = 0.04) in ducks fed l-Thr at 0.28 %.

Reproductive performance and ducklings' performance

The impact of dietary Thr on reproductive performance and ducklings' growth performance is illustrated in Fig. 3. No significant variations were observed in terms of fertility, hatchability, body weight at 7 days of age, feed consumption, and feed conversion ratio across all experimental groups. But, ducklings derived from eggs laid by breeders consuming l-Thr at a concentration of 0.28 % exhibited the highest ($P < 0.05$) body weight on day one of age, succeeded by groups that received Thr at concentrations of 0.14, 0.21, and 0.35 %, whereas the lowest body weights were observed at 0.0 % and 0.07 % as compared with those group fed Thr at 0.28 %.

Discussion

In the present study, ducks fed l- Thr at 0.28 % increased egg production, egg mass, egg weight and FCR. Previous investigations have demonstrated that l-Thr supplementation enhances laying performance in breeder avian breeds through several physiological mechanisms, primarily by optimizing amino acid balance, improving immune

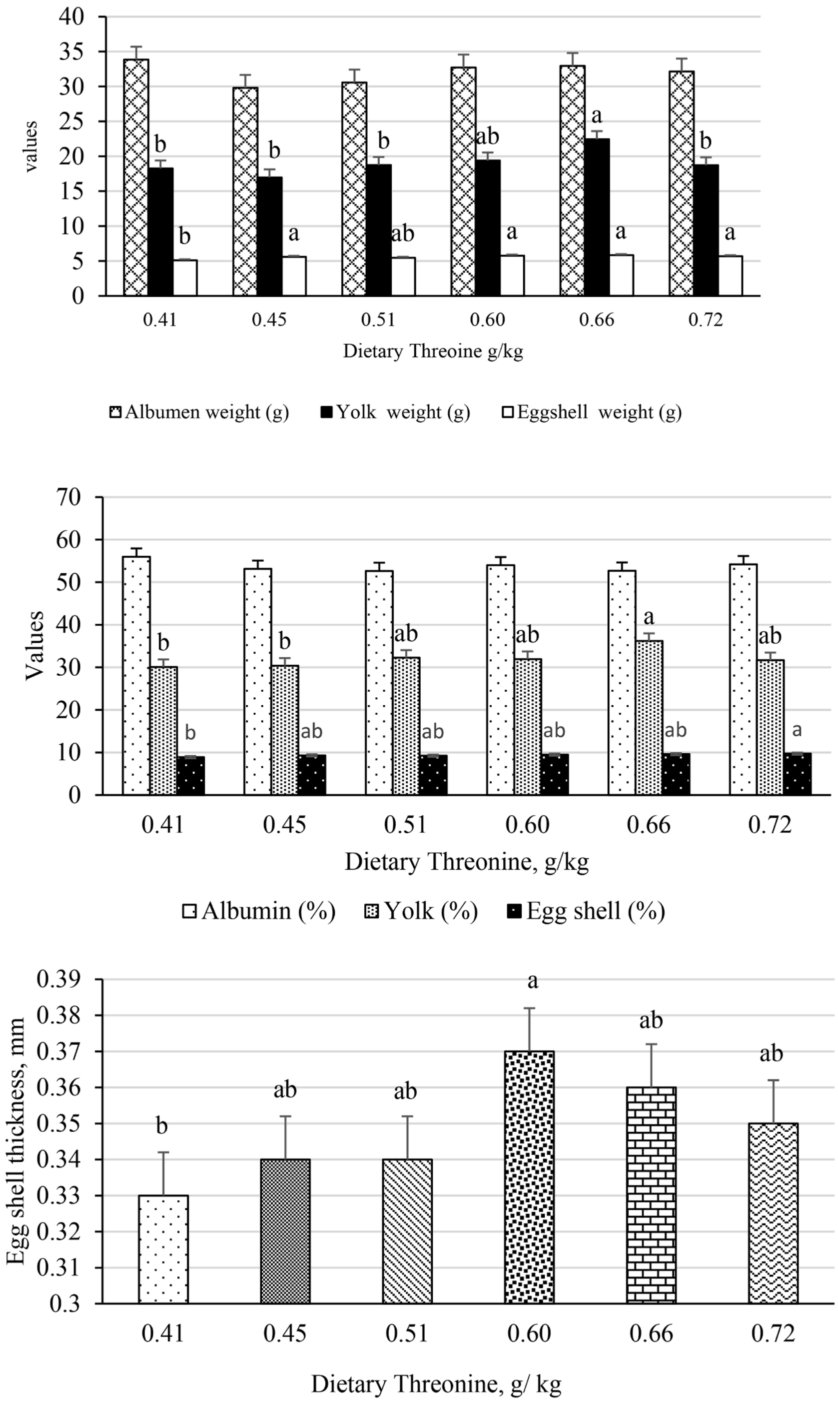

Fig. 2. Effects of the dietary threonine levels on egg quality at week 43 of age. Data ± SEM and means on each bar with no common letter differ significantly at $P < 0.05$.

function, enhancing nutrient absorption, and structural integrity of the intestinal architecture. The fact that Thr is a significant component of mucin, a glycoprotein that acts as a barrier, shields intestinal linings from damage, and aids in digestion and absorption, could be one reason for the improved egg production and FCR (Smirnov et al., 2006). In the present study, the highest ileum villus width was observed in ducks fed 0.21 % and 0.28 % Thr diet and the inclusion of l-Thr at 0.07 %, 0.14 %, 0.28 % and 0.35 % decreased jejunum crypt depth compared to the control diet. Further, plasma tyrosine elevated at 0.28 % l-Thr and plasma citrulline increased linearly at 0.28 % l-Thr as compared to the control diet. The relationship between blood tyrosine levels and egg production is significant, as tyrosine plays a crucial role in the reproductive performance of laying hens. Tyrosine, along with phenylalanine, is critical for synthesizing egg proteins, indicating its importance in meeting the amino acid requirements during egg formation (Murphy, 1994). Changes in plasma amino acid levels, including tyrosine, correlate with egg formation, suggesting that optimal blood levels are necessary for efficient egg production (Taylor et al., 1970). Research indicates that dietary supplementation with tyrosine can enhance egg production metrics, including egg number and mass, particularly in post-peak laying periods (Ali, 2018). Additionally, tyrosine is linked to improved fertility and reproductive outcomes in various animal models, suggesting its potential benefits in avian species as well (Loutfi et al., 2018; Spankowsky et al., 2011). Citrulline as a precursor to arginine,

Table 4
Effects of maternal dietary threonine concentrations on the plasma-free amino acids.

AAs	Thr levels,%						SEM	P-Value		
	0.41	0.45	0.51	0.60	0.66	0.72		Thr	Linear	Quadratic
Taurine	0.237	0.298	0.312	0.348	0.356	0.265	0.01	0.25	0.29	0.03
Aspartic acid	0.083	0.069	0.069	0.083	0.083	0.073	0.005	0.67	0.89	0.79
Threonine	0.201	0.236	0.221	0.200	0.257	0.176	0.01	0.22	0.69	0.19
Serine	0.859	0.731	0.702	0.745	0.816	0.602	0.03	0.11	0.08	0.96
Glutamic acid	0.197	0.219	0.181	0.167	0.195	0.214	0.01	0.59	0.99	0.24
Glycine	0.474	0.437	0.419	0.369	0.441	0.400	0.01	0.47	0.21	0.32
Alanine	1.307	1.099	1.088	1.163	1.167	1.125	0.04	0.53	0.37	0.24
Citrulline	0.004	0.003	0.007	0.008	0.011	0.011	0.001	0.06	0.003	0.88
Valine	0.247	0.269	0.257	0.275	0.286	0.243	0.008	0.50	0.75	0.16
Cysteine	0.010	0.012	0.016	0.011	0.012	0.010	0.001	0.64	0.88	0.23
Isoleucine	0.167	0.192	0.226	0.273	0.286	0.218	0.030	0.81	0.31	0.38
Methionine	0.006	0.005	0.004	0.003	0.006	0.005	0.001	0.90	0.81	0.51
Leucine	0.210	0.191	0.185	0.225	0.258	0.218	0.008	0.20	0.12	0.80
Tyrosine	0.210^{bc}	0.205^{bc}	0.230^{abc}	0.241^{ab}	0.261^{a}	0.196^{c}	0.007	0.03	0.38	0.02
Phenylalanine	0.162	0.193	0.170	0.200	0.180	0.184	0.008	0.66	0.48	0.46
Ornithine	0.029	0.035	0.036	0.030	0.031	0.040	0.003	0.61	0.39	0.74
Lysine	0.378	0.376	0.441	0.355	0.442	0.356	0.024	0.64	0.99	0.46
Histidine	0.095	0.102	0.082	0.077	0.080	0.103	0.005	0.36	0.77	0.09
Arginine	0.244	0.264	0.300	0.276	0.232	0.266	0.017	0.70	0.96	0.38
Proline	0.214	0.219	0.217	0.219	0.181	0.209	0.010	0.76	0.43	0.89

SEM, standard error of mean. [a,b] Mean values within a column with unlike superscript letters were significantly different ($P < 0{\cdot}05$).

Table 5
Effects of maternal dietary threonine concentrations on amino acid composition (g/100 CP) of egg yolk.

AAs	Thr levels,%						SEM	P-Value		
	0.41	0.45	0.51	0.60	0.66	0.72		Thr	Linear	Quadratic
Aspartic acid	2.493	2.640	2.525	2.795	2.724	2.848	0.05	0.24	0.02	0.95
Threonine	1.316	1.504	1.415	1.545	1.501	1.658	0.03	0.09	0.01	0.97
Serine	1.695^{b}	2.001^{b}	1.892^{b}	2.001^{b}	1.946^{b}	2.506^{a}	0.06	0.006	0.001	0.23
Glutamic acid	3.652	3.623	3.436	3.833	3.744	3.835	0.06	0.44	0.19	0.48
Glycine	1.219^{a}	0.895^{b}	0.855^{b}	0.949^{b}	0.918^{b}	0.949^{b}	0.04	0.04	0.08	0.02
Alanine	1.520	1.450	1.372	1.538	1.502	1.543	0.02	0.19	0.32	0.14
Cysteine	0.256^{b}	0.233^{b}	0.213^{b}	0.286^{b}	0.278^{b}	0.384^{a}	0.01	0.004	0.001	0.01
Valine	1.448	1.688	1.550	1.720	1.688	1.366	0.06	0.35	0.83	0.06
Methionine	0.580	0.548	0.521	0.621	0.611	0.582	0.02	0.83	0.54	0.88
Isoleucine	1.283	1.556	1.421	1.552	1.520	1.212	0.06	0.33	0.76	0.05
Leucine	2.274	2.540	2.417	2.639	2.583	2.534	0.06	0.56	0.19	0.34
Tyrosine	0.882	0.862	0.861	1.014	0.951	1.165	0.03	0.05	0.007	0.19
Phenylalanine	1.248	1.374	1.322	1.459	1.405	1.394	0.03	0.51	0.15	0.34
Lysine	2.149	2.381	2.272	2.489	2.439	2.399	0.06	0.53	0.15	0.37
Histidine	0.790	0.835	0.809	0.897	0.866	0.846	0.02	0.60	0.23	0.40
Arginine	1.898	1.881	1.828	2.013	1.932	1.988	0.03	0.55	0.23	0.71
Proline	1.125	1.083	1.076	1.180	1.162	1.219	0.01	0.12	0.02	0.23

SEM, standard error of mean. [a,b] Mean values within a column with unlike superscript letters were significantly different ($P < 0{\cdot}05$).

which is crucial for various physiological processes. Citrulline supplementation has been shown to influence nitric oxide synthesis, antioxidant status in laying hens (Uyanga et al., 2020). This suggests potential areas for further research, such as the direct measurement of tyrosine and citrulline levels in ducks and their correlation with physiological traits.

Studies have shown that the production offspring in poultry sector can be boosted by modifying the maternal diet. The effects of maternal nutrition on embryonic development were demonstrated by Fan et al. (2018). Jiang et al. (2019) clarified that Thr supplementation in breeder hens had affirmative influences on the offspring performance. In the current study, ducklings derived from eggs laid by breeders feeding l-Thr at a concentration of 0.28 % exhibited the highest body weight on day one of age. Assaf et al. (2009) displayed that the optimal hatchability per total and fertile eggs was found at 0.2 % dietary Thr levels which is consistent with herein study. On the other hand, Jiang et al. (2019) exhibited that the fertilization rate, hatchability rate and hatching weight of chicks were not affected by adding extra Thr in feed. Likewise, Thr supplementation did not significantly affect the hatchability of broiler breeders, according to Ashrafi et al. (2011). In a study involving Longyan duck breeders, increasing Thr concentrations improved egg production and hatchling weight, indicating a positive correlation between Thr levels and reproductive performance (Azzam et al., 2023).

Dietary Thr at 0.28 % increased yolk weight and its ratio to total egg weigh. Assaf et al. (2009) reported that Thr improved yolk weight and egg internal quality. In contrast, it has been found Increasing Thr levels in the diet of laying ducks resulted in a significant decrease in yolk weight and its proportion, while albumen weight and its proportion increased significantly (Fouad et al., 2017). In our study, increasing the Thr level increased the yolk weight and proportion, without affecting the albumen weight and proportion, which may explain why ducklings' hatch body weights increased. This finding could be due to the yolk serves as a primary source of nutrients for ducklings immediately post-hatch, influencing their initial body weight. The yolk contains proteins and other nutrients essential for the initial growth and development of ducklings (Peach and Thomas, 1986). Variability in yolk composition and utilization can be influenced by factors such as egg size, which affect the initial body weight and growth potential of ducklings (Hepp et al., 1987).

Gül et al. (2024) elucidated that dietary Thr level quadratically

Table 6
Effects of maternal dietary threonine concentrations on amino acid composition (g/100 CP) of egg albumen.

AAs	Thr levels,%						SEM	P-Value		
	0.41	0.45	0.51	0.60	0.66	0.72		Thr	Linear	Quadratic
Aspartic acid	7.839[a]	7.880[a]	7.871[a]	7.832[a]	7.774[a]	7.401[b]	0.03	0.002	0.001	0.005
Threonine	5.144[a]	5.124[a]	5.113[a]	5.088[a]	5.015[a]	4.842[b]	0.02	0.003	< 0.001	0.04
Serine	7.036[a]	7.030[a]	7.012[a]	7.021[a]	6.880[ab]	6.685[b]	0.03	0.008	0.001	0.03
Glutamic acid	11.83[a]	11.82[a]	11.76[a]	11.76[a]	11.54[ab]	11.20[b]	0.05	0.004	< 0.001	0.04
Glycine	2.982[a]	2.982[a]	2.962[a]	2.924[a]	2.900[a]	2.758[b]	0.01	< 0.001	< 0.001	0.01
Alanine	3.937[a]	3.890[a]	3.888[a]	3.897[a]	3.843[a]	3.722[b]	0.01	0.015	0.001	0.13
Cysteine	2.020	1.967	1.996	1.930	1.961	1.860	0.02	0.211	0.027	0.57
Valine	4.265[a]	4.130[abc]	4.117[abc]	4.155[ab]	4.044[bc]	3.975[c]	0.02	0.045	0.003	0.917
Methionine	4.088	4.114	4.289	4.151	4.026	4.139	0.03	0.273	0.820	0.28
Isoleucine	2.516	2.430	2.398	2.482	2.354	2.352	0.02	0.098	0.020	0.93
Leucine	5.854[a]	5.827[a]	5.783[a]	5.815[a]	5.673[ab]	5.505[b]	0.03	0.005	< 0.001	0.07
Tyrosine	3.226[a]	3.224[a]	3.250[a]	3.228[a]	3.169[a]	3.039[b]	0.01	0.011	0.003	0.01
Phenylalanine	5.873[a]	5.872[a]	5.796[a]	5.923[a]	5.719[ab]	5.561[b]	0.03	0.014	0.004	0.05
Lysine	5.306[a]	5.314[a]	5.301[a]	5.277[a]	5.164[ab]	5.007[b]	0.02	0.007	0.001	0.03
Histidine	1.587[a]	1.580[a]	1.572[a]	1.556[ab]	1.523[b]	1.466[c]	0.008	< 0.001	< 0.001	0.02
Arginine	3.622[a]	3.586[a]	3.572[ab]	3.580[a]	3.459[bc]	3.412[c]	0.02	0.005	< 0.001	0.20
Proline	3.105[bc]	3.135[bc]	3.197[ab]	3.273[a]	3.165[abc]	3.067[c]	0.02	0.033	0.94	0.00

SEM, standard error of mean.
[a,b] Mean values within a column with unlike superscript letters were significantly different ($P < 0{\cdot}05$).

Table 7
Effects of maternal dietary threonine concentrations on intestinal structure.

Items	Thr levels,%						SEM	P-Value		
	0.41	0.45	0.51	0.60	0.66	0.72		Thr	Linear	Quadratic
Ileum VH	267.1	228.2	230.0	239.5	284.5	248.2	10.53	0.56	0.72	0.50
Ileum VW	62.44[ab]	52.11[bc]	45.38[c]	67.30[a]	70.38[a]	48.93[bc]	2.30	0.004	0.82	0.73
Ileum CD	56.59	50.91	57.19	63.95	74.14	66.29	3.32	0.30	0.09	0.95
Ileum VH/CD	4.840	4.538	4.033	3.987	4.098	3.867	0.19	0.71	0.17	0.59
Jejunum VH	280.4	265.3	225.8	275.1	320.0	281.1	8.61	0.08	0.22	0.29
Jejunum VW	62.11	60.73	60.18	61.91	62.18	61.47	1.62	0.99	0.94	0.88
Jejunum CD	72.09[a]	60.45[bc]	49.02[c]	63.04[ab]	59.16[bc]	58.78[bc]	1.85	0.03	0.12	0.05
Jejunum VH/CD	3.911	4.375	4.677	4.717	5.424	4.804	0.17	0.11	0.04	0.37

SEM, standard error of mean.
[a,b] Mean values within a column with unlike superscript letters were significantly different ($P < 0{\cdot}05$).

affected eggshell thickness. In the present study, the improvement in eggshell thickness at 0.21 % Thr may be due to its positive effect on calcium metabolism. It has been found that Thr increases the nucleation rate of calcium carbonate crystals, which may be due to its interaction with calcium ions, highlighting its role in mineral metabolism beyond traditional acidic macromolecules (Liu et al., 2024). In addition, Thr metabolism is linked to broader metabolic processes, including protein turnover and deoxyribonucleotide biosynthesis. These processes can indirectly influence calcium metabolism by affecting the availability of metabolic substrates and energy, which are essential for maintaining calcium homeostasis (Hartman, 2007). While threonine's direct role in calcium metabolism is not as extensively documented as other amino acids, its influence on calcium absorption and biomineralization suggests a significant, albeit indirect, relationship. Additionally, the interplay between Thr and other metabolic pathways may further impact calcium homeostasis, indicating a complex network of interactions that warrant further studies.

The nutrition and general health of laying birds affects the amino acid content of blood and eggs. In the current study, there were no statistically significant differences detected between treatments in terms of the amino acid composition of blood plasma, except tyrosine. Several factors can affect the levels of amino acids in the blood of avian species, which in turn affect the birds' production performance. Compared to chicken blood, duck blood exhibits different mineral contents, such as higher levels of magnesium and manganese, which may influence amino acid metabolism (Sorapukdee and Narunatsopanon, 2017). The efficiency of amino acid utilization decreases with age, which can impact the overall amino acid concentration in the blood (Adeola et al., 2012). Positive alterations in amino acid composition of egg can potentially enhance the nutritional value of eggs and then health benefits of embryos. Efficient yolk utilization supports higher body weights at hatch and during the early growth phase (Jamroz et al., 2009). It's crucial to use nutritional approaches to improve the quality of eggs, especially the amino acid composition. In the current study, amino acid compositions (except Ser, Gly and Cys) of egg yolk were not affected by adding Thr. A shift in the hen's metabolism may be the cause of all these modifications to the amino acid profile in the yolk of laying chickens given Thr supplements. Larbier (1973) explained that when Thr and Lys increased, the concentrations of these nutrients increased linearly in the egg yolk, increasing both the yolk's weight and the total weight of the egg. In addition, Al-Obaidi et al. (2016) stated that duck eggs have high nutritional value due to its essential amino acids. The nutritional value and quality of egg albumen are impacted by changes in its amino acid content. Albumen proteins may function as a storehouse for bioactive proteins and peptides.

According to Moreira Filho et al. (2019), the *in ovo* injection of Thr promotes the morphological and functional development of the intestinal mucosa at hatching and at 21 days of age, as well as the performance of the chicks. Digestive enzymes have a Thr concentration of 5–11 % (Block et al., 1966); consequently, Thr might have an impact on how well nutrients are absorbed. Abbasi et al. (2014) illuminated that Thr supplementation, especially 110 % level; in the low CP diets increased CD. These alterations were mainly owing to the noticeable increase in VH and significant reduction in CD. According to Geyra et al. (2001), CD formation is critical for intestinal cell renewal and for accelerating gut maturation. Rasheed et al. (2018) revealed that reduced

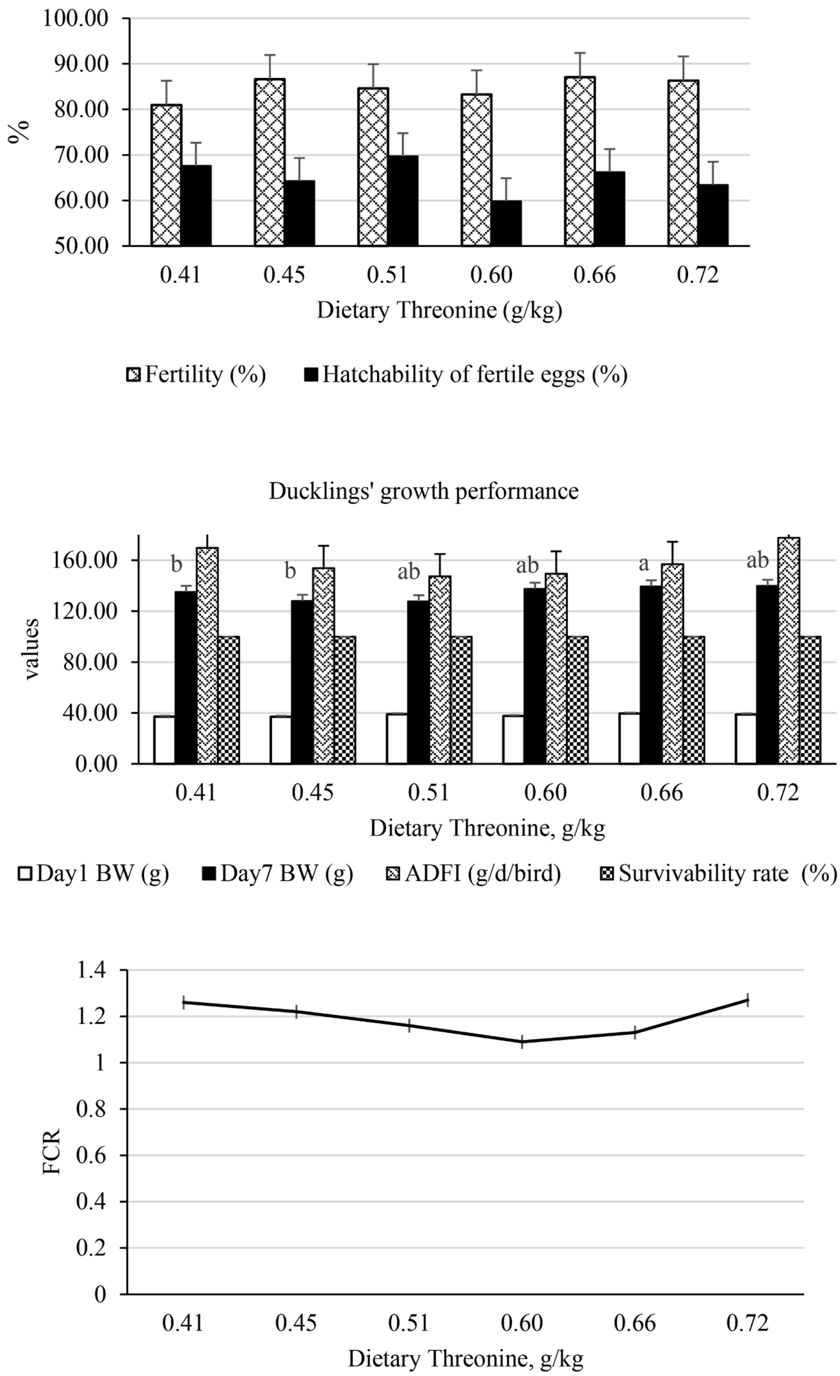

Fig. 3. Effects of the dietary threonine levels on reproductive performance and ducklings' growth performance in duck breeders. Data ± SEM and means on each bar with no common letter differ significantly at $P < 0.05$.

CD in Thr-supplemented birds could be linked to an abundance of dietary Thr causing a fast turnover of intestinal tissues. However, in broiler chicken, Eftekhari et al. (2015) reported that the increase of Thr levels (100, 110, 120 and 130 %) did not influence VH. Tanure et al. (2015) clarified that dietary Thr levels during the starter phase did not promoted any morphological alterations in the intestinal mucosa or affect nutrient metabolism. Finally, Thr's role in mucin formation may be connected to improvements in gut morphology. Mucin functions as a filtering barrier against external infections and shields the intestinal epithelium from acids and digesting enzymes (Kim and Ho, 2010). The augmented proliferation of enterocytes (as a result of Thr) secretion of intestinal mucin (as a result of developed crypt that contains mucin secreting goblet cell) as well as induces nutrients absorption (Ospina-Rojas et al., 2013).

Conclusion

These findings suggest that l-Thr supplementation at 0.28 % (constituting total Thr content of 0.66 %) is an effective strategy to optimize the performance of duck breeders and the quality of their offspring, providing valuable insights for dietary formulations in poultry production and emphasizing the importance of balanced amino acid nutrition for maximizing breeder performance and offspring quality. Further studies are needed to determine whether dietary protein levels can be reduced with amino acid supplementation in ducks without compromising productive and reproductive performance.

Disclosures

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgments

This work was funded by the National Key Research and Development Programs of China (2022YFD1300502, 2022YFD1300503, 2021YFD1300405), Guangdong Feed Industry Technology System (2024CXTD14), the Science and Technology Program of Guangdong Province (2023B1212060039) and State Key Laboratory of Swine and Poultry Breeding Industry (GDNKY-ZQQZ-K3, GDNKY-ZQQZ-K22). The Acknowledgement was extended to Researchers Supporting Project (RSPD2025R731), King Saud University (Riyadh, Saudi Arabia).

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