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Estimation of L-threonine requirements for Longyan laying ducks

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Objective: A study was conducted to test six threonine (Thr) levels (0.39%, 0.44%, 0.49%, 0.54%, 0.59%, and 0.64%) to estimate the optimal dietary Thr requirements for Longyan laying ducks from 17 to 45 wk of age.
Methods: Nine hundred Longyan ducks aged 17 wk were assigned randomly to the six dietary treatments, where each treatment comprised six replicate pens with 25 ducks per pen.
Results: Increasing the Thr level enhanced egg production, egg weight, egg mass, and the feed conversion ratio (FCR) (linearly or quadratically; $p<0.05$). The Haugh unit score, yolk color, albumen height, and the weight, percentage, thickness, and breaking strength of the eggshell did not response to increases in the Thr levels, but the albumen weight and its proportion increased significantly ($p<0.05$), whereas the yolk weight and its proportion decreased significantly as the Thr levels increased.
Conclusion: According to a regression model, the optimal Thr requirement for egg production, egg mass, and FCR in Longyan ducks is 0.57%, while 0.58% is the optimal level for egg weight from 17 to 45 wk of age.

Keywords: Laying Duck, Requirement, Threonine

INTRODUCTION

Threonine (Thr) is a crucial amino acid for poultry nutrition because it is the third most limiting amino acid and its metabolites are required for normal metabolism [1]. Thr is essential for mucin production, which plays a major role in intestinal health and nutrient absorption [2], as well as for the production of antibodies [3] and feather development [4]. In poultry, Thr deficiency has negative effects on the growth performance, carcass yield, and quality by reducing the thigh and breast yield, increasing body fat deposition, inhibiting feather development, and decreasing antibody formation, as well as having positive effects on the mortality rate [5-9]. In broiler chickens and quails, increasing the Thr levels during embryonic development improves the growth performance, mucin secretion, and the humoral immune response after hatching and feeding the supplemented diet [10-12]. In laying hens, dietary supplementation with L-Thr enhances egg production, the egg weight, feed conversion ratio (FCR), and antibody production during different phases of production and at different ambient temperature ranges [13-19]. However, to the best of our knowledge, no previous study has estimated the Thr requirements for laying ducks, and thus the Thr requirements for laying ducks are not available in the NRC [20]. Thus, the main aim of the present study was to estimate the Thr requirements of Longyan laying ducks, which are the most common laying ducks in South China, where they account for more than 60% of the laying ducks with a population size of more than 300 million birds. Longyan ducks exhibit early maturation, high egg production (280 to 300 eggs/yr), medium size, acclimatization to high ambient temperatures and disease resistance, and their eggs are popular. We estimated the Thr requirements from 17 to 45 wk based on the laying performance and egg quality.

MATERIALS AND METHODS

Bird management and experimental diet

A total of 900 Longyan ducks with similar body weights (1.2±0.01 kg) and the same genetic background were allocated randomly to six dietary treatments at 17 wk of age, where each treatment comprised six replicates with 25 ducks (each replicate was housed with an indoor area of 3×1.8 m, outdoor area of 3×1.8 m, and pool area of 3×1.8 m), and they were studied for 28 wk. Birds were fed the same basal diet in pellet form (Table 1), which was supplemented with 0.0% (control), 0.05%, 0.10%, 0.15%, 0.20%, or 0.25% of Thr in the form of L-Thr (98.5% L-Thr, Tongliao Meihua Biological Sci-Tech Co. Ltd, Inner Mongolia, China). The control diet was formulated to meet the nutritional requirements of egg-laying ducks [21], with the exception of Thr. Fresh water was provided *ad libitum* and feed was offered twice daily with an average of 160 g/bird/d, excluding any unconsumed remains. The ducks had access to the outdoor and pool areas during the daytime, but they were housed indoors at night. Natural light was available in the daytime with 4 h of incandescent lighting at 15 lx from 1,830 to 2,230. All of the experimental procedures were approved by the Animal Care and Use Committee of Guangdong Academy of Agriculture Science, China.

Laying performance and egg quality parameters

Feed intake, eggs, and broken and shell-less eggs were recorded daily on a per replicate basis. Feed intake, egg production, egg weight, egg mass, and FCR (g feed/g egg) were calculated daily on a per replicate basis, and then expressed as averages for the complete 28-wk study period.

Eight eggs were collected at random from each replicate between 37 and 45 wk of age (three times) and the average of 48 eggs per replicate was used to evaluate the egg quality each time. An Egg Analyzer (model EA-01, 133 ORKA Food Technology, Ramat HaSharon, Israel) was used to determine the yolk color and Haugh unit score on the day of collection, and an Egg Force Reader (model EFR-01, ORKA Food Technology,

Table 1. Composition of the experimental diets and nutrient levels

Items	Diet					
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Ingredients						
Corn (%)	30.58	30.58	30.58	30.58	30.58	30.58
Wheat (%)	30.00	30.00	30.00	30.00	30.00	30.00
Wheat bran (%)	11.50	11.50	11.50	11.50	11.50	11.50
Peanut meal (%)	15.60	15.60	15.60	15.60	15.60	15.60
Limestone (%)	8.50	8.50	8.50	8.50	8.50	8.50
Calcium hydrogen phosphate (%)	1.18	1.18	1.18	1.18	1.18	1.18
Sodium chloride (%)	0.3	0.3	0.3	0.3	0.3	0.3
Premix[1)] (%)	1.0	1.0	1.0	1.0	1.0	1.0
DL-Methionine (%)	0.21	0.21	0.21	0.21	0.21	0.21
L-Lysine-HCl (%)	0.61	0.61	0.61	0.61	0.61	0.61
L-Tryptophan (%)	0.04	0.04	0.04	0.04	0.04	0.04
L-Isoleucine (%)	0.23	0.23	0.23	0.23	0.23	0.23
Zeolite (%)	0.25	0.25	0.25	0.25	0.25	0.25
Total	100.0	100.0	100.0	100.0	100.0	100.0
Nutritional value						
Metabolizable energy (Kcal/kg)	2,500	2,500	2,500	2,500	2,500	2,500
Crude protein (%)	16.0	16.0	16.0	16.0	16.0	16.0
Calcium (%)	3.6	3.6	3.6	3.6	3.6	3.6
Total P (%)	0.59	0.59	0.59	0.59	0.59	0.59
Available Phosphorus (%)	0.35	0.35	0.35	0.35	0.35	0.35
Lysine (%)	0.90	0.90	0.90	0.90	0.90	0.90
Methionine (%)	0.40	0.40	0.40	0.40	0.40	0.40
Threonine (%)	0.39	0.44	0.49	0.54	0.59	0.63
Tyrptophan (%)	0.59	0.59	0.59	0.59	0.59	0.59
Isoleucine (%)	0.65	0.65	0.65	0.65	0.65	0.65
Leucine (%)	0.94	0.94	0.94	0.94	0.94	0.94

[1)] Supplied per kilogram of diet: retinyl palmitate, 12,000 IU; cholecalciferol, 2,000 IU; DL-α-tocopheryl acetate, 38 mg; menadione sodium bisulphite, 1.0 mg; thiamin mononitrate, 3.0 mg; riboflavin, 9.6 mg; pyridoxine hydrochloride, 6.0 mg cobalamin, 0.03 mg; chloride choline, 500 mg; nicotinic acid, 25 mg; calcium-D-pantothenate, 28.5 mg; folic acid, 0.6 mg; biotin, 0.15 mg; Fe, 50 mg; Cu, 10 mg; Zn, 90 mg; Mn, 90 mg, I, 0.5 mg; Se, 0.4 mg.

Israel) was used to measure the eggshell breaking strength. The yolks were separated, weighed, and expressed as percentages relative to the egg weight. The eggshell thickness was measured using a digital micrometer based on the average of three pieces of shell with membranes from the blunt, mid-length, and pointed ends. The shells with membranes were dried to a constant weight at 105°C in an oven and then weighed.

Statistical analysis

The experimental data were analyzed by one-way analysis of variance using the general linear model procedure in SAS (version 9.1; SAS Institute, 22), where the pen was the experimental unit for laying performance and the individual egg served as the experimental unit for egg quality. Orthogonal polynomial contrasts were employed to test the linear and quadratic effects of Thr supplementation. A quadratic broken-line regression model was used to determine the dietary Thr requirements by performing the NLIN procedure in SAS [22], where the model used was that described by Xie et al [23].

RESULTS AND DISCUSSION

Productive performance improved in a linear or quadratic manner ($p<0.05$; Table 2) as functions of increasing Thr supplementation. Our results are consistent with an earlier study of laying hens by [13], who reported that increasing the dietary Thr level significantly enhanced egg production, egg weight, and FCR. In addition, Faria et al [14] found that increasing the Thr concentration from 0.39% to 0.53% significantly increased egg production and the egg mass. Gomez and Angeles [24] observed that increasing the Thr level from 0.42% to 0.48% improved the FCR in laying hens during the second cycle of production. Cardoso et al [19] recommended increasing the Thr level from 0.523% to 0.567% to enhance egg production, the egg mass, and FCR in White Leghorn from 60 to 76 wk of age. In addition, recent studies by Azzam et al [16,17] detected significant improvements in egg production when the Thr level increased from 0.47% to 0.67% from 40 to 48 wk of age, while increasing the Thr level from 0.57% to 0.74% from 56 to 64 wk improved egg production and the egg mass in laying hens. In laying quails, egg production, the egg weight, and FCR were improved by increasing the Thr concentration in the diet [25] Similar findings have been obtained in meat-type birds, including turkey toms [5], Japanese quails [6], and Pekin ducks from 1 to 21 days of age [8] and from 15 to 35 days of age [9], where the average daily weight gain and FCR increased significantly with the dietary Thr concentration. Furthermore, in chickens challenged with infectious bursal disease, the body weight gain and FCR were improved significantly by increasing the Thr concentration in the diet [7]. Moreover, fertilized eggs injected with Thr on day 14 of incubation exhibited significant improvements in their body weight gain and FCR after the chickens hatched and they were fed the same diet (without Thr supplementation) for 28 and 42 days of age [10,11]. Increasing the Thr level enhances the gut morphology and its normal development by increasing the villus height and villus height/crypt depth ratio in different parts of the intestine, thereby positively affecting nutrient absorption due to the increased villus surface area [8]. This may explain why increasing the Thr level improves egg production, the egg weight, egg mass, and FCR. By contrast, there were no effects on the FCR after increasing the Thr level from 0.81% to 1.06% in quail chicks from 1 to 35 days according to Baylan et al [26], or from 0.70% to 0.90% in broiler chickens from 22 to 42 days according to Ayasan et al [27]. These differences may be related to the Thr level in the basal diet, breed, age, and/or the experimental period.

Egg quality

As shown in Table 3, the Haugh unit score, yolk color, albumen height, egg shell quality (eggshell weight, percentage, thickness, and breaking strength) did not respond ($p>0.05$) to increases in the dietary Thr level. By contrast, the albumen weight and percentage increased ($p<0.05$) as the dietary Thr level increased, whereas the yolk weight and percentage decreased ($p<0.05$). Gomez and Angeles [24] reported that increasing the Thr level from 0.42% to 0.54% had no effects on the albumen or yolk proportions, but the egg shell percentage declined as the dietary Thr level increased when continued for 6 wks during the second cycle in laying hens. Cardoso et al [19] found that feeding white laying hens a diet containing 0.523%, 0.546%, 0.569%, 0.592%, or 0.615% Thr for 17 wk during the late laying period had no effects on the egg quality, including the eggshell thickness, albumen, and yolk proportion. Azzam et al [16] found that increasing the Thr level from 0.47% to 0.87% in laying hens

Table 2. Effects of dietary threonine (Thr) supplementation on the performance of laying ducks

Item	Dietary Thr (mg/ kg)						SEM	p-value		
	0.39	0.44	0.49	0.54	0.59	0.64		Thr	Linear	Quadratic
Egg production (%)	81.8	83.2	86.5	86.4	86.4	86.1	1.05	0.009	0.001	0.02
Egg weight (g)	64.9	65.9	66.6	67.0	66.5	67.0	0.3	0.000	0.000	0.005
Egg mass (g/d/d)	53.0	54.8	57.6	57.9	57.5	57.6	0.7	0.000	0.000	0.002
FCR[1)]	3.15	2.95	2.80	2.79	2.82	2.81	0.05	0.000	0.000	0.002

SEM, standard error of means (n = 25 birds/replicate; n = 6 replicates/treatment).
[1)] Feed conversion ratio, g of feed/g of egg mass.

Table 3. Effects of dietary threonine (Thr) supplementation on egg quality

Item	Dietary Thr (mg/kg)						SEM	p-value		
	0.39	0.44	0.49	0.54	0.59	0.64		Thr	linear	Quadratic
Haugh unit	73.8	75.1	71.1	70.1	75.3	76.1	2.06	0.2	0.5	0.08
Yolk color	5.83	6.08	6.08	6.33	5.75	5.41	0.32	0.3	0.4	0.09
Albumen height (mm)	5.91	5.91	5.51	5.66	6.05	6.25	0.22	0.2	0.2	0.06
Albumen weight (g)	38.1	41.8	40.9	40.0	41.2	41.4	0.5	0.001	0.005	0.07
Albumen (%)	58.8	63.4	61.3	59.7	62.0	61.7	0.7	0.001	0.1	0.3
Yolk weight (g)	20.7	18.2	19.7	20.6	19.3	19.6	0.5	0.01	0.6	0.5
Yolk (%)	32.0	27.6	29.6	30.8	29.0	29.2	0.7	0.005	0.2	0.2
Eggshell weight (g)	5.98	5.89	6.03	6.41	5.92	6.05	0.12	0.06	0.4	0.2
Eggshell (%)	9.21	8.94	9.05	9.56	8.90	9.03	0.18	0.1	0.7	0.5
Eggshell thickness (mm)	0.338	0.332	0.335	0.338	0.323	0.327	0.007	0.6	0.2	0.7
Breaking strength (N)	4.01	3.75	3.73	4.54	3.87	3.94	0.25	0.2	0.7	0.7

SEM, standard error of means (n = 8 eggs/replicate; n = 6 replicates/treatment).

aged 40 wk during the summer season for 8 wk had no effect on the Haugh unit score, yolk color, eggshell thickness, or strength. In addition, feeding laying hens diets supplemented with high levels of Thr (1%, 2%, or 3%) for two months during the post-peak period had no effects on the albumen height, Haugh unit score, yolk color, and egg shell strength, but the eggshell thickness declined in a linear manner. In brown laying hens, increasing the Thr level from 0.0% to 0.4% for 8 wks during the late laying period had no significant effects on the albumen height, eggshell percentage, or eggshell thickness, but the Haugh unit score improved significantly [18]. Valerio et al [28] found that increasing the Thr levels in white or brown laying hens from 21 to 36 wk of age did not change the Haugh unit score. Moreover, Sa et al [29] showed that the Haugh unit score was not affected by increasing the Thr levels in different strains of laying hens in an experiment conducted for 16 wk (from 34-50 wk of age). Previous studies indicate that the effects of the Thr levels on egg quality may vary according to the age, strain, and experimental period. In our experiment, we found that increasing the Thr level increased the albumen weight and proportion, but decreased the yolk weight and proportion, which may explain why the egg weight increased. This finding could be due to the involvement of Thr in protein synthesis [1]. In our experiment, the control group exhibited the worst laying performance compared with the other groups because this group was fed a diet deficient in Thr. Feeding laying birds with a diet deficient in amino acids generally reduces protein production throughout the whole body, but specifically in the liver and magnum, which is responsible for albumen secretion [30,31]. In a previous study, increasing the concentration of amino acids increased the albumen proportion in laying hens as well as decreasing the yolk proportion [32].

In conclusion, we estimate that the dietary Thr requirement is 0.57% to achieve the maximum egg production, egg mass, and feed utilization relative to the egg mass, while a level of 0.58% is required to obtain the maximum egg weight in Longyan ducks from 17-45 wk of age.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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