

Estimation of dietary tryptophan requirement for laying duck breeders: effects on productive and reproductive performance, egg quality, reproductive organ and ovarian follicle development and serum biochemical indices

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ABSTRACT This study aimed to determine the dietary tryptophan (**Trp**) requirement for laying duck breeders. A total of 504 Longyan duck breeders (body weight: 1.20 ± 0.02 kg) aged 22 wk were randomly allocated to 6 treatments, each with 6 replicates of 14 ducks. During the next 16 wk, birds were fed the basal diet with total Trp contents of 1.00, 2.00, 3.00, 4.00, 5.00 and 6.00 g/kg, respectively. Dietary Trp levels increased egg production, egg mass and feed intake of duck breeders from 22 to 37 wk ($P < 0.05$), and there were linear and quadratic effects of Trp level ($P < 0.05$). The feed conversion ratio (**FCR**) quadratically decreased with dietary Trp levels ($P < 0.05$). Dietary Trp levels decreased ($P < 0.05$) egg albumen height and Haugh unit at wk 8 or 12, and the responses were linear and quadratic ($P < 0.05$). The body weight of breeders, absolute and relative weight of oviduct, number and total weight of preovulatory follicles (**POF**), and its proportion relative to

ovarian weight were increased ($P < 0.05$), and the responses were linear ($P < 0.01$) and quadratic ($P < 0.001$). Ovarian weight increased quadratically ($P < 0.05$), and the mean weight of POF increased ($P < 0.05$), linearly and quadratically. The proportion of small yellow follicles relative to ovary weight decreased ($P < 0.01$) linearly and quadratically. At wk 16 of the trial period, the serum albumin content and alanine aminotransferase activity decreased ($P < 0.05$) and the creatinine content increased ($P < 0.01$) linearly and quadratically. The Trp requirements were estimated to be 3.14 g/kg for optimizing egg production, 2.93 g/kg for egg mass, and 2.92 g/kg for FCR. Overall, dietary Trp levels (1 to 6 g/kg) affected productive performance, egg quality, reproductive organ and ovarian follicle development, and serum biochemical indices of layer duck breeders, and a diet containing 2.9 to 3.1 g Trp per kg feed was adequate during the laying period (22 to 37 wk of age).

Key words: laying duck breeder, laying performance, requirement, tryptophan

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INTRODUCTION

L-tryptophan is an essential amino acid for poultry, required for protein synthesis, affecting appetite, regulating lipid metabolism and immune responses, and plays vital roles in intestinal function (Emadi et al., 2010). Broiler chicks fed Trp-deficient diets had poor body weight gain, feed intake and feed conversion (Corzo et al., 2005). Supplementation of Trp in the diet increased growth performance of young broiler chicks (Shan et al., 2003), and higher Trp (0.3% to 0.5%) than the NRC recommended

level (0.2%) could give better growth, immune response and antioxidant status in broiler chickens (Mund et al., 2020), and alleviate oxidative stress and improve welfare and feed efficiency in broilers reared in cages with a high stocking density (Wang et al., 2014). In laying hens, the supplementation of Trp could increase egg weight (Antar et al., 2004) and egg production (Ohtani et al., 1989), alleviate fatty liver and modify microsomal mixed function oxidase in the liver (Akiba et al., 1992); excess dietary Trp could improve shell quality and immune function under conditions of high temperature and humidity (Dong et al., 2012). In addition, Trp is highly related to feather pecking, which is one of the most prevalent causes of mortality for commercial laying hens (Birkl et al., 2017; 2019). In meat ducks, dietary Trp supplementation could alleviate stress and improve growth performance, antioxidative activity and meat quality (Liu et al., 2015). Furthermore, dietary

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tryptophan (0.11% to 0.27%) addition affected the productive and reproductive performance, the development of follicles of broiler breeders (Jiang et al., 2018). Therefore, it is necessary to add Trp to poultry diets to achieve normal performance, antioxidant and immune status, and production quality, and excess Trp may even alleviate the negative effects of stress.

It was reported that absolute requirement of the chick for Trp varied with age and was significantly different for male and female birds (Freeman, 1979). Wang et al. (2016) suggested that for male broilers 22 to 42 d of age, the apparent ideal digestible Trp requirement was approximately 0.15%, based on performance and meat quality, while it was, or no higher than, 0.11% for female broilers. The dietary Trp requirement of the young (0 to 21 d of age) broiler chicken was no greater than 0.16% (Smith and Waldroup, 1988). Supplementation of dietary Trp at 0.16% has no positive effect on broiler chickens (21 to 42 d of age), including growth performance and intestinal barrier function (Goo et al., 2019). In a previous study, dietary Trp increased egg production, mass and egg content, and dietary Trp at 0.16% or 157 mg per hen as the requirement for maximum production of laying hens from 53 to 59 wk of age (Russell and Harms, 1999). Similarly, dietary Trp requirement was around 0.15% to 0.16% or 143 to 155 mg per hen for laying hens from 41 to 60 wk of age (Wen et al., 2019). The optimal dietary Trp level was 0.203% or 254 mg per hen per day, for Chinese yellow-feathered broiler breeder hens aged from 28 to 37 wk of age (Jiang et al., 2018). The Trp requirement for laying duck breeders is unknown and needed to be established.

The present study, therefore, has examined the effects of Trp supplementation on productive and reproductive performance, egg quality, reproductive organ and ovarian follicle development, serum biochemical indices in laying duck breeders, and has used productive performance as a relevant indicator to estimate dietary Trp requirement.

MATERIALS AND METHODS

Experimental Design and Diets

The use of the ducks and the experimental protocol were approved by the Animal Care and Use Committee of the Animal Science Institute of Guangdong Academy of Agriculture Sciences (No. GAASIAS-2016-017). The experiment was completely randomized design. A total of 504 Longyan duck breeders (22-wk) were randomly divided into 6 treatment groups with 6 replicates of 14 birds each and fed for 16 wk corresponding to the peak laying period. The 6 treatments were the basal diet supplemented with 0 (control), 1.02, 2.04, 3.06, 4.08, or 5.10 g L-Trp/kg (98%, Evonik Industries AG, Essen, Germany) respectively, which resulted in total contents of 1.00, 2.00, 3.00, 4.00, 5.00 and 6.00 g Trp/kg of diet. Birds were housed singly in cages (42 cm × 30 cm × 50 cm) with a nipple drinker and feeder (Guangzhou Huanan Poultry Equipment, Guangzhou, PRC). Fresh drinking water was available ad libitum, and 80 g of

Table 1. Composition and nutrient levels of the diets.

Items	Dietary Trp levels (g/kg)					
	1.0	2.0	3.0	4.0	5.0	6.0
Ingredients (%)						
Corn (CP, 7.8%)	61.19	61.19	61.19	61.19	61.19	61.19
Soybean meal (CP, 43.6%)	3.00	3.00	3.00	3.00	3.00	3.00
Wheat bran (CP, 15.7%)	10.0	10.0	10.0	10.0	10.0	10.0
Corn gluten meal (CP, 63.5%)	14.0	14.0	14.0	14.0	14.0	14.0
Limestone	8.60	8.60	8.60	8.60	8.60	8.60
Dicalcium phosphate	1.30	1.30	1.30	1.30	1.30	1.30
Salt	0.30	0.30	0.30	0.30	0.30	0.30
DL-Methionine (98%)	0.06	0.06	0.06	0.06	0.06	0.06
L-Lysine•HCl (98.5%)	0.55	0.55	0.55	0.55	0.55	0.55
Vitamin-mineral premix ¹	1.00	1.00	1.00	1.00	1.00	1.00
L-Tryptophan (98%, g/kg) ²	0.00	1.02	2.04	3.06	4.08	5.10
Total	100	100	100	100	100	100
Nutrients (%)						
Apparent metabolizable energy (AME, MJ/kg) ³	10.73	10.70	10.74	10.76	10.81	10.80
Crude protein (CP) ³	17.2	17.1	16.9	17.0	17.0	16.9
Calcium ³	3.62	3.60	3.65	3.69	3.65	3.66
Total phosphorus ³	0.68	0.63	0.65	0.64	0.66	0.63
Available phosphorus	0.38	0.38	0.38	0.38	0.38	0.38
Methionine (g/kg) ³	4.49	4.36	4.21	4.50	4.23	4.18
Lysine (g/kg) ³	8.86	8.60	8.67	8.79	8.38	8.64
Methionine + cysteine (g/kg) ³	7.65	7.48	7.27	7.75	7.51	7.41
Threonine (g/kg) ³	6.20	6.10	5.90	6.12	6.11	6.06
Tryptophan (g/kg) ³	0.90	1.92	3.10	3.92	4.89	5.79

¹Provided per kilogram of diet: VA 12 500 IU; VD₃ 4 125 IU; VE 15 IU; VK 2 mg; thiamine 1 mg; riboflavin 8.5 mg; calcium pantothenate 50 mg; niacin 32.5 mg; pyridoxine 8 mg; biotin 2 mg; folic acid 5 mg; VB₁₂ 5 mg; 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; zeolite carrier 5500 mg.

²Added L-Tryptophan in other 5 treatments except control replaced the corresponding content of zeolite carrier in the vitamin-mineral premix.

³Analyzed values.

pelleted feed per duck was introduced twice daily at 07:00 and 15:00. The basal diet (Table 1) was composed mainly of corn and soybean meal and was formulated to satisfy the nutritional requirements for Longyan laying duck breeders with the exception of Trp. The diets were made individually based on the basal diet. The analyzed nutrient levels of gross energy, calcium, phosphorus, crude protein and key amino acids are also listed in Table 1. The experimental diets were analyzed for gross energy, crude protein, calcium, and phosphorus according to AOAC (2003) procedures. The gross energy in diets was analyzed by Parr 6400 bomb calorimetry (Parr Instrument Inc., Moline, IL, USA). The crude protein in diets was analyzed by a Kjeltac 8400 Analyzer Unit (FOSS Analytical AB, Hoganas, Sweden). The amino acids in diets were analyzed after hydrolysis by an amino acid analyzer (HITACHI L-8900, Hitachi, Ltd., Tokyo, Japan). The lysine and threonine were

measured after acid hydrolysis with 6 N HCl (reflux for 24 h at 110°C), methionine and cystine were determined after oxidation with performic acid and subsequent hydrolysis with 6 M HCl, and the tryptophan content was determined after alkaline hydrolysis at 120°C for 16 h (Zhang et al., 2013).

Starting at 34 wk of age, each breeder was artificially inseminated twice weekly with 100 μ L of pooled semen to evaluate reproductive performance (fertility, hatchability, and proportion of healthy ducklings). In total, 1,440 eggs (40 eggs from each replicate) were collected over 5 sequential d between 34 and 35 wk, starting on the second day after the first artificial insemination. The eggs were weighed, labeled with number and date, and stored in a dark controlled-temperature room (18°C; 75% to 80% relative humidity), and then incubated (JXB2000; Dezhou Jingxiang Technology Co, Dezhou, PRC) for 28 d. Temperatures and humidity were: 38.4°C and 45% (d 0 to 5); 38.0°C and 50% (d 6 to 10); 37.5°C with 50% (d 11 to 15); 37.1°C and 55% (d 16 to 20); 36.8°C and 60% (d 21 to 25); then 36.5°C and 65% (d 26 to 28) (Zhang et al., 2020). The eggs were candled on d 6 and 18 to eliminate infertile eggs and dead embryos. After 28 d, the healthy hatched ducklings were counted and recorded, and eggs that failed to hatch were counted. Fertility was calculated as fertile eggs as a proportion of set eggs. Hatchability was calculated on the basis of fertile eggs. Healthy ducklings (clean and dry, free of deformities and with bright eyes) were assessed macroscopically (Zhang et al., 2020). The hatchling body weight was measured on a replicate basis.

Sample Collection

Five eggs per replicate were collected at 4-wk intervals during the treatment period for determining egg quality; measurements were made on the day of collection.

At the end of wk 8 (29 wk of age) and 16 (37 wk of age), 2 healthy ducks in each replicate were randomly selected and fasted for 12 h for sampling. Between 4:00 and 5:00 pm, approximately 3 mL blood was collected from a wing vein of each duck using noncoated evacuated tubes. The tubes were then incubated in a 37°C water bath while tilted at a 45° angle for 3 h, then centrifuged at 3,000 $\times g$ for 10 min to harvest serum. The serum samples were stored at -20°C for subsequent analysis (Zhang, et al., 2020).

At the end of the trial (37 wk of age), the sampled ducks were weighed and then killed by cervical dislocation. The liver was collected and weighed, the weight and length of the oviduct were measured, and the ovary was collected and weighed. The preovulatory follicles (POF, > 10 mm in diameter) and small yellow follicles (SYF, 6 to 10 mm in diameter) were dissected, counted, weighed, and recorded. The relative weights, or indices, of liver, oviduct, and ovary were calculated as percentages of duck body weight; the weight proportions of POF and SYF were calculated as percentages of ovarian weight (Zhang, et al., 2020).

Productive Performance and Egg Quality Measurement

The number and weight of all oviposited eggs, and feed consumption were recorded daily in each replicate, and then expressed as averages for their corresponding 4-wk laying period.

Eggshell thickness and breaking strength were separately determined using an Egg Shell Thickness Gauge and Egg Force Reader (Orka Food Technology Ltd., Ramat Hasharon, Israel). The shells with membranes were weighed after drying at 105°C for 6 h. The egg weight and shell weight of the 5 eggs for each treatment replicate were individually recorded. Eggshell proportion was calculated as the eggshell weight relative to egg weight. Egg albumen height, yolk color and Haugh unit were determined using an Egg Analyzer (Orka) (Zhang et al., 2020).

Serum Biochemical and Antioxidant Indices

The contents in serum of total protein (TP), albumin (ALB), creatinine (CRE), uric acid (UA), alanine aminotransferase (ALT), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), calcium (Ca), and phosphorus (P) were determined with kits in an automatic biochemistry analyzer (all from Shanghai Kehua Bio-Engineering Co., Ltd., Shanghai, China) (Zhang et al., 2020).

Statistical Analysis

Replicate (each replicate contained 14 cages, one duck in each cage) served as the experimental unit for analysis of performance and egg quality data; the average of the 2 sampled ducks in each replicate was the experimental unit for other variables. The normality of the data and homogeneity of variances were first verified by Explore procedure using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL). The effects of dietary Trp levels were analyzed by one-way ANOVA, and then polynomial regressions were fitted. Quadratic regressions ($Y = aX^2 + bX + c$) were fitted to the responses of the dependent variables to Trp supplementation. The dietary concentration of Trp at which the response first reached 95% of the maximum was used to estimate the requirement (Zhang et al., 2020). Data are expressed as means and pooled SEM.

RESULTS

Productive and Reproductive Performance

The effects of dietary Trp levels on productive performance of laying duck breeders are shown in Table 2. Dietary Trp levels increased (5 to 8 wk, 13 to 16 wk, and 1 to 16 wk; $P < 0.05$) egg production and mass of laying duck breeders, and the responses were linear (13 to 16 wk, and 1 to 16 wk; $P < 0.05$) and quadratic (5 to 8 wk,

Table 2. Effects of dietary different tryptophan levels on the productive performance of duck breeders in the laying period (22 to 37 wk of age).

Items	Trp level (g/kg) ¹						SEM	P value		
	1.0	2.0	3.0	4.0	5.0	6.0		ANOVA	Linear	Quadratic
Egg production (%)										
1–4 wk	87.2	90.9	93.1	87.7	90.3	91.2	0.73	0.112	0.393	0.622
5–8 wk	80.4	87.3	87.3	85.1	88.4	85.1	0.76	0.037	0.171	0.034
9–12 wk	84.0	84.6	85.6	80.8	84.6	87.8	0.83	0.271	0.410	0.308
13–16 wk	68.2	74.2	79.1	77.9	75.6	76.9	0.93	0.004	0.012	0.001
1–16 wk	79.6	83.0	84.9	83.5	84.0	84.2	0.53	0.044	0.019	0.009
Average egg weight (g)										
1–4 wk	58.1	59.2	58.1	58.7	58.4	57.8	0.18	0.253	0.414	0.311
5–8 wk	61.0	62.2	61.6	62.2	61.7	61.3	0.19	0.468	0.935	0.223
9–12 wk	63.2	63.2	63.0	63.5	63.2	62.6	0.19	0.852	0.591	0.658
13–16 wk	63.0	63.3	63.2	63.6	63.0	62.3	0.19	0.482	0.328	0.130
1–16 wk	61.4	62.0	61.6	62.1	61.6	61.1	0.17	0.546	0.537	0.236
Egg mass (g)										
1–4 wk	50.6	54.0	54.0	51.1	52.6	52.8	0.43	0.101	0.651	0.575
5–8 wk	49.2	54.2	53.8	52.9	54.4	52.2	0.48	0.014	0.228	0.012
9–12 wk	53.1	53.5	53.9	51.3	53.4	54.9	0.49	0.442	0.527	0.444
13–16 wk	42.9	47.1	49.9	49.7	47.7	47.8	0.59	0.003	0.027	<0.001
1–16 wk	48.8	51.5	52.3	51.8	51.7	51.4	0.32	0.023	0.049	0.004
Average daily feed intake										
1–4 wk	157	161	162	159	159	160	0.36	0.001	0.216	0.049
5–8 wk	155	158	158	158	158	158	0.31	0.069	0.071	0.010
9–12 wk	157	159	159	157	159	160	0.33	0.039	0.014	0.040
13–16 wk	154	156	158	158	157	157	0.33	0.002	0.002	<0.001
1–16 wk	156	158	159	158	158	159	0.25	0.001	0.006	0.002
FCR (g:g)										
1–4 wk	3.11	3.00	3.00	3.13	3.04	3.04	0.023	0.444	0.829	0.912
5–8 wk	3.16	2.91	2.95	2.99	2.90	3.03	0.024	0.019	0.308	0.024
9–12 wk	2.97	2.97	2.95	3.08	2.99	2.93	0.024	0.548	0.995	0.532
13–16 wk	3.60	3.33	3.15	3.19	3.30	3.29	0.038	0.004	0.031	<0.001
1–16 wk	3.19	3.07	3.04	3.06	3.06	3.08	0.016	0.089	0.129	0.015

¹Mean of 6 replicates (14 ducks per replicate) per treatment. ²FCR, feed conversion ratio.

13 to 16 wk, and 1 to 16 wk; $P < 0.05$) with increasing Trp levels. The average daily feed intake was increased (1 to 4 wk, 9 to 12 wk, 13 to 16 wk, and 1 to 16 wk; $P < 0.05$) and there were linear (9 to 12 wk, 13 to 16 wk, and 1 to 16 wk; $P < 0.05$) and quadratic ($P < 0.05$) effects. The FCR was decreased (5 to 8 wk, 13 to 16 wk; $P < 0.05$) and responses were linear (13 to 16 wk, $P < 0.05$) and quadratic (5 to 8 wk, 13 to 16 wk, 1 to 16 wk; $P < 0.05$) during the laying period from 22 to 37 wk.

Dietary Trp levels did not affect average egg weight, fertility, hatchability, or hatchling body weight, nor proportion of healthy ducklings (Table 3).

Egg Quality

Table 4 shows the effects of dietary Trp levels on egg quality in laying duck breeders during the trial period. Dietary Trp levels did not affect egg weight, eggshell

thickness, breaking strength or shell proportion during the treatment period. The albumen height was decreased (wk 8, $P < 0.05$) and the responses were linear (wk 4 and 8, $P < 0.01$) and quadratic (wk 4 and 8, $P < 0.05$). The yolk color was increased linearly ($P < 0.05$) and quadratically ($P < 0.05$) after ducks had been fed for 16 wk. The Haugh unit was decreased (wk 12 and 16; $P < 0.05$) and there were linear (wk 8 and 12; $P < 0.05$) and quadratic (wk 12; $P < 0.01$) effects with dietary Trp levels.

Reproductive Organs and Ovarian Follicle Development

As shown in Table 5, the body weight of breeders was affected ($P < 0.05$) and increased ($P < 0.05$) linearly and quadratically with increasing Trp levels in the diet. Weight and index of the oviduct were increased

Table 3. Effects of dietary different tryptophan levels on the reproductive performance of duck breeders in the laying period (33 to 34 wk).

Variables	Trp level (g/kg) ¹						SEM	P value		
	1.0	2.0	3.0	4.0	5.0	6.0		ANOVA	Linear	Quadratic
Average egg weight (g)	61.1	61.5	62.1	61.1	60.9	61.0	0.25	0.757	0.447	0.527
Fertility of set eggs (%)	86.5	82.4	77.9	76.3	81.3	76.9	1.48	0.327	0.081	0.119
Hatchability of fertile eggs (%)	49.8	67.2	76.6	65.7	70.4	70.8	2.97	0.058	0.088	0.065
Healthy duckling (%)	95.7	95.6	97.7	93.4	97.4	96.7	0.87	0.768	0.736	0.917
1-d hatchling body weight (g) ²	39.4	39.7	40.5	40.0	39.0	38.7	0.27	0.448	0.276	0.133

¹Mean of 6 replicates (50 eggs per replicate) per treatment.

Table 4. Effects of dietary different tryptophan levels on the egg quality of duck breeders in the laying period (22 to 37 wk of age).

Variables	Trp level (g/kg) ¹						SEM	P value		
	1.0	2.0	3.0	4.0	5.0	6.0		ANOVA	Linear	Quadratic
Egg weight (g)										
4 wk	63.8	63.7	63.9	64.4	64.1	63.8	0.35	0.995	0.843	0.917
8 wk	62.3	62.0	62.7	62.9	62.3	62.0	0.23	0.821	0.946	0.601
12 wk	63.5	64.3	63.1	63.4	63.2	63.3	0.34	0.936	0.590	0.865
16 wk	61.7	60.1	60.3	61.5	60.0	60.5	0.32	0.489	0.445	0.659
Albumen height (mm)										
4 wk	8.07	8.31	8.13	8.26	8.29	7.79	0.079	0.395	0.425	0.192
8 wk	7.77	7.62	7.88	7.56	7.25	7.33	0.067	0.036	0.006	0.019
12 wk	7.79	7.73	7.53	7.52	7.49	7.15	0.074	0.171	0.008	0.029
16 wk	7.63	7.83	7.97	7.56	7.73	7.44	0.063	0.168	0.210	0.107
Yolk color										
4 wk	9.33	10.1	9.72	9.78	9.33	9.61	0.073	0.021	0.637	0.244
8 wk	9.73	9.67	9.27	9.77	9.70	9.00	0.077	0.008	0.052	0.085
12 wk	9.50	9.97	9.63	9.87	9.90	9.57	0.051	0.017	0.725	0.082
16 wk	8.53	9.03	9.13	9.13	9.33	9.13	0.083	0.096	0.019	0.013
Haugh unit										
4 wk	88.6	90.1	89.4	89.7	90.0	87.2	0.435	0.419	0.428	0.161
8 wk	87.3	86.7	88.2	86.2	84.6	85.1	0.412	0.101	0.021	0.054
12 wk	87.1	86.8	85.8	85.7	85.1	82.7	0.458	0.049	0.002	0.006
16 wk	86.4	88.3	89.4	86.4	87.9	86.0	0.381	0.051	0.436	0.083
Eggshell thickness (mm)										
4 wk	0.316	0.314	0.317	0.318	0.317	0.321	0.001	0.894	0.284	0.508
8 wk	0.313	0.314	0.316	0.316	0.319	0.320	0.001	0.433	0.048	0.109
12 wk	0.330	0.331	0.325	0.335	0.321	0.328	0.002	0.298	0.403	0.709
16 wk	0.315	0.317	0.315	0.318	0.314	0.315	0.002	0.998	0.857	0.956
Eggshell breaking strength (N)										
4 wk	40.4	41.5	43.6	41.9	41.9	41.4	0.488	0.624	0.675	0.319
8 wk	40.9	40.8	41.4	41.3	41.7	42.4	0.555	0.974	0.379	0.661
12 wk	38.1	39.4	41.2	38.9	36.4	38.1	0.595	0.319	0.361	0.356
16 wk	40.7	40.2	39.8	39.7	40.4	40.4	0.547	0.995	0.935	0.835
Eggshell proportion (%) ²										
4 wk	9.69	9.61	9.78	9.72	9.68	9.75	0.037	0.873	0.559	0.840
8 wk	9.63	9.58	9.57	9.61	9.60	9.58	0.032	0.996	0.807	0.957
12 wk	9.27	9.50	9.37	9.38	9.30	9.43	0.042	0.705	0.789	0.927
16 wk	9.14	9.06	8.92	9.11	9.13	9.00	0.040	0.625	0.718	0.854

¹Mean of 6 replicates (5 eggs per replicate) per treatment.

²Eggshell proportion (%) = 100 × eggshell weight/egg weight.

Table 5. Effects of dietary different tryptophan levels on the reproductive organ and ovarian follicle development of duck breeders (37 wk of age).

Variables ²	Trp level (g/kg) ¹						SEM	P value		
	1.0	2.0	3.0	4.0	5.0	6.0		ANOVA	Linear	Quadratic
Body weight (g)	1.02	1.08	1.14	1.09	1.11	1.16	0.014	0.034	0.007	0.019
Liver (g)	35.1	37.5	38.1	38.6	35.7	36.7	0.716	0.721	0.850	0.427
Liver index (%)	3.48	3.49	3.33	3.54	3.22	3.20	0.063	0.495	0.127	0.266
Oviduct weight (g)	35.8	45.8	47.9	49.8	52.3	48.7	1.282	0.001	<0.001	<0.001
Oviduct index (%)	3.51	4.22	4.14	4.70	4.64	4.23	0.096	0.001	0.005	<0.001
Oviduct length (cm)	42.7	45.1	44.5	43.0	46.1	44.3	0.610	0.623	0.448	0.680
Ovarian weight (g)	33.5	39.8	38.6	39.2	39.3	38.7	0.660	0.047	0.057	0.019
Ovary index (%)	3.30	3.71	3.44	3.60	3.56	3.37	0.056	0.278	0.938	0.260
Number of POF	1.92	4.25	4.25	4.40	4.58	3.75	0.198	<0.001	0.009	<0.001
Total POF weight (g)	16.7	33.8	32.3	44.5	41.8	33.5	2.067	<0.001	0.003	<0.001
Mean POF weight (g)	6.15	7.45	8.12	9.94	9.15	8.95	0.413	0.094	0.011	0.012
POF proportion (%)	43.9	76.5	80.6	76.4	86.1	73.9	2.892	<0.001	0.002	<0.001
Number of SYF	11.3	11.9	6.58	8.17	11.5	7.92	0.518	0.002	0.116	0.142
Total SYF weight (g)	1.95	1.99	1.33	1.52	1.88	1.57	0.082	0.096	0.232	0.227
Mean SYF weight (g)	0.194	0.171	0.198	0.192	0.163	0.206	0.008	0.553	0.852	0.804
SYF proportion (%)	6.11	4.89	2.78	2.86	3.60	3.34	0.298	0.002	0.003	<0.001

¹Mean of 6 replicates (2 ducks per replicate) per treatment.

²POF, preovulatory follicles; SYF, small yellow follicles; LWF, large white follicles; liver, oviduct, and ovary index refer to their weight relative to body weight; POF and SYF proportion refers to their weight relative to ovarian weight.

($P < 0.01$) and the responses were linear ($P < 0.01$) and quadratic ($P < 0.001$). The ovarian weight was affected ($P < 0.05$) and increased quadratically ($P < 0.05$) with dietary Trp levels. The number, total and relative

weights of POF were increased ($P < 0.001$), and the responses were linear ($P < 0.01$) and quadratic ($P < 0.001$). The mean weight of POF was not affected but it increased ($P < 0.05$) linearly and quadratically with

Table 6. Effects of dietary different tryptophan levels on the serum biochemical indices of duck breeders at the end of wk 8 and 16.

Time	Variables ²	Trp level (g/kg) ¹						SEM	P value		
		1.0	2.0	3.0	4.0	5.0	6.0		ANOVA	Linear	Quadratic
8 wk	TP (g/L)	63.2	59.5	65.4	64.0	59.4	62.7	0.67	0.037	0.760	0.853
	ALB (g/L)	24.2	23.5	22.2	23.6	24.1	25.2	0.57	0.795	0.502	0.357
	UA (μ mol/L)	285	277	295	284	273	256	0.6	0.802	0.291	0.372
	CRE (μ mol/L)	34.7	34.3	36.2	36.1	33.3	37.0	0.40	0.060	0.326	0.613
	ALT (U/L)	30.5	32.5	30.9	28.6	31.2	30.6	0.64	0.683	0.671	0.878
	TG (mmol/L)	13.0	12.4	15.2	14.5	10.5	12.0	0.69	0.414	0.434	0.418
	TC (mmol/L)	3.92	3.37	3.41	3.27	2.91	3.55	0.113	0.210	0.152	0.080
	HDL-C (mmol/L)	1.56	1.50	1.49	1.67	1.38	1.54	0.034	0.263	0.705	0.931
	LDL-C (mmol/L)	0.67	0.47	0.52	0.63	0.46	0.51	0.023	0.022	0.134	0.229
	Ca (mmol/mL)	7.81	7.61	8.56	8.30	7.42	7.76	0.125	0.060	0.686	0.241
16 wk	P (mmol/mL)	3.19	2.99	3.56	3.19	2.75	3.17	0.130	0.659	0.668	0.870
	TP (g/L)	55.4	60.6	59.7	58.1	60.1	61.8	0.69	0.104	0.040	0.116
	ALB (g/L)	22.4	20.2	18.4	15.8	13.0	13.0	0.80	<0.001	<0.001	<0.001
	UA (μ mol/L)	190	183	186	208	203	150	6.26	0.097	0.366	0.113
	CRE (μ mol/L)	31.0	44.9	39.7	46.0	51.5	43.2	1.66	0.005	0.008	0.004
	ALT (U/L)	33.2	27.3	28.2	21.7	24.4	27.5	1.00	0.017	0.031	0.004
	TG (mmol/L)	6.81	9.28	9.15	10.5	9.99	5.79	0.547	0.069	0.889	0.013
	TC (mmol/L)	2.55	2.46	2.93	2.82	2.55	1.86	0.104	0.041	0.124	0.008
	HDL-C (mmol/L)	1.43	1.49	1.51	1.56	1.47	1.33	0.036	0.554	0.536	0.151
	LDL-C (mmol/L)	0.484	0.292	0.503	0.488	0.401	0.329	0.026	0.059	0.393	0.424
Ca (mmol/mL)	7.35	6.78	6.97	7.25	7.92	7.35	0.152	0.365	0.245	0.437	
P (mmol/mL)	3.18	2.82	3.99	3.45	3.84	3.22	0.150	0.202	0.381	0.289	

¹Mean of 6 replicates (2 duck samples per replicate) per treatment.

²TP, total protein; ALB, albumin; UA, uric acid; CRE, creatinine; ALT, alanine aminotransferase; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Ca, calcium; P, phosphorus.

dietary Trp levels. Dietary Trp levels did not affect the number, total weight, and mean weight of SYF, but the SYF proportion was affected ($P < 0.05$) and decreased ($P < 0.01$) linearly and quadratically with increasing dietary Trp levels.

Serum Biochemical Indices

Effects of dietary Trp level on the serum biochemical indices of duck breeders in the laying period are shown in Table 6. At wk 8, with the exception of serum TP and LDL-C contents being affected ($P < 0.05$), other biochemical indices were not affected by dietary Trp levels. At wk 16, the serum contents of ALB, CRE, and TC, and ALT activity were affected ($P < 0.05$) by the dietary Trp levels. The serum ALB content decreased ($P < 0.001$), the CRE content increased ($P < 0.01$), and the ALT activity decreased ($P < 0.05$) linearly and quadratically with increasing Trp levels. There were quadratic responses of TG ($P < 0.05$) and TC ($P < 0.01$) contents by dietary Trp levels. Other biochemical indices in serum were not influenced by dietary Trp levels.

Estimations of the Dietary TRP Requirements

Dietary Trp requirements of laying duck breeders, as estimated from the quadratic regression analyses of productive traits, are shown in Table 7. The Trp requirements in g/kg for Longyan duck breeders from 22 to 37 wk of age were estimated to be 3.14 for optimizing egg production, 2.93 for egg mass, and 2.92 for FCR.

DISCUSSION

Consistent with the findings in broiler breeders (Jiang et al., 2018) and laying hens (Khattak and Helmbrecht, 2019; Wen et al., 2019), dietary Trp levels affected the productive performance of duck breeders in this study, including increased egg production, egg mass and feed intake, and decreased FCR, and there were linear and quadratic responses with increasing Trp levels. It is well known that Trp is the obligate precursor of serotonin and melatonin (Liu et al., 2015; Yue et al., 2017), which play important roles in maintenance of normal

Table 7. Estimation of the dietary tryptophan requirements based on quadratic regressions of egg production, egg mass, and FCR on dietary Trp levels.

Variables	Regression equation ¹	R ²	P value	Trp requirement (g/kg) ²
Egg production (%)	$Y = -0.8128X^2 + 5.3825X + 75.975$	0.707	0.044	3.14
Egg mass (g/d)	$Y = -0.6761X^2 + 4.1676X + 46.014$	0.721	0.023	2.93
FCR (g:g) ³	$Y = 0.03X^2 - 0.1842X + 3.318$	0.734	0.089	2.92

¹Y is the dependent variable and X are the dietary Trp levels (g/kg).

²Dietary Trp requirement = X giving 95% of the maximal response (g/kg).

³FCR, feed conversion ratio.

physiological processes (Wang et al., 2015). Serotonin has long been considered to be an important neurotransmitter that mediates appetite and regulates the feed intake of broilers and laying hens (Bai et al., 2017; Wen et al., 2019). Melatonin increases secretion of adrenocorticotrophic hormone and hence corticosteroids as key components of the hypothalamic-pituitary-adrenal axis, and it affects the development of the reproductive system in mammals (Soares et al., 2003). Moreover, the changes in reproductive organs and ovarian follicles with dietary Trp levels found here in laying duck breeders were similar to the responses in egg production. In this respect, the increased egg production of duck breeders is mainly due to the roles of Trp on reproductive organ and follicles development. In addition, Trp was reported to be involved in protein synthesis to support egg production (Wu, 2009), and bird performance was highly related to protein digestibility (Cowieson and Roos, 2014). To some degree, the improved performance in response to dietary Trp supplementation of duck breeders in the current study possibly result from enhanced protein digestibility, but it needs to be further examined.

In the present study, dietary Trp levels did not affect the reproductive performance of laying duck breeders, but it affected egg quality. There were linear and quadratic responses of albumen height and Haugh unit of duck eggs to increasing dietary Trp levels, and they then decreased with higher levels (5 or 6 g/kg). This implies that more Trp may affect the quality of albumen, possibly due to the influences on ovalbumin, the major egg white protein, and is highly related to the egg quality (Chang et al., 2018). Essential amino acids are vital components of ovalbumin (Sun et al., 2019) and excess dietary Trp might disrupt the balance among amino acids. In contrast to the current study, dietary Trp levels did not influence the egg quality in laying hens (Khattak and Helmbrecht, 2019). This difference may be due to their using lower dietary Trp levels (1.0 to 3.1 g/kg) compared to the current study (1.0 to 6.0 g/kg), and also there were no differences of albumen height and Haugh unit with lower Trp levels (1.0 to 4.0 g/kg). The present authors are unaware of any previous study showing that excess dietary Trp supplementation decreases egg albumen height and Haugh unit. This finding emphasizes the need for limiting Trp supplementation to not more than 4 g/kg in laying duck breeders.

Supplementing the diet of duck breeders here with Trp increased the weight of oviduct and ovary, and the number and weight of POF, which accounted for the improved egg production. Similarly, Jiang et al (2018) reported that dietary Trp addition (1.1 to 2.7 g/kg) increased egg production, and total and average weight of POF in broiler breeders. The weight of SYF, relative to ovarian weight decreased linearly and quadratically with dietary Trp levels but the total and average weight of SYF was not affected. On the one hand, these results may result from the increase in ovarian weight, or, on the other hand, these may be due to the progressive development of SYF into POF, and thus increasing their

number and weight. In addition, dietary Trp supplementation increased body weight in breeders in this study, which implies the positive energy balance occurred with the supplementation of Trp, and possibly due to its effect on the metabolism of proteins, carbohydrates and lipids (Rogers and Pseti, 1992). Similarly, the body weight of broilers was increased linearly and quadratically by dietary Trp levels (1.3 to 2.5 g/kg, Corzo et al., 2005).

Serum biochemical indices are often used to monitor health and the functions of body organs, and also to reflect the nutritional status of animals. The CRE and UA contents in serum are widely used for monitoring renal function (Hokamp and Nabity, 2016). The serum UA content was un-affected but the CRE content was linearly and quadratically increased with Trp supplementation here, though the levels remained within normal physiological ranges for birds (Hrabcakova et al., 2014). Laborde et al. (1995) suggested that the albumin fraction of total protein is a better indicator of the long-term protein status. Dietary Trp levels (1.0 to 3.1 g/kg) linearly and quadratically increased serum ALB content in laying hens (Khattak and Helmbrecht, 2019). Castrogiovanni et al. (2014) found that the Trp could stimulate the liver to produce IGF-I to promote growth and development, and regulate protein degradation. Serum ALB content and ALT activity are reliable indicators of hepatic function and are closely correlated with the degree of hepatic lipidosis in animals. The current study also observed that Trp deficiency increased serum ALT activity, inferring that liver function was impaired with Trp deficiency. Akiba et al. (1992) found that Trp supplementation of laying hens alleviated fatty liver in laying hens and modified microsomal mixed function oxidase in the liver, which suggests that L-tryptophan affects lipid metabolism. In this respect, the decreased TC in layer duck breeder with the supplementation of TRP (6 g/kg) is possibly due to the enhancement effect of TRP on liver function and affected lipid metabolism in duck breeders.

For variables of egg production, egg mass and FCR measured here, it can be concluded that a diet containing 2.9 to 3.1 g/kg Trp, equivalent to 461 to 493 mg/d per duck, was adequate for laying duck breeders during the laying period. This is higher than the recommended levels of 2.03 g/kg or 254 mg per hen in Chinese yellow-feathered broiler breeder hens (28 to 37 wk of age, Jiang et al., 2018). It is also higher than for meat-duck breeders during the laying period (2.0 to 2.2 g/kg, Hou et al., 2012). The increased egg production compared to the broiler breeders or enhanced body weight to meat-duck breeders may account for the higher requirement of Trp. The higher requirement of Trp for laying duck breeders indicates that Trp is vital and may be considered to be the third-ranking essential amino acids for laying duck breeders, after methionine and lysine. As no feeding standard exists for Trp in laying duck breeders, the present data now provides the needed information.

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DISCLOSURES

The authors declare no conflicts of interest.

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