Estimation of dietary arginine requirements for Longyan laying ducks

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ABSTRACT This study aimed to establish the arginine requirements of Longyan ducks from 17 to 31 wk of age based on egg production, egg quality, plasma, and ovarian indices, as well as the expression of vitellogenesis-related genes. In total, 660 Longyan ducks with similar body weight at 15 wk of age were assigned randomly to 5 treatments, each with 6 replicates of 22 birds, and fed a corn-corn gluten meal basal diet (0.66% arginine) supplemented with either 0, 0.20%, 0.40%, 0.60%, or 0.80% arginine. Dietary arginine did not affect egg production by laying ducks, but it increased (linear, $P < 0.01$) the egg weight at 22 to 31 and 17 to 31 wk of age. Dietary arginine increased the yolk color score (linearly, $P < 0.05$) and the yolk percentage (quadratic, $P < 0.05$), where the maximum values were obtained with 1.26% arginine. Dietary arginine affected the total shell percentage and shell thickness, with the highest values using 1.46% arginine ($P < 0.01$). The weight and number of small yellow follicles (SYFs) increased (quadratic, $P < 0.05$) with the dietary arginine level and there was a quadratic response ($P < 0.05$) in terms of the SYFs weight/ovarian weight; the highest values were obtained in ducks fed 1.26% arginine. The plasma arginine concentration exhibited a quadratic ($P < 0.05$) response to dietary arginine. The plasma progesterone concentration decreased (linear, $P < 0.05$) as dietary arginine increased. The mRNA abundance of the very low density lipoprotein receptor-b increased in the second large yellow follicle membranes (quadratic, $P < 0.05$) with the dietary arginine level, where the highest value occurred with 1.26% arginine. According to the regression model, the dietary arginine requirements for Longyan laying ducks aged 17 to 31 wk are 1.06%, 1.13%, 1.22%, and 1.11% to obtain the maximum yolk percentage, SYFs number, SYFs weight, and SYFs weight/ovarian weight, respectively.

Key words: arginine, laying duck, productivity performance, egg quality, vitellogenesis

2017 Poultry Science 96:144–150
http://dx.doi.org/10.3382/ps/pew205

INTRODUCTION

Avian species exhibit uricotelism and they have a functionally incomplete urea cycle, so they are unable to synthesize arginine de novo (Tamir and Ratner, 1963). Therefore, they are highly dependent on dietary arginine and they have an absolute arginine requirement. Much is known about the requirement for arginine and the graded effects of dietary arginine supplementation on performance, physiology, and immunity in fast-growing broilers and meat-type ducks (Fouad et al., 2013; Wang et al., 2013; Tan et al., 2014), but almost no information is available regarding laying ducks and they are not covered by the NRC (1994) guidelines. Previous studies (Snyder et al., 1956; Hogan et al., 1957) indicate that the arginine requirement for growing chickens fed casein as the chief protein source is between 1.7% and 2.0% in the diet, depending on the specific conditions. The arginine requirement for broilers aged 1 to 21 d when fed soybean meal and corn gluten meal as the chief protein source in the diet is between 1.19% and 1.28%, depending on age and predicted variables (Cuca and Jensen, 1990; Chamruspollert et al., 2002). Wang et al. (2013) suggested that the arginine requirement for male White Pekin ducks aged 1 to 21 d is 0.95% to 1.16% to achieve the maximum weight gain, feed/gain, and breast meat yield, which is similar to the NRC (1994) recommendations of 1.1% and 1.0% arginine for White Pekin ducks aged 0 to 2 and 2 to 7 wk, respectively. As part of a systematic program to optimize the performance of highly productive egg-laying ducks, the aim of the present study was to estimate the arginine requirements of Longyan laying ducks aged 17 to 31 wk.

Received January 8, 2016.
Accepted May 9, 2016.
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MATERIALS AND METHODS

Animals and Treatments

In this study, 660 Longyan shelducks with similar BW (1.42 ± 0.04 kg) at 15 wk of age were assigned randomly to 5 treatments, each with 6 replicates of 22 birds (each replicate was housed with indoor 3 × 4 m, outdoor 3 × 5 m, and pool 3 × 5 m areas), and they were studied over the following 16 wk. Birds were fed a corn-corn gluten meal basal diet (0.66% Arginine, Table 1), supplemented with either 0, 0.20%, 0.40%, 0.60%, or 0.80% arginine in the form of L-arginine hydrochloride (99.6% L-arginine hydrochloride, Huaheng Biological Technology Co. Ltd, Hebei, China). Appropriate amounts of L-alanine were supplemented to formulate isonitrogenous diets using zeolite as a "filler", as described by Kim and Wu (2004). Excluding arginine, the other dietary nutrient levels were set according to Ruan et al. (2015). The contents of arginine and other amino acids in the basal diet were analyzed by ion-exchange chromatography with an amino acid analyzer (L-8900, Hitachi, Tokyo, Japan) after acid hydrolysis, according to the method recommended by the Standardization Administration of China (2000).

Fresh drinking water was available ad libitum throughout the study. The daily feed allowance was the maximum without leaving refusals and it was split into 2 feeds, which were provided at 7:00 a.m. and 3:00 p.m. Each replicate group of ducks had daytime access to the outdoor and pool areas, but they were all housed indoors at night, with 4 h of light (incandescent lighting at 15 lx) from 6:30 p.m. to 10:30 p.m, i.e., a light:dark regime of 16:8 h. All of the procedures were approved by the Animal Care and Use Committee of Guangdong Academy of Agriculture Sciences.

After feeding for 16 wk, 2 birds were selected randomly from each replicate and blood was collected from the left wing vein using 5 mL vacutainers at 6:00 p.m., approximately 6 h before ovulation when the plasma concentration of luteinizing hormone (**LH**) rises to a significant high peak (Etches and Cheng, 1981). After 30 min, the plasma was separated by centrifugation (1,200 × *g* for 10 min) and stored in 0.5 mL Eppendorf tubes at −20°C. These birds were then killed, exsanguinated, and the ovaries and livers were collected. Samples from the liver were rinsed quickly with phosphate-buffered saline (**PBS**), snap frozen in liquid nitrogen, and stored at −80°C, as described by Ruan et al. (2015).

Productivity Performance

The birds were fed the 5 diets for a 14-d adaptation period before any measurements were made. The numbers of total, broken, and shell-less eggs were recorded daily on a per replicate basis. All of the eggs produced were weighed individually and graded daily (European Economic Community, 1989). Egg production, egg weight, egg mass (average egg weight per day per duck), and the feed conversion rate (feed intake/egg weight) were calculated daily, and were expressed as averages for a 2-wk early laying period (average daily egg production by all ducks ranged from 50% to 80%), a 10-wk peak-laying period (average daily egg production by all ducks >80%), and a 12-week period from early to peak laying.

Egg Quality

Egg quality was measured based on 3 eggs collected randomly from each replicate every 4 wk, and the average of the 18 eggs was calculated per treatment to determine the quality traits. The length and breadth of each egg were measured to calculate the shape index, i.e., (breadth/length) × 100. The breaking strength of uncracked eggs was determined on the vertical axis using an Egg Force Reader (model EFR-01, ORKA Food Technology, Ramat HaSharon, Israel). After weighing

Table 1. Composition and nutrient levels in the basal diet (%, as fed basis).

Ingredients	Percentage (%)	Nutrient composition[2]	Level
Corn	44.1	AME, MJ/kg	10.4
Wheat middling	13.7	CP, %	17.0
Distillers dried grains with solubles	16.1	Ca, %	3.60
Corn gluten meal	11.0	Total P, %	0.53
Calcium hydrogen phosphate	1.13	Available P, %	0.35
Limestone	9.22	Total Lys, %	0.80
Salt	0.30	Total Met, %	0.40
Premix[1]	1.00	Total Met+Cys, %	0.72
DL-methionine	0.04	Total Thr, %	0.60
L-lysine·HCl	0.46	Total Try, %	0.21
Tryptophan	0.08	Total Arg, %	0.66
L-alanine	1.64		
Zeolite powder	1.23		
Total	100		

[1]The premix provided the following per kilogram diet: vitamin A 12,000 IU, vitamin D_3 1,800 IU, vitamin E 26 IU, vitamin K 1.0 mg, vitamin B_1 3.0 mg, vitamin B_2 9.6 mg, vitamin B_6 6.0 mg, vitamin B_{12} 0.03 mg, choline 500 mg, D-calcium pantothenate 28.5 mg, folic acid 0.6 mg, biotin 0.15 mg, Fe 50 mg, Cu 10 mg, Mn 90 mg, Zn 90 mg, I 0.50 mg, and Se 0.40 mg. [2]Total Lys, Met, Met+Cys, Thr, Try, Arg, and CP were measured in the mixed feed. Other nutrient levels are calculated values.

each egg individually, it was broken onto a flat surface to measure the yolk color, albumen height, and Haugh units with an Egg Analyzer (model EA-01, ORKA Food Technology). Next, the yolk was separated and weighed. Shells with attached membranes were removed from their internal contents, washed under a gentle flow of water to remove any adhering albumen, dried in an oven to a constant weight at 65°C, and weighed. The thickness was measured based on 3 pieces of shell without membranes from the blunt, mid-length, and pointed ends using a digital micrometer, and the average was calculated. The weight of the albumen was calculated as the difference between the total egg weight and the weights of the shell and yolk for each egg.

Ovary-related Indices

The collected ovaries, small yellow follicles (**SYFs**; 3 mm < diameter < 8 mm) and large yellow follicles (**LYFs**; diameter ≥ 8 mm) were weighed using an electronic balance (AB-S/PH, Mettler Toledo, Switzerland). The numbers of SYFs and LYFs were recorded. The percentage weights of SYFs and LYFs were calculated relative to the ovarian weight. SYF and LYF membranes were separated, rinsed quickly with PBS, snap frozen in liquid nitrogen, and stored at –80°C.

Chemical Analysis of Plasma

Arginine in the plasma was analyzed by HPLC using precolumn derivatization with o-phthaldialdehyde, as described by Wu et al. (1997). The plasma concentrations of estradiol-17β (**E_2**), LH, follicle-stimulating hormone (**FSH**), and progesterone were determined by radioimmunoassays using kits (Beijing North Institute of Biological Technology, Beijing, China; Yang et al., 2005). Each sample was assayed in duplicate.

mRNA Expression Levels of Vitellogenesis-related Genes

Total RNA was extracted from the frozen liver, SYF, and LYF membranes using an extraction kit (Invitrogen, Carlsbad, CA). All of the RNA samples were treated with DNAase (Takara, Biotechnology Co. Ltd, Dalian, China), qualified by determination at $OD_{260:280}$, and evaluated after gel electrophoresis.

Complementary DNA (**cDNA**) was prepared by reverse transcription from 2.5 μg of high-quality RNA in a final volume of 25 μL according to the manufacturer's instructions (Promega, Madison, WI). Primers in the current study were designed based on GenBank sequences using Primer Premier 6.0 and prepared by Shanghai Shenggong Biological Company (Shanghai, China). Primer sequences for vitellogenin-II (**VTG-II**, GeneBank: XM_005022289.2), very low density apolipoprotein-II (**ApoVLDL-II**, GeneBank: GQ180104.1), very low density lipoprotein receptor-b (**VLDLR-b**, GeneBank: JF950612.1), and β-actin (GeneBank: EF667345.1) were shown as follows: 5′-CCCTAGTGCTCACCCTTGTA-3′ and 5′-CGGACCTTGAGGAGGTAAGT-3′, 5′-TGGTCAGTTCTTGGCGGATG-3′ and 5′-TCACTGCTCATTGGGTCTCC-3′, 5′-GCCATATTCCAGCCACAACT-3′ and 5′- CTATTGCCATTGCCCCCACTA-3′, 5′-GCTATGTCGCCCTGGATTT-3′ and 5′- GGATGCCACAGGACTCCATAC-3′. The quantity of cDNA was amplified by PCR under the optimal conditions, which comprised initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 30 s, annealing for 30 s at 59 or 60°C, and extension for 30 s at 72°C, with a final extension for 10 min at 72°C. Aliquots of the PCR products were evaluated by electrophoresis on 1.5% agarose gel and the products excised from the gels were sequenced to verify their authenticity.

Quantitative real time PCR was performed using an MXPro 3500 system (Stratagene, La Jolla, CA) with 1 μL of the cDNA product in a total volume of 20 μL, which contained 10 μL of SYBR-green PCR master Mix (Takara, Biotechnology Co. Ltd, Dalian, China) and 0.5 μL (10 mM) of each primer. The specificity of the reaction was monitored by determining the product melting curve. The following protocol was used: denaturation for 30 s at 95°C, followed by 35 cycles for 20 s at 95°C, 30 s at 59 or 60°C, and 20 s at 72°C. The transcripts were quantified using a standard curve based on 10-fold serial dilutions of cDNA. Each sample was assayed in triplicate and the standard deviations of the threshold cycle value did not exceed 0.5.

The relative mRNA expression level of each target gene was calculated by the ΔCt method [$R = 2^{-(\Delta\Delta\ Ct)}$, where R is the relative expression level of the target gene and ΔCt is the value obtained by subtracting the Ct value for β-actin mRNA from the Ct value for the target mRNA] as described previously (Ruan et al., 2015).

Statistical Analysis

Replicate (n = 6) was taken as the experimental unit, where two birds were sampled per replicate unless stated otherwise. Data were analyzed using the GLM procedure in SAS 9.1 (SAS Institute Inc., Gary NC, 2004). Polynomial contrasts were used to test for linear and quadratic effects in response to the dietary arginine level (Eisemann et al., 2014). A quadratic regression equation based on 95% of the maximum or minimum response was used to estimate the optimal arginine requirement whenever a significant quadratic response ($P < 0.05$) was observed (Corzo et al., 2006).

RESULTS

Productivity Performance

As shown in Table 2, the egg weights during the peak and whole laying period were the only productivity

Table 2. Effects of dietary arginine on the productivity performance of early and peak-laying ducks.[1]

	Dietary arginine (%)						*P*-value[3]		
Variable	0.66	0.86	1.06	1.26	1.46	SEM[2]	Arg	L	Q
Early laying period (50% < egg production < 80%, 20 to 21 wk of age)									
Egg production, %	70.5	68.5	69.4	71.7	73.0	2.28	0.66		
Egg weight, g	52.8	52.7	52.8	53.2	53.3	0.26	0.30		
Egg mass, g/d	37.3	36.3	37.0	38.3	37.3	1.54	0.92		
Feed conversion[4], g/g	4.52	4.65	4.58	4.43	4.59	0.20	0.95		
Peak-laying period (egg production>80%, 22 to 31 wk of age)									
Egg production, %	86.0	86.6	87.0	85.9	82.4	2.11	0.56		
Egg weight, g	60.4	61.0	60.7	60.6	61.5	0.17	<0.01	<0.01	0.51
Egg mass, g/d	51.7	53.0	53.9	52.7	51.6	0.77	0.22		
Feed conversion[4], g/g	3.10	3.04	2.98	3.05	3.11	0.05	0.32		
Whole laying period (20 to 31 wk of age)									
Egg production, %	82.4	82.7	85.5	83.3	82.2	1.72	0.67		
Egg weight, g	59.0	59.5	59.4	59.2	60.0	0.17	<0.01	<0.01	0.56
Egg mass, g/d	49.0	50.0	49.8	49.4	49.3	1.00	0.94		
Feed conversion, g/g	3.30	3.24	3.26	3.23	3.29	0.06	0.90		

[1]Data represent the means based on 6 replicates (22 birds/replicate).
[2]Derived from ANOVA error mean square.
[3]Arg = treatment effect; when significant, linear (L) and quadratic (Q) effects were tested.
[4]All birds were given 160 g/day during the early and peak-laying periods.

Table 3. Effects of dietary arginine on the egg composition and quality of ducks during the laying period (17 to 31 wk of age).[1]

	Dietary arginine (%)						*P*-value[3]		
Variable	0.66	0.86	1.06	1.26	1.46	SEM[2]	Arg	L	Q
Egg composition									
Yolk[4], %	28.4	28.6	29.0	29.2	28.4	0.19	<0.05	0.19	<0.01
Albumen, %	61.2	61.0	60.7	60.8	61.8	0.31	0.17		
Shell, %	10.1	10.3	10.2	10.0	10.3	0.05	<0.01	0.64	0.88
Egg quality									
Shape index, %	72.4	72.6	73.2	72.3	72.5	0.28	0.19		
Breaking strength, N	4.17	4.01	4.09	4.01	4.22	0.12	0.65		
Shell thickness, mm	0.323	0.329	0.325	0.321	0.334	0.002	<0.01	<0.05	0.09
Yolk color score	9.38	9.20	9.42	9.42	9.57	0.07	<0.05	<0.05	0.12
Haugh unit	79.6	79.9	80.5	78.4	77.5	0.78	0.08		

[1]Data represent the means based on 6 replicates (3 eggs/replicate).
[2]Derived from ANOVA error mean square.
[3]Arg = treatment effect; when it was significant, linear (L) and quadratic (Q) effects were tested.
[4]Regression equation based on dietary arginine level (%); quadratic equation: $Y = 24.3 + 8.59$ (arginine) – 3.86 (arginine)^2; $R^2 = 0.29$; *P*-value = 0.010, which yielded an optimized total dietary arginine value of 1.06%.

performance indices affected by the dietary arginine level (linear, $P < 0.01$), where the highest value was obtained with 1.46% arginine.

Egg Composition and Quality

The effects of dietary arginine on the egg composition and quality of ducks during the laying period are shown in Table 3. The yolk percentage was affected by the dietary arginine level and the quadratic response was significant ($P < 0.05$). The shell percentage was also affected by the dietary arginine level (the highest values were obtained with 0.86% and 1.46%, $P < 0.01$). The shell thickness and yolk color score increased (linear, $P < 0.05$) with the dietary arginine content, where the highest values for the shell thickness and yolk color score were obtained with 1.46% arginine.

Ovary-related Indices

As shown in Table 4, the SYFs number and SYFs weight of laying ducks at 31 wk of age exhibited quadratic ($P < 0.05$) responses, to the dietary arginine level. The SYFs weight/ovarian weight was also affected by the dietary arginine level and the quadratic response was significant ($P < 0.01$).

Chemical Analysis of Plasma

The plasma variables are summarized in Table 5. The plasma arginine concentration exhibited a quadratic ($P < 0.05$) response to the dietary arginine level. The plasma progesterone concentration decreased (linear, $P < 0.05$) as the dietary arginine level increased. The concentrations of the other plasma indices were unaffected by the dietary arginine level.

Table 4. Effects of dietary arginine on ovarian indices for laying ducks at the end of the study (31 wk of age).[1]

	Dietary arginine (%)						*P*-value[4]		
Variable[2]	0.66	0.86	1.06	1.26	1.46	SEM[3]	Arg	L	Q
LYFs number	5.50	5.00	5.67	5.50	5.83	0.23	0.17		
SYFs number[5]	11.3	15.3	20.0	20.8	17.3	1.97	<0.05	<0.05	<0.05
Ovarian weight, g/kg BW	41.8	44.7	43.8	41.6	41.0	2.82	0.86		
LYFs weight, g	38.9	40.5	39.3	39.5	47.4	2.40	0.11		
SYFs weight[5], g	1.36	1.73	2.01	2.52	2.08	0.17	<0.01	<0.01	<0.05
LYFs weight/ovarian weight	78.8	82.5	78.2	82.0	83.3	2.42	0.48		
SYFs weight/ovarian weight[5]	2.67	4.00	4.33	4.83	3.50	0.40	<0.05	0.06	<0.01

[1]Data represent the means based on 6 replicates (2 birds/replicate).
[2]LYFs = large yellow follicles, follicles with mean diameter > 8 mm; SYFs = small yellow follicles, follicles with mean diameter of 3 to 8 mm.
[3]Derived from ANOVA error mean square.
[4]Arg = treatment effect; when it was significant, linear (L) and quadratic (Q) effects were tested.
[5]Regression equation based on dietary arginine level (%); quadratic equation: Y(SYFs number) = –27.4 + 80.0 (arginine) – 33.6 (arginine)^2, R^2 = 0.36, P = 0.002; Y (SYFs weight) = –1.86 + 6.42 (arginine) – 2.50 (arginine)^2, R^2 = 0.46, $P < 0.001$; and Y (SYFs weight/ovarian weight) = –0.07 + 0.21 (arginine) – 0.09 (arginine)^2, R^2 = 0.40, $P < 0.001$, which yielded optimized total dietary arginine values of 1.13%, 1.22%, and 1.11%, respectively.

Table 5. Effects of dietary arginine on the plasma chemical indices of laying ducks at the end of the study (31 wk of age).[1]

	Dietary arginine (%)						*P*-value[4]		
Variable[2]	0.66	0.86	1.06	1.26	1.46	SEM[3]	Arg	L	Q
Arginine (μmol/L)	161	183	205	198	171	10.8	<0.05	0.31	<0.01
E_2 (pg/mL)	511	433	365	371	316	92.3	0.62		
LH (mIU/mL)	2.06	2.13	2.51	2.44	2.23	0.19	0.42		
FSH (mIU/mL)	1.06	1.00	1.28	1.07	0.92	0.11	0.28		
Progesterone (pg/mL)	187	196	161	172	164	6.82	<0.01	<0.01	0.66

[1]Data represent the means based on 6 replicates (2 birds/replicate).
[2]E_2 = Estradiol-17β, LH = luteinizing hormone, FSH = follicle-stimulating hormone.
[3]Derived from ANOVA error mean square.
[4]Arg = treatment effect; when it was significant, linear (L) and quadratic (Q) effects were tested.

Table 6. Effects of dietary arginine on the mRNA expression levels of vitellogenesis-related genes in the liver and follicle membranes at the end of the study (31 wk of age).[1]

	Dietary arginine (%)						*P*-value[4]		
Variable[2]	0.66	0.86	1.06	1.26	1.46	SEM[3]	Arg	L	Q
Liver									
VTG-II	1.13	1.10	1.64	1.63	1.31	0.19	0.14		
ApoVLDL-II	0.88	1.17	1.34	0.97	1.36	0.28	0.66		
LYF1 membrane									
VLDLR-b	0.96	0.98	1.04	1.05	0.94	0.13	0.97		
LYF2 membrane									
VLDLR-b	0.66	0.94	0.96	1.11	0.93	0.10	0.05	<0.05	<0.05
SYF membrane									
VLDLR-b	0.99	0.97	0.96	1.02	1.11	0.09	0.76		

[1]Data represent the means based on 6 replicates (2 birds/replicate).
[2]VTG-II = vitellogenin-II, ApoVLDL-II = very low density apolipoprotein-II, VLDLR-b = very low density lipoprotein receptor-b, LYF1 = the first large yellow follicle, LYF2 = the second large yellow follicle, SYF = small yellow follicle.
[3]Derived from ANOVA error mean squared.
[4]Arg = treatment effect; when it was significant, linear (L) and quadratic (Q) effects were tested.

mRNA Expression Levels of Vitellogenesis-related Genes

As shown in Table 6, the mRNA abundance of very low density VLDLR-b, which is expressed in the second large yellow follicle membranes, was the only vitellogenesis-related index affected by the dietary arginine levels (quadratic, $P < 0.05$), where the highest value was obtained with 1.26% arginine.

DISCUSSION

In many previous studies, a diet formulated with a large percentage of corn gluten meal due to its low arginine content was used successfully to determine the requirement for arginine (Labadan et al., 2001; Wang et al., 2013; Yuan et al., 2015). In the current study, we also used corn gluten meal as the major dietary protein source and we found that the weights of egg obtained

from ducks aged 22 to 31 and 17 to 31 wk increased linearly as the dietary arginine content increased from 0.66% to 1.46%, which is consistent with previous findings in hens (Yuan et al., 2015) and Japanese quails (Atakisi et al., 2009). This suggests that a higher dietary arginine content might yield superior productivity performance in ducks during the peak laying period. A metabolite of arginine (nitric oxide) is considered to regulate follicular development, maturation, and even egg production (Manwar et al., 2006), which probably explains the mechanism that allows dietary arginine to increase the egg weight. In the present study, there was no difference in egg production by laying ducks, whereas the dietary arginine level affected egg production by laying hens and broiler breeders in previous investigations (Silva et al., 2012; Duan et al., 2015; Lieboldt et al., 2015). The results of previous studies using laying hens and broiler breeders indicate that the effect of dietary arginine on egg production is variable, which may be related to the experimental diet, breed, and/or age of birds.

Furthermore, we examined the composition and quality of eggs obtained from laying ducks, which showed that the yolk percentage exhibited a quadratic response to dietary arginine, where the highest value occurred with 1.26% arginine. It is known that yolk precursor-very low density ApoVLDL and VTG are synthesized by the liver, secreted into bloodstream, transported to the ovary, and absorbed into follicles via the VLDLR (Speake et al., 1998). Thus, we determined the transcript abundances of ApoVLDL-II and VTG-II in the liver, as well as VLDLR-b in the follicle membranes. However, only the abundance of VLDLR-b transcripts expressed in the second large follicle membranes increased in a quadratic manner as the dietary arginine levels increased in the present study, thereby indicating that dietary arginine affects vitellogenesis in ducks mainly via the rapidly enlarging preovulatory follicle. In addition, the shell thickness exhibited a linearly increasing response to increases in the dietary arginine levels, where the highest shell percentage was obtained in ducks fed a diet containing 1.46% arginine, which indicates that a higher dietary arginine content is beneficial for the shell quality because arginine metabolites (proline and polyamine) are involved in collagen production (Flynn et al., 2002). Collagen is essential for the initial phase of eggshell calcification (Brionne et al., 2014). Liposoluble pigments in the yolk are reflected by the yolk color score (Sunde, 1962). In the present study, the yolk color score increased in a linear manner in response to increases in the dietary arginine levels. Atakisi et al. (2009) reported that the inclusion of L-arginine in the diet of laying quails reduced lipid peroxidation and enhanced the total antioxidant capacity. In addition, Duan et al. (2015) found that the addition of dietary arginine improved the total antioxidant capacity and reduced lipid peroxidation in the yolk. This may explain why increasing the concentration of L-arginine in the diet improved the yolk color score in the present study.

It has been demonstrated that nitric oxide plays a role in the physiology of the reproductive system, where it acts during control of the activity of reproductive organs including the regulation of follicular development, ovulatory mechanisms, and egg production (Manwar et al., 2006). Thus, we considered ovarian indices as reliable indicators to evaluate ovarian development and its functions, as described previously by Sun et al. (2015). In the present study, increases in the dietary arginine levels led to quadratic increases in the SYFs weight, SYFs number, and plasma arginine concentration in laying ducks, thereby indicating that the peak stage for laying ducks probably depends on the dietary arginine and/or nitric oxide concentration. Among the plasma hormones detected in the current study, we found that the concentration of progesterone, which is produced mostly by granular cells in small maturing follicles, was decreased when the dietary arginine level exceeded 1.06%. In terms of ovary development and hormone secretion, it is still unclear whether these effects are attributable to the generation of nitric oxide in the vasculature and neurons within the ovary, or if they are directly attributable to nitric oxide generated by various cells within the ovary. Further studies are needed to investigate the role of nitric oxide during reproductive system development.

In conclusion, the addition of dietary arginine increased the egg weight due to increases in the shell weight and yolk deposition, as well as improving SYFs development. According to the regression model, the dietary arginine requirements for Longyan laying ducks aged 17 to 31 wk are 1.06%, 1.13%, 1.22%, and 1.11% to obtain the maximum yolk percentage, SYFs number, SYFs weight, and SYFs weight/ovarian weight, respectively.

ACKNOWLEDGMENTS

This work was supported by the Earmarked Fund for China Agriculture Research System (Grant No. CARS-43-13), the Special Foundation of President Fund of the Guangdong Academy of Agricultural Sciences (Grant No. 201521), the Science and Technology Program of Guangdong Province (Grant No. 2011A020102009, 2016A020210043), Operating Funds for Guangdong Provincial Key Laboratory of Animal Breeding and Nutrition (Grant No. 2014B030301054), and the Natural Science Foundation of Guangdong Province (Grant No. 2014A030310054).

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