

Effects of dietary threonine supplementation on productivity and expression of genes related to protein deposition and amino acid transportation in breeder hens of yellow-feathered chicken and their offspring

Shouqun Jiang,^{*,1} HebatAllah Kasem El-Senousey,^{*,†} Qiuli Fan,^{*} Xiajing Lin,^{*} Zhongyong Gou,^{*} Long Li,^{*} Yibing Wang,^{*} Ahmed Mohamed Fouad,^{*,†} and Zongyong Jiang^{*,1}

^{*}*Institute of Animal Science, Guangdong Academy of Agricultural Sciences, State Key Laboratory of Livestock and Poultry Breeding, Key Laboratory of Animal Nutrition and Feed Science in South China, Ministry of Agriculture, Guangdong Key Laboratory of Animal Breeding and Nutrition, Guangdong Public Laboratory of Animal Breeding and Nutrition, 510640 Guangzhou, P. R. China; and* [†]*Department of Animal Production, Faculty of Agriculture, Cairo University, Giza 12613, Egypt*

ABSTRACT This study investigated the effects of the dietary threonine (Thr) levels on the performance, offspring traits, embryo amino acid transportation, and protein deposition in breeder hens of yellow-feathered chickens. In total, 720 breeder hens of Lingnan yellow-feathered chickens were randomly assigned to 1 of 6 dietary treatments, with 6 replicates per treatment (20 birds per replicate). The breeder hens were fed either basal diet (Thr = 0.38%) or basal diet supplemented with 0.12, 0.24, 0.36, 0.48, or 0.60% Thr from 197 to 266 D. There was a positive response in terms of the laying rate after adding different levels of Thr to the diet, but no significant effects on the average daily gain, average daily egg weight, feed conversion ratio, average broken eggs, and unqualified egg rate ($P > 0.05$). However, the eggshell strength and eggshell percentage decreased in a linear manner as the dietary Thr concentration increased ($P = 0.05$). Dietary supplementation with Thr had significant effects on the expression of mucin 2 (*MUC2*) in the uterus and zonula occludens

protein 1 (*ZO-1*) in the duodenum of breeders ($P < 0.05$). In chick embryos at embryonic age 18 D, significant upregulation of poultry target of rapamycin (*pTOR*) occurred in the liver and breast muscle, as well as threonine dehydrogenase (*TDH*) in the thigh, and aminopeptidase (*ANPEP*) ($P < 0.05$) in the duodenum and ileum due to dietary Thr supplementation, but there were no effects on *MUC2* expression in the duodenum and ileum ($P > 0.05$). The livability of the progeny broilers tended to increase with the dietary Thr concentration (quadratic, $P = 0.08$). Thus, dietary supplementation with Thr had positive effects on the laying production by breeder hens and offspring performance, and it also regulated the expression levels of genes related to amino acid transportation and protein deposition. The optimal dietary Thr concentration that maximized the laying rate in yellow-feathered chicken breeders aged 197 to 266 D was 0.68% according to quadratic regression analysis.

Key words: breeder hens of yellow-feathered chicken, offspring, poultry target of rapamycin, threonine, threonine dehydrogenase

2019 Poultry Science 98:6826–6836
<http://dx.doi.org/10.3382/ps/pez420>

INTRODUCTION

Studies have shown that the growth and development of breeders and their offspring can be improved by manipulating the maternal diet. Thus, Fan et al. (2018)

showed that maternal nutrition can affect embryonic development and the expression of genes related to embryonic and muscle development. Threonine (Thr) is the third limiting amino acid in poultry (Kidd and Kerr, 1996), and it has a vital role in improving growth, intestinal morphology, barrier function, the mRNA expression level of mucin 2 (*MUC2*), immune system functions, antioxidant ability (Chen et al., 2016; Bi et al., 2018), and the production of antibodies such as IgA and IgG (Azzam et al., 2011; Dong et al., 2017).

In meat-type birds, depression of the growth rate, feed intake, and carcass yield are consequences of Thr deficiency, whereas the effects in laying birds are a low laying rate, egg weight, egg mass, and feed conversion ratio (Azzam et al., 2011; Zhang et al., 2014; Fouad

© The Author 2019. Published by Oxford University Press on behalf of Poultry Science Association. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com.

Received December 13, 2018.

Accepted August 30, 2019.

¹Corresponding authors: jsqun3100@sohu.com (SJ); jiangz28@qq.com (ZJ)

et al., 2017). In addition, Thr is required to improve the secretion of digestive enzymes and to maintain normal populations of useful bacteria when the diet contains a low level of crude protein (Dong et al., 2017). Thus, Thr can maintain productivity when birds are fed diets with low crude protein contents.

Previous studies have highlighted the critical effect of Thr supplementation on improving egg production in laying hens and ducks (Azzam et al., 2014, 2017; Fouad et al., 2017). Furthermore, the in ovo injection of Thr improves the performance of chicks and enhances the morphological and functional development of the intestinal mucosa at hatching and at 21 D (Moreira Filho et al., 2019).

Yellow-feathered chickens are the most important poultry farming and meat resources, with more than 4 billion produced annually, which is comparable to the production of white-feathered broilers in China (Jiang et al., 2017). However, few studies have investigated the effects of dietary supplementation with nutrients on breeder hens of yellow-feathered chickens. Moreover, although previous studies showed that increasing the levels of limiting amino acids improved the productivity of breeder hens of yellow-feathered chickens (Jiang et al., 2017), none investigated the specific effects of Thr supplementation. We hypothesized that the optimal level of Thr could optimize the performance of breeders and their offspring by regulating amino acid transport. Thus, the present study investigated the effects of different dietary Thr levels on the performance, embryo amino acid transportation, and protein deposition in breeder hens of yellow-feathered chickens as well as the traits of their offspring.

MATERIALS AND METHODS

Birds, Diet, and Management

The experimental protocol was approved by the Animal Care and Use Committee of the Institute of Animal Science, Guangdong Academy of Agricultural Sciences, China.

In total, 720 Lingnan yellow-feathered 197-day-old broiler breeder hens were obtained from a local hatchery (Guangdong Wiz Agricultural Science & Technology Co. Ltd, Guangzhou, China). Hens with similar weights and laying rates were randomly assigned to 1 of 6 dietary treatments, with 6 replicates per treatment (20 birds per replicate). Before the experiment, all of the breeder hens were fed the same typical diet, which provided the complete nutrient requirements for yellow-feathered broiler breeders, as described in the Chinese Feeding Standard of Chicken (2004). During the experiment from 197 to 266 D, the basal diet (Table 1) provided was formulated to meet the standard nutritional requirements except for Thr (0.38%), and 5 supplemented diets were fed with Thr added at 0.12, 0.24, 0.36, 0.48, or 0.60% so the total dietary contents were 0.50, 0.62, 0.74, 0.86, and 0.98%, respectively.

Table 1. Composition and nutrient levels of the basal diet (as-fed basis, g/kg).

Item	Content
Ingredient	
Corn	413
Wheat	325
Peanut meal	141
L-Lysine HCl	5.3
DL-Methionine	4.1
L-Threonine	0.0
Tryptophan	0.8
Isoleucine	1.9
Limestone	68.5
Dicalcium phosphate	16.8
Zeolite (carrier)	10.6
Salt (NaCl)	3
Vitamin-mineral premix ¹	10
Total	1000
Nutrient composition	
AME (MJ/kg) ²	11.50
CP ³	168.10
Ca ³	28.33
Total phosphorus ³	6.14
Non-phytate phosphorus ²	4.10
Lysine ³	8.61
Methionine ³	5.41
Met+Cys ³	7.30
Threonine ³	3.80
Tryptophan ³	2.10
Isoleucine ³	5.90

¹To provide the following per kilogram of diet: vitamin A, 15,000 IU; vitamin D₃, 3,600 IU; vitamin E, 53 IU; vitamin K₃, 6 mg; thiamin, 3 mg; riboflavin, 9 mg; pyridoxine, 6 mg; cyanocobalamin, 0.03 mg; pantothenic acid, 18 mg; niacin, 60 mg; folic acid, 1.5 mg; biotin, 0.18 mg; choline, 600 mg; Fe, 72 mg; Cu, 7.2 mg; Mn, 90 mg; Zn, 72 mg; I, 0.9 mg; Se, 0.48 mg. The carrier was zeolite.

²Values were calculated based on the data provided by the Feed Database in China (2016).

³Measured values.

All of the birds were housed in laying cages with 2 hens per cage in the same house, and with 16 h of lighting daily from 0600 h to 2200 h. The room temperature and humidity were maintained at 29 ± 3°C and 65 ± 5%, respectively. All breeders received and consumed 125 g of diet per bird each day and they had ad libitum access to fresh water. The egg number, total egg weight, broken eggs, and unqualified eggs for each replicate were recorded daily. The unqualified eggs included excessively large or small eggs, sand shell eggs, misshapen eggs, dirty eggs, and eggs without a shell (but with an intact membrane). Breeder hens were artificially inseminated with 25 μL of pooled semen per bird every 3 D.

Four settable eggs per replicate with a total of 24 eggs per treatment were selected to determine the concentrations of Thr and total amino acids using an automatic amino acid analyzer (L-8900, Hitachi Ltd, Tokyo, Japan). Another 4 settable eggs per replicate with a total of 48 eggs per treatment were selected to determine the egg quality, including the egg weight, egg shape index, eggshell strength, egg yolk color, Haugh unit, egg yolk ratio, albumen ratio, eggshell ratio, and eggshell thickness.

From 253 D of age, 1,800 settable eggs (50 eggs per replicate with equal numbers from each day) were

collected for 7 consecutive days, labeled, and weighed individually, and stored at 15°C until their incubation. The settable eggs comprised all eggs except for small, double-yolked, and abnormal eggs. After disinfection with 30 mL of 10% formalin and 15 g of potassium permanganate per m³ for 30 min in a confined room, the eggs were incubated in a commercial tunnel incubator (XDZ90720, Xingyi Electronic Equipment Co. Ltd, Qingdao, China) with randomized locations. After incubation for 19 D, infertile eggs and those with dead embryos were removed. The liver, thigh and breast muscle, duodenum, and ileum mucosa were sampled promptly from 2 live embryos from each replicate of the treatments with Thr added at 0, 0.24, and 0.48%, where the samples were snap-frozen in liquid N₂ and stored at -80°C until their analysis. The remaining eggs were incubated for 3 more days in another commercial hatcher (XDM15120, Xingyi Electronic Equipment Co. Ltd, Qingdao, China). The fertilization rate, hatchability of fertilized eggs, and hatchling weight were determined at hatching.

At 259 D of age, 2 randomly selected breeders from each replicate were weighed and blood samples were obtained via the wing vein. Heparin was used as an anticoagulant and the samples were placed immediately on ice. Plasma was obtained by centrifugation at 860 × *g* for 20 min. The birds were then killed by cervical dislocation. Liver, duodenum, ileum, and uterus mucosa samples were collected promptly, snap-frozen in liquid N₂, and stored at -80°C until their analysis.

The ovaries and oviducts were removed and weighed, and the number and weights of the total large follicles (>8 mm) were determined (Jiang et al., 2017).

Biochemical Determinations of Plasma and Liver

The plasma concentrations of urea nitrogen, uric acid, total protein, and albumin, and the activities of glutamic oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) were determined using kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) with a spectrophotometer (Biomate 5, Thermo Electron Corporation, Rochester, NY).

Quantitative RT-PCR

Total RNA was isolated from the frozen liver, intestine, and uterus mucosa samples, before reverse transcription into complementary DNA using standard procedures. Subsequently, PCR amplification, recovery, cloning, and sequencing were performed to prepare standards from positive clone plasmids of the target fragments. In this study, we selected β -actin as the housekeeping gene for normalization purposes from 3 possible reference genes comprising glyceraldehyde-3-phosphate dehydrogenase, β -actin, and 18 ribosomal RNAs. The mRNA expression level of β -actin was used

as an internal control (Hu et al., 2008; Chen et al., 2015) because it did not differ significantly between the experimental groups. Primers and probes were designed for each transcript, and they were obtained from Shanghai SanGon Biological Technology and Services Company (Shanghai, PR China). The primers based on chicken sequences are listed in Table 2. Specific transcripts were quantified by quantitative PCR with an ABI 7500 Real-Time Detection System (Applied Biosystems, Carlsbad, CA) using a SYBR Premix Ex Taq II kit (Takara, Dalian, China). Amplification was performed in a total volume of 25 μ L containing 12.5 μ L of 2 × SYBR Premix, 0.5 μ L internal reference dye 6-carboxy-X-rhodamine, 2.5 μ L of 5 × diluted cDNA (5 ng/ μ L), 1.25 μ L of each primer (10 mmol/L), and 7 μ L double-distilled H₂O. The real-time PCR program started with denaturation at 95°C for 1 min, followed by 35 cycles at 95°C for 15 s and 60°C for 60 s. Dissociation analysis of the amplification products was performed after each PCR run to confirm that a single PCR product was amplified and detected. Data were analyzed with ABI 7500 SDS software (Applied Biosystems) where the baseline was set automatically by the software and average dCt values (normalized using β -actin) were used to calculate the relative expression levels based on the comparative Ct method calculated as $2^{-\Delta\Delta C_t}$. Results were expressed as relative abundances, i.e., $\log(2^{-\Delta\Delta C_t})$.

Statistical Analysis

Data were subjected to 1-way analysis of variance using SPSS statistical software (SPSS, 2008). Linear and quadratic effects were tested and considered significant at $P < 0.05$. Differences among treatments were examined using Duncan's multiple range tests and considered significant when $P < 0.05$. Data were expressed as means and their pooled standard errors. Quadratic regression ($Y = c + bx + ax^2$) was fitted to determine the linear and quadratic effects of Thr concentration on the laying rate.

RESULTS AND DISCUSSION

Performance of Breeders and Progeny Broilers

Chinese Yellow-feathered chickens are native and unique breeds, and they are raised throughout China and Southeast Asian countries. China is the third largest producer of chickens, and the annual production of Chinese Yellow-feathered chickens is more than 4 billion (Sarsenbek et al., 2013; Jiang et al., 2017, Gou et al., 2019). The 3 types of Chinese Yellow-feathered broilers are slow, medium, and fast growing. Slow-growing Chinese Yellow-feathered broilers have a slower growth rate compared with fast-growing broilers such as Arbor Acres, Ross, and Cobb, with a market age of 63 D and a body weight of about 2 kg

Table 2. Sequences of real-time PCR primers.

Gene name	Sequence	GenBank no.
<i>GOT</i>	F-5'-CGTATAGGTGCCGAGTTCCT-3' R-5'-CGTATAGGTGCCGAGTTCCT-3'	NM_205321.1
<i>GPT</i>	F-5'-GGGAAATGTCTTGCGCCTAG-3' R-5'-GCTCTGGACAAAACCTGAAGCC-3'	XM_015292423.1
<i>TDH</i>	F-5'-AAACCTGTGACCAACGATGC-3' R-5'-AACAGCCGCAACACTCAAAT-3'	XM_420039.5
<i>MUC2</i>	F-5'-CATTCAACGAGGAGAGCTGC-3' R-5'-TTCCCTGCAGCAGGAACAAC-3'	NM_001318434.1
<i>β-gal 9</i>	F-5'-ACCACGCAGTTAAGAGCCCT-3' R-5'-CACCAGCAAATGTCAGCAATG-3'	NM_001001611.2
<i>ZO-1</i>	F-5'-CCAAAGACAGCAGGAGGAGA-3' R-5'-TGGCTAGTTTCTCTCGTGCA-3'	XM_015278981.1
<i>pTOR</i>	F-5'-GGAATGAACCGTGATGACCG-3' R-5'-AGCATTTGACTGAGAGGGCT-3'	NC_006108.3
<i>PepT1</i>	F-5'-TAGACTGGGCAAGCGAGAAG-3' R-5'-AGCAGCAGCAACGAAAGC-3'	KF366603
<i>B⁰AT1</i>	F-5'-TCTGCCTGGGTTTGTTCATCT-3' R-5'-AGCCAGTAATTGCCAGACCT-3'	XM_419056.4
<i>ANPEP</i>	F-5'-TGGTTGAATGAGGGCTTTGC-3' R-5'-TGCTGGGGTGTTCATCTCAT-3'	NM_204861.1
<i>β-actin</i>	F-5'-GAGAAATTGTGCGTGACATCA-3' R-5'-CCTGAACCTCTCATTGCCA-3'	NM_205518

GOT: glutamic oxaloacetic transaminase; *GPT*: glutamic-pyruvic transaminase; *TDH*: threonine dehydrogenase; *MUC2*: mucin 2; *β-gal 9*: β-defensin-9; *ZO-1*: zonula occludens protein 1; *pTOR*: poultry target of rapamycin; *PepT1*: peptide transporter 1; *B⁰AT1*: neutral amino acid transporter; *ANPEP*: aminopeptidase.

Table 3. Effects of dietary threonine levels on production performance of breeder hens of yellow-feathered chickens.

Variable	Thr supplementation (bold) and (total dietary content, %)						SEM ¹	P-value ²		
	0.00 (0.38)	0.12 (0.50)	0.24 (0.62)	0.36 (0.74)	0.48 (0.86)	0.60 (0.98)		Thr	L	Q
Productive performance										
Initial BW (kg)	2976.3	2990.0	2975.0	3003.0	2966.3	2967.5	4.4	0.34		
Final body weight (kg)	3134.7	3162.5	3159.1	3123.8	3062.7	3145.5	1.6	0.53		
Average daily gain (g)	2.4	2.7	2.8	2.2	2.7	2.7	0.14	0.87		
Average daily egg production (g)	41.7	43.0	41.3	42.0	41.9	41.3	0.29	0.60		
Average daily feed intake (g)	125	125	125	125	125	125	0.0	1.00		
Feed conversion ratio (kg feed/kg egg)	3.12	3.03	3.16	3.10	3.11	3.15	0.02	0.59		
Laying performance										
Laying rate (%) ³	70.0 ^b	73.3 ^a	71.9 ^{a,b}	72.5 ^a	72.4 ^a	70.3 ^b	0.33	0.01		0.001
Average egg weight (g)	62.5	61.3	61.2	62.9	62.0	62.5	0.30	0.52		
Broken egg rate (%)	3.6	4.3	3.7	4.3	4.3	4.0	0.23	0.92		
Unqualified egg rate (%)	0.95	1.1	0.94	0.77	1.2	0.93	0.07	0.65		
Fertility and hatchability										
Fertilization rate (%)	95.7	96.7	95.6	95.6	95.7	95.9	0.44	0.98		
Hatchability (%)	90.0	89.7	88.0	81.7	82.0	75.7	1.82	0.14		
Hatchling weight (g)	40.5	40.9	40.2	40.6	40.6	41.1	0.16	0.65		

¹Means within each row with different superscripts are significantly different (P < 0.05). Standard error of the mean from ANOVA (n = 6).

²Thr = treatment effect; when significant, linear (L) and quadratic (Q) effects were tested.

³Regression equation based on dietary threonine concentration (%); quadratic equation: Y = 60.40 + 36.9X - 27.28X²; R² = 0.26; P = 0.01. This equation yielded an optimized total dietary threonine concentration of 0.68%.

(Zhu et al., 2012; Ruan et al., 2017). Chinese yellow-feathered chickens are popular because consumers prefer their flavor. Many studies have been conducted to maximize the production and quality of the chickens produced to cover the requirements of the Chinese yellow-feathered broiler chicken industry in China. However, few studies have investigated the nutritional requirements of broiler breeders. Thus, the present study investigated the effect of different dietary threo-

nine levels in Chinese yellow-feathered broiler breeders, which has not been investigated previously.

As shown in Table 3, Thr supplementation had no significant effects on the growth performance of breeder hens of yellow-feathered chickens (P > 0.05). Similarly, Chen et al. (2016) found that dietary supplementation with Thr in broilers did not affect growth performance (final body weight, daily feed intake, and feed conversion ratio) over 21 D. Moreover, Bi et al. (2018)

Table 4. Effects of dietary threonine levels on growth performance and biochemical variables in the plasma and liver of broiler progeny aged from 1 to 21 D.

Variable	Thr supplementation (bold) and (total dietary content, %)						SEM ¹	<i>P</i> -value ²		
	0.00 (0.38)	0.12 (0.50)	0.24 (0.62)	0.36 (0.74)	0.48 (0.86)	0.60 (0.98)		Thr	L	Q
Initial weight (g)	40.3 ^{a,b}	40.7 ^a	40.2 ^{a,b}	39.9 ^b	40.8 ^a	40.6 ^{a,b}	0.10	0.05		
Final weight (g)	382.6 ^b	391.2 ^{a,b}	410.7 ^a	379.1 ^b	393.9 ^{a,b}	400.4 ^{a,b}	3.22	0.04		
ADG (g)	16.3 ^b	16.7 ^{a,b}	17.7 ^a	16.2 ^b	16.8 ^{a,b}	17.1 ^{a,b}	0.15	0.04		
ADFI (g)	29.6	29.7	30.7	29.2	28.4	29.6	0.36	0.64		
FCR (kg feed/kg egg)	1.78	1.78	1.79	1.81	1.69	1.73	0.02	0.60		
Livability (%)	93.0	99.5	99.5	97.7	96.3	97.9	0.78	0.14		0.08
In plasma										
Uric acid (umol/L)	394.0	315.1	341.6	373.4	352.7	346.1	9.10	0.19		
In liver										
GOT (U/g prot)	20.4	20.4	21.9	22.0	23.7	20.3	0.47	0.23		
GPT (U/g prot)	6.03	4.63	5.06	4.85	4.88	4.75	0.17	0.20		

¹Means within each row with different superscripts are significantly different ($P < 0.05$). Standard error of the mean by ANOVA ($n = 6$).

²Thr = treatment effect; when significant, linear (L) and quadratic (Q) effects were tested.

GOT: glutamic oxaloacetic transaminase; GPT: glutamic-pyruvic transaminase.

showed that high levels of dietary Thr had no significant effect on the feed conversion ratio in Pekin ducks at 21 D. By contrast, in the present study, the progeny of the broilers (Table 4) was significantly affected by the dietary Thr concentration in terms of the final body weight and average daily gain. Bi et al. (2018) and Jiang et al. (2017) also found that dietary Thr had significant effects on the body weight gains by Pekin ducklings at 21 D. Furthermore, Moreira Filho et al. (2019) showed that the in ovo injection of Thr increased the final weight and weight gain as the Thr level increased in broiler chicks during periods of 1 to 7, 1 to 14, and 1 to 21 D. In the present study, the livability of the progeny broilers tended to increase with the dietary Thr concentration provided to the maternal yellow-feathered breeder hens (quadratic, $P = 0.08$, Table 4). Therefore, supplementing the maternal diet with Thr had beneficial effects on their progeny.

The present study showed that the laying rate increased due to the addition of Thr in the diet of breeder hens of yellow-feathered chickens, although there were no significant effects of Thr supplementation on the egg weight ($P > 0.05$) (Table 3). Similarly, Azzam et al. (2014, 2017) found that the addition of Thr to the diet of laying hens increased egg production but did not significantly affect the egg weight. In addition, Fouad et al. (2017) showed that a diet containing 0.57% Thr maximized egg production in laying ducks. Ashrafi et al. (2011) reported that egg production increased with the dietary threonine level up to 0.67% Thr in the diet, whereas the egg weight decreased as the dietary threonine level increased in broiler breeders (Cobb strain) at 60 wk of age. Samadi and Liebert (2006) found that the optimal Thr concentration was influenced by the genotype, age, dietary efficiency of Thr utilization, and the level of feed intake, which could explain the difference between our results and those obtained in previous studies.

In the current study, the optimal dietary Thr concentration that maximized the laying rate in yellow-feathered broiler breeder chickens was 0.68% according to quadratic regression analysis ($y = -27.28x^2 + 36.9 \times 60.40$; $R^2 = 0.26$; $P = 0.01$). The hatchability rate, fertilization rate of the total eggs, and hatchling weight were not affected by Thr supplementation ($P > 0.05$; Table 3). Similarly, Ashrafi et al. (2011) found that dietary Thr supplementation had no significant effects on the hatchability of broiler breeders from 60 to 63 wk.

Egg Quality

As shown in Table 5, Thr supplementation had no effects on the egg quality parameters (egg shell index, egg yolk color, Haugh unit, and egg yolk %; $P > 0.05$). However, the eggshell strength, eggshell thickness, and eggshell percentage decreased in a linear manner as the dietary Thr concentration increased ($P \leq 0.05$). These results agree with those obtained by Al Bustany and Elwinger (1987) and Azzam et al. (2014), who found that increasing the protein and amino acid levels reduced the eggshell percentage. The albumen percentage increased (linear, $P = 0.05$) with the dietary Thr concentration in the yellow-feathered chicken breeder hens. Cardoso et al. (2014) and Azzam et al. (2017) found that dietary Thr supplementation had no effects on the egg quality in laying hens, but Azzam et al. (2014) found that the addition of dietary Thr (from 56 to 64 wk of age) increased the Haugh units and albumen height in laying hens. Furthermore, Fouad et al. (2017) showed that dietary Thr decreased the yolk weight and yolk weight % in laying ducks, whereas the albumen weight and percentage increased, and the Haugh unit score, yolk color, albumen height, and egg shell quality were not affected. In brown laying hens, the albumen height, eggshell percentage, and eggshell thickness were not improved by the addition of

Table 5. Effects of dietary threonine levels on egg quality in breeder hens of yellow-feathered chickens.

Variable	Thr supplementation (bold) and (total dietary content, %)						SEM ¹	<i>P</i> -value ²		
	0.00 (0.38)	0.12 (0.50)	0.24 (0.62)	0.36 (0.74)	0.48 (0.86)	0.60 (0.98)		Thr	L	Q
Egg weight (g)	62.5	61.3	61.3	62.9	62.0	62.5	0.30	0.52		
Egg shape index	1.3	1.3	1.3	1.3	1.3	1.3	0.003	0.94		
Eggshell strength (N)	4.0	3.6	4.1	3.7	3.5	3.6	0.07	0.07	0.05	
Egg yolk color	6.6	7.0	6.7	7.0	6.8	6.8	0.08	0.82		
Haugh unit	74.7	77.8	73.4	74.6	73.7	75.5	0.66	0.46		
Egg yolk (%)	30.2	30.1	30.7	29.7	29.8	29.8	0.15	0.36		
Albumen (%)	60.8	61.1	60.3	61.5	61.6	61.6	0.17	0.14	0.05	
Eggshell (%)	9.1	8.8	9.0	8.8	8.7	8.6	0.06	0.16	0.02	
Eggshell thickness (mm)	0.33	0.32	0.32	0.32	0.32	0.31	0.002	0.25	0.02	
In egg										
Threonine (%)	4.4 ^{a,b}	4.4 ^{a-c}	4.3 ^{b,c}	4.3 ^{a-c}	4.3 ^c	4.4 ^a	0.01	0.02		0.009
Total amino acids (%)	94.7	94.6	95.0	95.6	93.4	96.1	0.34	0.32		

¹Means within each row with different superscripts are significantly different ($P < 0.05$). Standard error of the mean from ANOVA ($n = 6$).

²Thr = treatment effect; when significant, linear (L) and quadratic (Q) effects were tested.

Table 6. Effects of dietary threonine levels on reproductive organs and follicle development in breeder hens of yellow-feathered chickens.

Variable	Thr supplementation (bold) and (total dietary content, %)						SEM ¹	<i>P</i> -value ²		
	0.00 (0.38)	0.12 (0.50)	0.24 (0.62)	0.36 (0.74)	0.48 (0.86)	0.60 (0.98)		Thr	L	Q
Ovary weight (g)	62.3	55.4	55.6	55.9	59.4	63.3	1.6	0.54	0.60	0.07
Oviduct weight (g)	63.74	55.08	58.5	55.3	58.0	60.7	1.2	0.29		
Large follicle number	6.1	5.3	5.3	5.0	5.3	5.9	0.2	0.20		
Total large follicle weight (g)	52.3	43.1	44.1	43.0	46.8	51.1	1.6	0.38		

¹Means within each row with different superscripts are significantly different ($P < 0.05$). Standard error of the mean from ANOVA ($n = 6$).

²Thr = treatment effect; when significant, linear (L) and quadratic (Q) effects were tested.

Thr, but the Haugh unit score improved significantly (Abdel-Wareth and Esmail, 2014).

Reproductive Organs and Follicle Development

Threonine is the third limiting amino acid, but its effects on reproductive organs have not been investigated previously. Thus, we recorded the ovary weight, oviduct weight, number of large follicles, and their weight in order to evaluate the reproductive status (Xia et al., 2017; Ruan et al., 2018). We found no differences in the weights of the ovary and oviduct, large follicle number, and total large follicle weight ($P > 0.05$) (Table 6). Recently, Meng et al. (2017) and Ruan et al. (2018) showed that the first limiting amino acid (methionine) affects the growth of the reproductive organs in duck breeders during the laying production period and pre-laying period in laying hens. Similar results were obtained in laying ducks when arginine (one of the essential amino acids in poultry nutrition) was added to their diets (Xia et al., 2017). However, the addition of lysine to the diet of laying duck diets did not affect their reproductive organs (Fouad et al., 2018). Previous studies and the current results indicate that the effects of amino acids on the development of reproductive organs may differ according to age, the levels of amino acids in the basal diet, and the production phase.

Biochemical Variables Measured in the Plasma and Livers From Chinese Yellow-Feathered Broiler Breeder Hens and Their Chick Embryos

Threonine is involved in various metabolic processes such as protein synthesis and uric acid formation (Eftekhari et al., 2015). In poultry, the amino acid requirements can be estimated by determining the plasma uric acid concentration (Miles and Featherston, 1974; Min et al., 2017). The concentrations of urea nitrogen, uric acid, GOT, and GPT have been employed to determine the amino acid status (excess or deficiency) (Hikami et al., 1988; Gong et al., 2005; Wang et al., 2006; Azzam et al., 2011). Albumin and α -, β -, and γ -globulins represent the total protein content (Lumeij, 1997), and thus significant increases in the levels of albumin and globulins can reflect the total protein concentration (Hunt and Hunsaker, 1965). In the current study, we found that the dietary Thr concentration had no effects on the urea nitrogen, uric acid, total protein, and albumin contents of the plasma as well as the activities of GOT and GPT in the livers ($P > 0.05$) of breeder hens of yellow-feathered chickens (Table 7). Azzam et al. (2014) reported that Thr supplementation had no significant effects on the serum total protein, albumin, and uric acid levels in laying hens from 56 to 64 wk of age. However, in another

Table 7. Effects of dietary threonine levels on biochemical variables in the plasma and liver from breeder hens of yellow-feathered chickens.

Variable	Thr supplementation (bold) and (total dietary content, %)						SEM ¹	P-value ²		
	0.00 (0.38)	0.12 (0.50)	0.24 (0.62)	0.36 (0.74)	0.48 (0.86)	0.60 (0.98)		Thr	L	Q
In plasma										
Urea nitrogen (mmol/L)	1.18	0.87	0.77	0.97	0.78	0.96	0.05	0.24	0.28	0.09
Uric acid (umol/L)	296.3	284.9	299.4	280.9	283.1	312.0	5.16	0.50		
Total protein (mg/mL)	72.34	71.77	68.22	74.35	71.04	71.00	0.78	0.38		
Albumin (g/L)	27.77	27.02	27.75	27.49	26.18	29.15	0.41	0.46	0.62	0.26
In liver										
GOT (U/g prot)	62.42	61.31	61.36	66.77	65.56	63.71	0.85	0.31		
GPT (U/g prot)	4.51	4.54	4.83	4.73	4.93	5.29	0.12	0.50		

¹Means within each row with different superscripts are significantly different ($P < 0.05$). Standard error of the mean from ANOVA ($n = 6$).

²Thr = treatment effect; when significant, linear (L) and quadratic (Q) effects were tested.

GOT, glutamic oxaloacetic transaminase; GPT, glutamic-pyruvic transaminase.

study, Azzam et al. (2017) found that the serum level of uric acid decreased significantly as the dietary Thr increased in laying hens during the post-peak period of egg production. Thus, the effects may vary according to different breeds, production periods, and experimental conditions. In the present study, the activities of GOT and GPT in the liver indicated that dietary Thr supplementation did not reach the level that induces liver damage.

Gene Expression Levels in Chinese Yellow-Feathered Broiler Breeder Hens and Chick Embryos

In general, the target of rapamycin (TOR), ribosomal protein S6 kinase, and eukaryotic initiation factor 4E binding protein 1, which participate in protein translation and protein biogenesis, are highly sensitive to dietary amino acid supplementation (Cota et al., 2006; Lee and Aggrey, 2016). Thus, previous studies showed

that amino acids such as methionine and tryptophan are implicated in the regulation of TOR. However, it is unclear whether dietary Thr is implicated in the regulation of the TOR gene in chickens. To the best of our knowledge, the present study is the first to investigate the effects of dietary Thr on the expression of the pTOR gene in yellow-feathered chicken breeder hens. We found no significant effect ($P > 0.05$) of dietary Thr on the expression of pTOR in the livers of breeder hens of yellow-feathered chickens, as shown in Table 8. However, we found that dietary Thr significantly up-regulated the expression of the pTOR gene in the livers and breast muscle from chick embryos at an embryonic age of 19 D (Table 9), thereby indicating the beneficial effect of dietary Thr on protein metabolism in chick embryos from breeder hens of yellow-feathered chickens.

According to previous studies, GOT and GPT are indicator enzymes of damage to the liver tissues (Jung et al., 2010). Adriani et al. (2014) showed that the concentrations of GOT and GPT increased as the degree

Table 8. Effects of dietary threonine levels on gene expression levels in the liver, intestine, and uterus of breeder hens of yellow-feathered chickens.

Variable	Thr supplementation (bold) and (total dietary content, %)						SEM ¹	P-value ²		
	0.00 (0.38)	0.12 (0.50)	0.24 (0.62)	0.36 (0.74)	0.48 (0.86)	0.60 (0.98)		Thr	L	Q
In liver										
<i>pTOR</i>	0.43	0.47	0.32	0.41	0.26	0.31	0.04	0.69		
<i>GOT</i>	0.56	0.41	0.51	0.48	0.49	0.45	0.02	0.58		
<i>GPT</i>	0.78	0.65	0.89	0.82	0.92	0.81	0.05	0.81		
<i>TDH</i>	2.37	2.39	2.51	2.51	2.47	2.62	0.04	0.50		
In duodenum										
<i>MUC2</i>	0.73	0.66	0.59	0.69	0.96	0.69	0.04	0.20		
<i>ZO-1</i>	0.57 ^{a,b}	0.60 ^a	0.62 ^a	0.44 ^b	0.42 ^b	0.27 ^c	0.03	0.0001	0.0001	0.04
In uterus										
<i>MUC2</i>	0.73 ^a	0.63 ^{a,b}	0.39 ^c	0.53 ^{b,c}	0.35 ^c	0.44 ^{b,c}	0.03	0.001	0.0001	0.04
<i>β-gal9</i>	0.90	0.79	0.92	0.98	1.19	1.20	0.07	0.42	0.05	

¹Means within each row with different superscripts are significantly different ($P < 0.05$). Standard error of the mean from ANOVA ($n = 6$).

²Thr = treatment effect; when significant, linear (L) and quadratic (Q) effects were tested.

pTOR, poultry target of rapamycin; GOT, glutamic oxaloacetic transaminase; GPT, glutamic-pyruvic transaminase; TDH, threonine dehydrogenase; MUC2, mucin 2; ZO-1, zonula occludens protein 1; β-gal 9, β-defensin-9.

Table 9. Effects of dietary threonine levels on gene expression levels in chick embryos from breeder hens of yellow-feathered chickens.

Variable	Thr supplementation (bold) and (total dietary content, %)			SEM ¹	P-value ²		
	0.00 (0.38)	0.24 (0.62)	0.48 (0.86)		Thr	L	Q
In liver							
<i>pTOR</i>	1.46 ^b	1.82 ^a	1.70 ^a	0.05	0.001	0.003	0.001
<i>GOT</i>	0.69	0.94	0.75	0.05	0.08		
<i>GPT</i>	0.30	0.36	0.39	0.03	0.63		
<i>TDH</i>	1.74	1.70	1.63	0.04	0.85		
In thigh muscle							
<i>pTOR</i>	1.25	1.21	1.30	0.02	0.21		
<i>TDH</i>	0.59 ^b	0.68 ^a	0.70 ^a	0.02	0.03	0.02	
In breast muscle							
<i>pTOR</i>	0.18 ^b	0.20 ^b	0.30 ^a	0.02	0.04	0.02	
<i>TDH</i>	0.60	0.65	0.61	0.01	0.44		
In ileum							
<i>ANPEP</i>	0.63 ^b	0.69 ^{a,b}	0.75 ^a	0.02	0.05	0.02	
<i>B⁰AT1</i>	0.18	0.14	0.21	0.02	0.27		
<i>MUC2</i>	0.67	0.67	0.65	0.03	0.94		
<i>PepT1</i>	0.59	0.57	0.59	0.03	0.96		
In duodenum							
<i>ANPEP</i>	0.90 ^b	0.95 ^b	1.10 ^a	0.03	0.002	0.001	
<i>B⁰AT1</i>	0.76	0.73	0.68	0.02	0.35		
<i>MUC2</i>	0.93	0.94	0.96	0.02	0.82		
<i>PepT1</i>	0.47	0.48	0.42	0.03	0.71		

¹Means within each row with different superscripts are significantly different (P < 0.05). Standard error of the mean from ANOVA (n = 6).

²Thr = treatment effect; when significant, linear (L) and quadratic (Q) effects were tested.

pTOR, poultry target of rapamycin; GOT, glutamic oxaloacetic transaminase; GPT, glutamic-pyruvic transaminase; TDH, threonine dehydrogenase; ANPEP, aminopeptidase; B⁰AT1, neutral amino acid transporter; MUC2, mucin 2; PepT1, peptide transporter 1.

of liver damage increased. In the present study, dietary supplementation with Thr had no positive effects on the mRNA expression levels of GOT and GPT in the livers of broiler breeders or their offspring at the embryonic stage (19 D) (Tables 8 and 9).

Threonine dehydrogenase (**TDH**) is considered the major enzyme responsible for Thr catabolism. The TDH activity increases significantly under conditions with dietary Thr imbalance in chickens (Davis and Austic, 1997). Lee et al. (2014) found that the TDH levels in chickens can be measured to evaluate the utilization of Thr. The present study is the first to investigate the effects of dietary Thr levels on the expression of the TDH gene in broiler breeders. We found that dietary supplementation with Thr had no significance effects on TDH gene expression in the livers of breeder hens of yellow-feathered chickens (Table 8) or the liver and breast muscles of chick embryos at 19 D (Table 9). However, the addition of Thr significantly increased the TDH gene expression level in the thigh muscles of chick embryos (P < 0.05). Therefore, supplementation of the maternal diet with Thr may be beneficial for embryonic chicks.

Zonula occludens (**ZO**) is one of the tight junction proteins that regulate intestinal permeability (Gilani et al., 2018). Chen et al. (2016) showed that Thr supplementation had no significant effect on the mRNA expression level of ZO-1 in the intestines of broilers at 21 D of age. By contrast, Chen et al. (2018) found

that dietary supplementation with Thr normalized the mRNA level of ZO-1 when decreased by lipopolysaccharide in the ileum of broilers. In the present study, the mRNA expression level of ZO-1 decreased with the high concentration of Thr (0.98% Thr) in the duodenum of broiler breeders. As dynamic structures, tight junctions are not static and the regulation of the tight junctions is influenced by various stimuli such as physiological stimuli, which contribute to nutrient transport, absorption, and intracellular homeostasis (Ulluwishewa et al., 2011; Guo et al., 2017). Further research is needed to investigate the effects of dietary Thr on the mRNA expression levels of tight junction proteins.

Threonine is an essential amino acid in poultry (Kidd and Kerr, 1996), and it is essential for the synthesis of mucin and improving the intestinal structure (Horn et al., 2009; Zhang et al., 2016; Jiang et al., 2017). Threonine represents approximately 11% of the mucin structure (*MUC2*) in humans (Gum, 1992). The chicken *MUC2* ortholog was identified as the protein most similar to the human *MUC2* (Lang et al., 2006; Jiang et al., 2013). Previous studies related to *MUC2* across species (including chickens) have shown that *MUC2* is an intestine-specific gene (Woodfint et al., 2017; Quintana-Hayashi et al., 2018). The protective role of *MUC2* in innate host defense has been demonstrated in chickens (Horn et al., 2009; Jiang et al., 2013; Zhang et al., 2014). Ariyadi et al. (2012) reported that the mRNA expression level of mucin is important for the

formation of the mucosal barrier in the oviduct of laying hens, which is responsible for defensive functions that are essential for maintaining laying hens with good health during the laying phase. In the present study, supplementation with Thr significantly decreased the expression of *MUC2* in the uterus of Chinese yellow-feathered broiler breeder hens ($P < 0.05$). In addition, the mRNA expression level of *MUC2* had no significant effects on the duodenum of breeder hens of yellow-feathered chickens (Table 8) and their offspring (Table 9). Moreira Filho et al. (2019) found that in ovo feeding with Thr increased the mRNA expression level of *MUC2* in the ileum of broiler chicks on the day of hatching, but there was no effect of Thr on the expression of *MUC2* in chicks at 21 D of age. In contrast to the results obtained in the present study, Azzam et al. (2017) found that dietary Thr supplementation increased the *MUC2* mRNA expression in the ileum and the highest expression level of *MUC2* was obtained with 0.66% Thr in laying hens. Furthermore, Bi et al. (2018) showed that the *MUC2* gene expression level in Pekin ducks at 22 D of age increased with the Thr supplementation level in the ileum. The differences between our results and those obtained previously may be attributable to the experimental conditions, bird type, or bird age, where Azzam et al. (2017) employed semi-controlled environmental conditions and Bi et al. (2018) used ducks aged 22 D. No information is available about the effects of Thr on the gene expression levels of *MUC2* in broiler breeder hens. Thus, further investigations are needed to determine the effects of Thr on *MUC2* mRNA gene expression in different parts of the oviducts of breeders.

It is known that chicken β -defensins have important antimicrobial activities against bacteria and fungi (Cuperus et al., 2013). In addition, the β -defensins in chickens have vital roles in the host innate immune system in the oviduct, as well as in the defense of the eggshell membrane and eggshell (Abdel Mageed et al., 2009). In the present study, we showed that the addition of Thr had no significant effect on the expression of the β -defensin-9 gene in breeder hens of yellow-feathered chickens. In fact, many physiological factors influence the expression of β -defensin genes such as the bird age, bird breed, and type of tissue tested (Sadeyen et al., 2006; Yoshimura et al., 2006).

The mRNA expression levels of aminopeptidase (*ANPEP*), amino acid transporters (such as B^0AT^1), and peptide transporters (such as *PepT1* and *PepT2*) control the absorption of amino acids in the small intestine of chickens (Mott et al., 2008; Zeng et al., 2011). In chickens, the highest distribution of *PepT1* is found in the small intestine (Chen et al., 1999, 2002). The duodenum has the highest expression levels of *PepT1*, followed by the jejunum and ileum (Chen et al., 2002). Zwarycz and Wong (2013) demonstrated the important role of *PepT1* in the absorption of dietary amino acids. Thus, the high expression level of *PepT1* in the intestine indicates that *PepT1* plays an important role in amino acid uptake. In the present study, dietary Thr had no

effects on the expression levels of the *PepT1* gene in the duodenum or ileum of Chinese yellow-feathered embryonic chicks ($P > 0.05$). However, Moreira Filho et al. (2019) found that in ovo feeding with Thr increased the mRNA expression level of *PepT1* in the ileum of broiler chicks on the day of hatching, although Thr had no effect on the expression of *PepT1* in chicks aged 21 D. The results obtained in the present study could be explained by the Thr percentage in the eggs and total amino acid percentage in the eggs not changing after the addition of Thr to the diet of breeder hens of yellow-feathered chickens.

The B^0AT^1 transporter is an Na^+ -independent transporter of neutral amino acids in the intestine and kidney, where it is responsible for the uptake of neutral and cationic amino acids, such as Gly, Ser, Thr, Cys, Tyr, Asn, Gln, His, Lys, and Arg (Bröer et al., 2004; Bröer, 2008). Dietary Thr supplementation had no significant effects on the mRNA expression levels of B^0AT^1 in the ileum and duodenum of Chinese yellow-feathered broiler breeder chick embryos ($P > 0.05$), whereas the mRNA expression levels of *ANPEP* increased significantly in the ileum and duodenum of the chick embryos ($P < 0.05$). *ANPEP* is a digestive enzyme that cleaves amino acid from the N-terminus of peptides, and it is found at the brush border membrane of enterocytes (Sanderink et al., 1988). In the current study, the increased expression of *ANPEP* may be attributed to the final digestion and absorption of dietary nutrients by digestive enzymes and the transporter proteins expressed in the apical membrane of enterocytes in the small intestine (Gilbert et al., 2007). Moreira Filho et al. (2019) found that in ovo supplementation with 3.5% Thr increased the mRNA expression levels of *ANPEP* in the ileum of broiler chicks on the day of hatching and 21 D of age. By contrast, Jiang et al. (2017) showed that dietary tryptophan upregulated the gene expression level of B^0AT^1 but it had no effect on the mRNA expression levels of *ANPEP* in Chinese broiler breeders. These differences may be explained by the different types of amino acids, ages, and experimental conditions.

In conclusion, we found that dietary supplementation with Thr had beneficial effects on breeder hens of yellow-feathered chickens and their offspring by regulating the expression levels of genes related to amino acid transportation and protein deposition. The optimal dietary Thr concentration that maximized the laying rate for yellow-feathered chicken breeders aged from 197 to 266 D was 0.68% according to quadratic regression analysis. The findings obtained in the present study may facilitate practical changes to the diet of breeder hens of yellow-feathered chickens.

ACKNOWLEDGMENTS

This study was supported by the National Key R&D Project (2018 YFD0500600), National Natural Science Foundation of China (31802104), “Twelve-Five” National Science and Technology Support Program

(2014BAD13B02), and Earmarked Fund for Modern Agro-industry Technology Research System (CARS-41-G10) from the Ministry of Agriculture, Scientific and Technological Project (2017B020202003) from the Department of Science and Technology of Guangdong Province, and Grant No. 201804020091 from the Guangzhou Science Technology and Innovation Commission, the Outstanding Talents Training Program of Guangdong Academy of Agricultural Sciences, Presidential Foundation of the Guangdong Academy of Agricultural Sciences (201620, 201805, 201807B, 201809B, and 201908), P. R. China.

REFERENCES

- Abdel-Wareth, A. A. A., and Z. S. H. Esmail. 2014. Some productive, egg quality and serum metabolic profile responses due to l-threonine supplementation to laying hen diets. *Asian J. Poult. Sci.* 8:75–81.
- Abdel Mageed, A. M., N. Isobe, and Y. Yoshimura. 2009. Immunolocalization of avian beta-defensins in the hen oviduct and their changes in the uterus during eggshell formation. *Reproduction* 138:971–978.
- Adriani, L., A. Rochana, A. Yulianti, A. Mushawwir, and N. Indrayani. 2014. Profil serum glutamate oxaloacetat transaminase (SGOT) and glutamate pyruvate transaminase (SGPT) level of broiler that was given noni juice (*Morinda citrifolia*) and palm sugar (*Arenga pita*). *Luc. Stii. Ser. Zoo.* 62:101–105.
- Al Bustany, Z., and K. Elwinger. 1987. Shell and interior quality and chemical composition of eggs from hens of different strains and ages fed different dietary lysine levels. *Acta Agric. Scand.* 37:175–187.
- Ariyadi, B., N. Isobe, and Y. Yoshimura. 2012. Differences in the mucosal surface barrier formed by mucin in the lower oviductal segments between laying and molting hens. *Poult. Sci.* 91:1173–1178.
- Ashrafi, S., H. A. Shahryar, A. Gorbani, P. P. Moghaddam, and M. Salmazadeh. 2011. The impact of supplemental L-threonine in broiler breeder molting hen diets on egg production, egg weight, and hatching traits. *Euro. J. Exp. Bio.* 1:169–172.
- Azzam, M. M. M., X. Y. Dong, P. Xie, C. Wang, and X. T. Zou. 2011. The effect of supplemental L-threonine on laying performance, serum free amino acids, and immune function of laying hens under high-temperature and high-humidity environmental climates. *J. Appl. Poult. Res.* 20:361–370.
- Azzam, M. M. M., X. Y. Dong, and X. T. Zou. 2017. Effect of dietary threonine on laying performance and intestinal immunity of laying hens fed low-crude-protein diets during the peak production period. *J. Anim. Physiol. Anim. Nutr.* 101:55–66.
- Azzam, M. M. M., C. Yuan, G. H. Liu, and X. T. Zou. 2014. Effect of excess dietary threonine on laying performance, egg quality, serum free amino acids, and digestive enzymes activities of laying hens during the postpeak period. *J. Appl. Poult. Res.* 23:605–613.
- Bi, Y., X. M. Nan, S. S. Zheng, L. S. Jiang, and B. H. Xiong. 2018. Effects of dietary threonine and immune stress on growth performance, carcass trait, serum immune parameters, and intestinal muc2 and NF- κ b gene expression in Pekin ducks from hatch to 21 days. *Poult. Sci.* 97:177–187.
- Broer, S. 2008. Amino acid transport across mammalian intestinal and renal epithelia. *Physiol. Rev.* 88:249–286.
- Bröer, A., K. Klingel, S. Kowalczyk, J. E. Rasko, J. Cavanaugh, and S. Bröer. 2004. Molecular cloning of mouse amino acid transport system B⁰, a neutral amino acid transporter related to Hartnup disorder. *J. Biol. Chem.* 279:24467–24476.
- Cardoso, A. S., F. G. P. Costa, M. R. Lima, E. T. Nogueira, C. S. Santos, R. B. Sousa, and D. V. G. Vieira. 2014. Nutritional requirement of digestible threonine for white egg layers of 60 to 76 weeks of age. *J. Appl. Poult. Res.* 23:724–728.
- Chen, F., Z. Jiang, S. Jiang, L. Li, X. Lin, and Q. Gou. 2015. Dietary vitamin A supplementation improved reproductive performance by regulating ovarian expression of hormone receptors, caspase-3 and Fas in broiler breeders. *Poult. Sci.* 95:30–40.
- Chen, Y. P., Y. F. Cheng, X. H. Li, W. L. Yang, C. Wen, S. Zhuang, and Y. M. Zhou. 2016. Effects of threonine supplementation on the growth performance, immunity, oxidative status, intestinal integrity, and barrier function of broilers at the early age. *Poult. Sci.* 96:405–413.
- Chen, H., Y. Pan, E. A. Wong, J. R. Bloomquist, and K. E. Webb. 2002. Molecular cloning and functional expression of a chicken intestinal peptide transporter (cPepT1) in *Xenopus* oocytes and Chinese hamster ovary cells. *J. Nutr.* 132:387–393.
- Chen, H., E. A. Wong, and K. E. Webb. 1999. Tissue distribution of a peptide transporter mRNA in sheep, dairy cows, pigs, and chickens. *J. Anim. Sci.* 77:1277–1283.
- Chen, Y., H. Zhang, Y. Cheng, Y. Li, C. Wen, and Y. Zhou. 2018. Dietary l-threonine supplementation attenuates lipopolysaccharide-induced inflammatory responses and intestinal barrier damage of broiler chickens at an early age. *Br. J. Nutr.* 119:1254–1262.
- Cota, D., K. Proulx, K. A. Blake Smith, S. C. Kozma, G. Thomas, S. C. Woods, and R. J. Seeley. 2006. Hypothalamic mTOR signaling regulates food intake. *Science.* 312:927–930.
- Cuperus, T. M., Coorens, A. van Dijk, and H. P. Haagsman. 2013. Avian host defense peptides. *Dev. Comp. Immunol.* 41:352–369.
- Davis, A. J., and R. E. Austic. 1997. Dietary protein and amino acid levels alter threonine dehydrogenase activity in hepatic mitochondria of *Gallus domesticus*. *J. Nutr.* 127:738–744.
- Dong, X. Y., M. M. M. Azzam, and X. T. Zou. 2017. Effects of dietary threonine supplementation on intestinal barrier function and gut microbiota of laying hens. *Poult. Sci.* 96:3654–3663.
- Eftekhari, A., V. Rezaei-pour, and R. Abdollahpour. 2015. Effects of acidified drinking water on performance, carcass, immune response, jejunum morphology, and microbiota activity of broiler chickens fed diets containing graded levels of threonine. *Livest. Sci.* 180:158–163.
- Fan, H., LV. Zengpeng, L. Gan, and Y. Guo. 2018. Transcriptomics-related mechanisms of supplementing laying broiler breeder hens with dietary daidzein to improve the immune function and growth performance of offspring. *J. Agric. Food Chem.* 66:2049–2060.
- Feed Database in China. 2016. Tables of feed composition and nutritive values in China- fifteenth edition. *Chinese Feed.* 21:33–43. (in Chinese)
- Fouad, A. M., W. Chen, D. Ruan, S. Wang, W. Xia, and C. T. Zheng. 2018. Effects of dietary lysine supplementation on performance, egg quality, and development of reproductive system in egg-laying ducks. *J. Appl. Anim. Res.* 46:386–391.
- Fouad, A. M., H. X. Zhang, W. Chen, W. G. Xia, D. Ruan, S. Wang, and C. T. Zheng. 2017. Estimation of L-threonine requirements for Longyan laying ducks. *Asian-Austral J. Anim. Sci.* 30:206–210.
- Gilani, S., G. S. Howarth, G. Natrass, S. M. Kitessa, R. Barekain, R. E. A. Forder, C. D. Tran, and R. J. Hughes. 2018. Gene expression and morphological changes in the intestinal mucosa associated with increased permeability induced by short-term fasting in chickens. *J. Anim. Physiol. Anim. Nutr.* 102:653–661.
- Gilbert, E. R., H. Li, D. A. Emmerson, K. E. Webb, Jr., and E. A. Wong. 2007. Developmental regulation of nutrient transporter and enzyme mRNA abundance in the small intestine of broilers. *Poult. Sci.* 86:1739–1753.
- Gong, L. M., C. H. Lai, S. Y. Qiao, D. Li, Y. X. Ma, and Y. L. Liu. 2005. Growth performance, carcass characteristics, nutrient digestibility and serum biochemical parameters of broilers fed low protein diets supplemented with various ratios of threonine to lysine. *Asian-Austral J. Anim. Sci.* 18:1164.
- Gou, Z., Q. Fan, L. Li, Z. Jiang, X. Lin, X. Cui, Y. Wang, C. Zheng, and S. Jiang. 2019. Effects of dietary iron on reproductive performance of Chinese Yellow broiler breeder hens during the egg-laying period. *Poult. Sci.* pez006, <https://doi.org/10.3382/ps/pez006>.
- Guo, Y., D. Liu, and B. Zhang. 2017. Modification of the chicken intestinal epithelial physical barrier in poultry by dietary factors. *Proc. Aust. Poult. Sci. Symp.* 28:12–18.

- Gum, J. R. 1992. Mucin genes and the proteins they encode: structure, diversity, and regulation. *Am. J. Respir. Cell Mol. Biol.* 7:557–564.
- Hikami, Y., T. Yasunori, T. Tanzan, and S. Hasegawa. 1988. Feed intake and plasma amino acid level in chicks selected for high and average body weight on a methionine-deficient diet. *Jpn. Poult. Sci.* 25:288–295.
- Horn, N. L., S. S. Donkin, T. J. Applegate, and O. Adeola. 2009. Intestinal mucin dynamics: response of broiler chicks and White Pekin ducklings to dietary threonine. *Poult. Sci.* 88:1906–1914.
- Hu, Y., Y. Ni, L. Ren, J. Dai, and R. Zhao. 2008. Leptin is involved in the effects of cysteamine on egg laying of hens, characteristics of eggs, and posthatch growth of broiler offspring. *Poult. Sci.* 87:1810–1817.
- Hunt, J. R., and W. G. Hunsaker. 1965. Physiology of the growing and adult goose. 3. Some nitrogen constituents of blood. *Br. Poult. Sci.* 6:15–21.
- Jiang, Z., T. J. Applegate, and A. C. Lossie. 2013. Cloning, annotation and developmental expression of the chicken intestinal MUC2 gene. *PLoS ONE* 8:e53781.
- Jiang, S. Q., Z. Y. Gou, X. J. Lin, and L. Li. 2017. Effects of dietary tryptophan levels on performance and biochemical variables of plasma and intestinal mucosa in yellow-feathered broiler breeders. *J. Anim. Physiol. Anim. Nutr.* 102:387–394.
- Jiang, Y., J. Tang, M. Xie, Z. G. Wen, S. Y. Qiao, and S. S. Hou. 2017. Threonine supplementation reduces dietary protein and improves lipid metabolism in Pekin ducks. *Br. Poult. Sci.* 58:687–693.
- Jung, S., J. H. Choe, B. Kim, H. Yun, Z. A. Kruk, and C. Jo. 2010. Effect of dietary mixture of gallic acid and linoleic acid on antioxidative potential and quality of breast meat from broilers. *Meat Sci.* 86:520–526.
- Kidd, M. T., and B. J. Kerr. 1996. L-threonine for poultry: a review. *J. Appl. Poult. Res.* 5:358–367.
- Lang, T., G. C. Hansson, and T. Samuelsson. 2006. An inventory of mucin genes in the chicken genome shows that the mucin domain of Muc13 is encoded by multiple exons and that ovomucin is part of a locus of related gel-forming mucins. *BMC Genomics* 7:197.
- Lee, J., and S. E. Aggrey. 2016. Transcriptomic differences of genes in the avian target of rapamycin (mTOR) pathway in a divergent line of meat-type chickens selected for feed efficiency. *Genet. Mol. Res.* 15:gmr-15027120.
- Lee, C. W., I. J. Cho, Y. J. Lee, Y. S. Son, I. Kwak, Y. T. Ahn, S. C. Kim, and W. G. An. 2014. Effects of dietary levels of glycine, threonine and protein on threonine efficiency and threonine dehydrogenase activity in hepatic mitochondria of chicks. *Asian-Austral J. Anim. Sci.* 27:69–76.
- Lumeij, J. 1997. Avian clinical biochemistry. *Clin. Biochem. Domest. Anim.* 5:857–883.
- Mageed, A. A., N. Isobe, and Y. Yoshimura. 2009. Immunolocalization of avian β -defensins in the hen oviduct and their changes in the uterus during eggshell formation. *Reproduction* 138:971–978.
- Meng, G. H., D. Song, L. B. Li, C. J. Yang, Z. X. Qu, and Y. P. Gao. 2017. Dietary methionine requirement of Jing Brown layer hens from 9 to 17 weeks of age. *J. Anim. Physiol. Anim. Nutr.* 101:925–935.
- Ministry of Agriculture, China. 2004. Feeding Standard of Chicken. Standards Press of China, Beijing.
- Min, Y. N., S. G. Liu, Z. X. Qu, G. H. Meng, and Y. P. Gao. 2017. Effects of dietary threonine levels on growth performance, serum biochemical indexes, antioxidant capacities, and gut morphology in broiler chickens. *Poult. Sci.* 96:1290–1297.
- Miles, R. D., and W. R. Featherston. 1974. Uric acid excretion as an indicator of the amino acid requirement of chicks. *Exp. Biol. Med.* 145:686–689.
- Moreira Filho, A. L. B., P. R. Ferket, R. D. Malheiros, C. J. B. Oliveira, P. C. Aristimunha, D. E. Wilsmann, and P. E. N. Givissiez. 2019. Enrichment of the amnion with threonine in chicken embryos affects the small intestine development, ileal gene 770 expression and performance of broilers between 1 and 21 days of age. *Poult. Sci.* 98:1363–1370.
- Mott, C. R., P. B. Siegel, K. E. Webb, Jr., and E. A. Wong. 2008. Gene expression of nutrient transporters in the small intestine of chickens from lines divergently selected for high or low juvenile body weight. *Poult. Sci.* 87:2215–2224.
- Quintana-Hayashi, M. P., M. Padra, J. T. Padra, J. Benktander, and S. K. Linden. 2018. Mucus-Pathogen Interactions in the Gastrointestinal Tract of Farmed Animals. *Microorganisms* 6:55.
- Ruan, D., A. M. Fouad, Q. Fan, W. Xia, S. Wang, W. Chen, C. Lin, Y. Wang, L. Yang, and C. T. Zheng. 2018. Effects of dietary methionine on productivity, reproductive performance, antioxidant capacity, ovalbumin and antioxidant-related gene expression in laying duck breeders. *Br. J. Nutr.* 119:121–130.
- Ruan, D., S. Q. Jiang, Y. J. Hu, F. Y. Ding, Q. L. Fan, F. Chen, X. J. Lin, L. Li, and Y. Wang. 2017. Effects of corn distillers dried grains with solubles on performance, oxidative status, intestinal immunity and meat quality of Chinese Yellow broilers. *J. Anim. Physiol. Anim. Nutr.* 101:1185–1193.
- Sadeyen, J. R., J. Trotter, J. Protais, C. Beaumont, N. Sellier, G. Salvat, P. Velge, and A. C. Lalmanach. 2006. Salmonella carrier-state in hens: study of host resistance by a gene expression approach. *Microb Infect.* 8:1308–1314.
- Samadi, and F. Liebert. 2006. Modeling of threonine requirement in fast-growing chickens, depending on age, sex, protein deposition, and dietary threonine efficiency. *Poult. Sci.* 85:1961–1968.
- Sarsenbek, A., T. Wang, J. K. Zhao, and W. Jiang. 2013. Comparison of carcass yields and meat quality between Baicheng-You chickens and Arbor Acres broilers. *Poult. Sci.* 92:2776–2782.
- Sanderink, G. J., Y. Artur, and G. Siest. 1988. Human aminopeptidases: A review of the literature. *J. Clin. Chem. Clin. Biochem.* 26:795–807.
- SPSS, 2008. SPSS 16. 0 for Windows. SPSS Inc., Chicago, IL, USA.
- Ulluwishewa, D., R. C. Anderson, W. C. McNabb, P. J. Moughan, J. M. Wells, and N. C. Roy. 2011. Regulation of tight junction permeability by intestinal bacteria and dietary components. *J. Nutr.* 141:769–776.
- Wang, X., S. Y. Qiao, M. Liu, and Y. X. Ma. 2006. Effects of graded levels of true ileal digestible threonine on performance, serum parameters and immune function of 10–25 kg pigs. *Anim. Feed Sci. Tech.* 129:264–278.
- Woodfint, R. M., P. R. Chen, J. Ahn, Y. Suh, S. Hwang, S. S. Lee, and K. Lee. 2017. Identification of the MUC2 promoter as a strong promoter for intestinal gene expression through generation of transgenic quail expressing GFP in gut epithelial cells. *Int. J. Mol. Sci.* 18:196; doi:10.3390.
- Xia, W., A. M. Fouad, W. Chen, D. Ruan, S. Wang, Q. Fan, Y. Wang, Y. Cui, and C. T. Zheng. 2017. Estimation of dietary arginine requirements for Longyan laying ducks. *Poult. Sci.* 96:144–150.
- Yoshimura, Y., H. Ohashi, K. Subedi, M. Nishibori, and N. Isobe. 2006. Effects of age, egg-laying activity, and Salmonella-inoculation on the expressions of gallinacin. *J. Reprod. Dev.* 52:211–218.
- Zeng, P. L., X. G. Li, X. Q. Wang, D. X. Zhang, G. Shu, and Q. B. Luo. 2011. The relationship between gene expression of cationic and neutral amino acid transporters in the small intestine of chick embryos and chick breed, development, sex, and egg amino acid concentration. *Poult. Sci.* 90:2548–2556.
- Zhang, Q., L. Xu, A. Doster, R. Murdoch, P. Cotter, A. Gardner, and T. J. Applegate. 2014. Dietary threonine requirement of Pekin ducks from 15 to 35 days of age based on performance, yield, serum natural antibodies, and intestinal mucin secretion. *Poult. Sci.* 93:1972–1980.
- Zhang, Q., Q. F. Zeng, P. Cotter, and T. J. Applegate. 2016. Dietary threonine response of Pekin ducks from hatch to 14 d of age based on performance, serology, and intestinal mucin secretion. *Poult. Sci.* 95:1348–1355.
- Zhu, C., Z. Y. Jiang, S. Q. Jiang, G. L. Zhou, Y. C. Lin, F. Chen, and P. Hong. 2012. Maternal energy and protein affect subsequent growth performance, carcass yield, and meat color in Chinese Yellow broilers. *Poult. Sci.* 91:1869–1878.
- Zwarycz, B., and E. A. Wong. 2013. Expression of the peptide transporters PepT1, PepT2, and PHT1 in the embryonic and posthatch chick. *Poult. Sci.* 92:1314–1321.