

# The application of reduced dietary crude protein levels supplemented with additional amino acids in laying ducks

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**ABSTRACT** This study was aimed at studying use of reduced dietary crude protein (**CP**) level supplemented with additional amino acids in laying ducks. A total of 720 Jingjiang ducks (50 wk) were randomly assigned to 5 treatments and fed 5 basal diets with CP levels at 17.5, 16.5, 15.5, 14.5, or 13.5%, with additional amino acids added to each diet for 12 wk. Each treatment had 6 replicates of 24 ducks each. Dietary CP levels affected ( $P < 0.05$ ) egg production and mass of laying ducks, and there was a linear and quadratic decrease with decreasing CP levels ( $P < 0.05$ ). Dietary CP levels did not affect egg weight and feed conversion ratio (**FCR**), but egg weight decreased linearly ( $P < 0.05$ ); FCR increased linearly and quadratically ( $P < 0.05$ ) with decreasing CP levels. There were no significant differences in egg quality among the different CP levels ( $P > 0.05$ ). Ovarian weight, total and mean weight of preovulatory follicles, and total weight of small yellow follicles (**SYF**) were decreased by dietary CP levels (linear,  $P < 0.01$  and quadratic,  $P < 0.05$ ). The oviductal weight decreased linearly ( $P < 0.05$ ), and the number of SYF decreased linearly and quadratically with

decreasing CP levels ( $P < 0.05$ ). The serum estradiol content decreased linearly with dietary CP levels ( $P < 0.05$ ). The serum contents of luteinizing hormone, prolactin, and progesterone decreased ( $P < 0.05$ ), linearly and quadratically (both  $P < 0.01$ ) with decreasing CP levels. The serum contents of creatinine (**CRE**), triglycerides (**TG**), total cholesterol (**TC**), and alanine aminotransferase (**ALT**) activity were affected ( $P < 0.05$ ) by different dietary CP levels. The total protein content increased linearly ( $P < 0.05$ ), TC content increased quadratically ( $P < 0.05$ ), and contents of albumin, CRE, TG, and phosphorus, and activities of aspartate aminotransferase and ALT increased linearly and quadratically (both  $P < 0.05$ ) with decreasing CP levels. Overall, reduced dietary CP levels with addition of amino acids affected the laying performance, the development of reproductive organs and ovarian follicles, serum hormones, and biochemical indices of laying ducks. Dietary CP levels can be reduced to 14.5% with additional amino acid supplementation for 12 wk in laying ducks without negative effect on laying performance and egg quality.

**Key words:** crude protein, laying performance, egg quality, laying duck

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## INTRODUCTION

In poultry, feeding low crude protein (**CP**) diets is an effective way of decreasing nitrogen excretion and

ammonia emissions, thereby reducing nitrogen loss to the environment (Summers, 1993; Burley et al., 2013; Hofmann et al., 2019). Dietary CP levels can be reduced, and specific requirements still met by adding crystalline amino acids. Currently, as crystalline amino acids are available at affordable prices, the use of low-CP diets with accurate amino acid supplementation is readily accomplished in formulating poultry diets. The reduction in performance and feathering that resulted from feeding broilers up to 3% reduced dietary CP can be corrected when the appropriate amino acids are supplemented (Ospina-Rojas et al., 2012), and there is additional need for nonessential nitrogen when feeding chicks a starter-phase diet reduced by 4% points in CP

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(Corzo et al., 2005). The CP in laying hen diets can be reduced by 2% units if diets are fortified with synthetic amino acids (methionine, lysine, threonine, and tryptophan) and isoleucine (Parenteau et al., 2020). In the long term, adequacy of amino acids, particularly methionine and the branched-chain amino acids, was necessary to sustain normal immunocompetence and to achieve maximal production performance when the dietary CP level of laying hens was reduced by 4% (Poosuwan et al., 2010). Similarly, reducing dietary CP from 16.5 to 12.0% and supplementing the diets with the essential amino acids maintained acceptable production performance of laying hens under high ambient temperature conditions (Torki et al., 2015). Taken together, it appears that the CP level of poultry diets can be reduced by 3 to 4% provided amino acids are supplemented.

In addition to chicken eggs, duck eggs are another source of animal protein for humans, and duck eggs supplied approximately 15% of all eggs for human consumption in 2009 (Pingel, 2011). Contemporary production uses corn–soybean–based duck diets, and the emission of nitrogen in the excreta of laying ducks is an important issue, as for other components of the poultry industry. Given the cost of feed proteins and this environmental

aspect, the current research aimed to evaluate low CP levels, supplemented with crystalline amino acids, in laying ducks. The effects of different reduced CP levels, with added amino acids to meet nutrient requirements, on laying performance, egg quality, reproductive organ and ovarian follicle development, and serum biochemical indices were assessed in laying ducks.

## 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). A total of 720 Jingjiang ducks (50 wk) were randomly assigned to 5 treatments consisting of 5 basal diets with CP levels of 17.5, 16.5, 15.5, 14.5, 13.5% fed for 12 wk, respectively. Additional amino acids were added to each diet, as detailed in Table 1. Each treatment had 6 replicates of 24 ducks, with 2 ducks housed in a cage (45 cm × 30 cm × 50 cm) provided with a nipple drinker and feeder (Guangzhou Huanan Poultry Equipment, Guangzhou, PRC). Fresh drinking water was

**Table 1.** Dietary composition and nutrient levels of the diets (kg).

CP levels	17.5%	16.5%	15.5%	14.5%	13.5%
Ingredients (g)					
Corn (CP, 7.8%) <sup>2</sup>	555	580	570	610	630
Soybean meal (CP, 43%) <sup>2</sup>	295	250	205	165	125
Limestone	86.0	86.0	87.0	87.0	87.0
Dicalcium phosphate	11.0	11.0	10.0	11.0	11.0
Wheat flour (CP, 13.97%) <sup>2</sup>	25.0	32.0	32.0	34.0	38.0
Soybean oil	20.0	20.0	20.0	20.0	20.0
Rice bran (CP, 12.5%) <sup>2</sup>	0.00	5.00	55.0	45.0	55.0
L-lysine sulfate (70%)	0.00	2.00	3.50	5.40	7.00
DL-methionine (Solid, 99%)	1.60	2.10	2.50	2.90	3.30
Phytase (heat-resistant)	0.20	0.20	0.20	0.20	0.20
Vitamin premix <sup>1</sup>	0.15	0.15	0.15	0.15	0.15
Mineral premix <sup>1</sup>	1.00	1.00	1.00	1.00	1.00
Salt	4.00	4.00	4.00	4.00	4.00
Rice husk	0.55	0.95	0.25	0.55	0.45
L-threonine (98%)	0.00	0.80	1.40	2.10	2.70
L-tryptophan (99%)	0.00	0.30	0.60	0.80	1.10
L-arginine (99%)	0.00	1.30	2.20	3.40	4.40
L-valine (99%)	0.50	1.30	1.80	2.50	3.10
L-isoleucine (99%)	0.00	0.70	1.20	1.80	2.40
L-leucine (99%)	0.00	1.20	2.20	3.20	4.20
Total	1,000	1,000	1,000	1,000	1,000
Nutrients (%) <sup>2</sup>					
AME (MJ/kg)	11.12	11.08	11.08	11.11	11.11
Crude protein (CP)	17.5 (17.3)	16.5(16.4)	15.5 (15.4)	14.5 (14.7)	13.5 (13.6)
Calcium	3.62	3.60	3.61	3.62	3.61
Total phosphorus	0.50	0.50	0.50	0.50	0.50
Available phosphorus	0.29	0.29	0.28	0.28	0.28
Lysine	0.82 (0.83)	0.82 (0.80)	0.82 (0.81)	0.82 (0.84)	0.82 (0.85)
Methionine + cysteine	0.76 (0.76)	0.76 (0.74)	0.76 (0.75)	0.76 (0.78)	0.76 (0.78)
Threonine	0.63 (0.63)	0.63 (0.63)	0.63 (0.62)	0.63 (0.63)	0.63 (0.63)
Tryptophan	0.25 (0.24)	0.25 (0.25)	0.25 (0.25)	0.25 (0.26)	0.25 (0.26)
Isoleucine	0.66 (0.67)	0.66 (0.63)	0.66 (0.65)	0.66 (0.68)	0.66 (0.68)
Leucine	1.22 (1.28)	1.22 (1.23)	1.22 (1.27)	1.22 (1.22)	1.22 (1.20)
Arginine	1.00 (1.02)	1.00 (0.96)	1.00 (1.01)	1.00 (1.03)	1.00 (1.01)
Valine	0.75 (0.77)	0.75 (0.74)	0.75 (0.77)	0.75 (0.77)	0.75 (0.76)

<sup>1</sup>Provided per kilogram of diet: VA 12,500 IU; VD<sub>3</sub> 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; VB<sub>12</sub> 5 mg; Mn 100 mg; I 0.5 mg; Fe 60 mg; Cu 8 mg; Se 0.2 mg; Co 0.26.

<sup>2</sup>The number in parentheses are analyzed values.

available *ad libitum*, and pelleted feed was introduced to each cage twice daily at 07:00 and 15:00. There was no mortality during the 12-wk duration of the study.

### Sample Collection

Five eggs with the average egg weight per replicate were randomly collected at the end of week 3, 6, 9, and 12 during the treatment period for determining egg quality; measurements were made on the day of collection.

At the end of the trial, 2 healthy ducks in each replicate were randomly selected and fasted for 12 h for sampling. Blood samples were taken from the wing vein into noncoated evacuated tubes, then incubated in a 37°C water bath while tilted at a 45° angle for 3 h, and then centrifuged at  $3,000 \times g$  for 10 min to harvest serum. Serum samples were kept at -20°C until analysis. The sampled ducks were then killed by cervical dislocation.

The initial and final body weight of all ducks were measured. The weight and length of the oviduct were measured, and the liver and ovary were collected and weighed. The preovulatory follicles (POF, > 10 mm in diameter), small yellow follicles (SYF, 6–10 mm in diameter), and large white follicles (LWF; 2–5 mm in diameter) were dissected, counted, weighed, and recorded. The weight proportions of POF, SYF, and LWF were calculated as percentages of ovarian weight (Zhang et al., 2020).

### Measurement of Crude Protein and Amino Acids

The CP and total amino acids contents of each treatment diet were analyzed after diets were assembled and pelleted before beginning the experiment. The CP was analyzed by a Kjeltac 8400 Analyzer Unit (FOSS Analytical AB, Hoganas, Sweden). The amino acids in the mixed diets were analyzed after hydrolysis by an amino acid analyzer (HITACHI L-8900, Hitachi, Ltd., Tokyo, Japan).

### Performance and Egg Quality Measurement

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

### Serum Hormone Analyses

After overnight thawing at 4°C, serum samples were used to measure concentrations of estradiol (E<sub>2</sub>),

prolactin (PRL), follicle-stimulating hormone, luteinizing hormone (LH), and progesterone (P<sub>4</sub>) using ELISA kits for ducks (Shanghai Enzyme-linked Biotechnology Co., Ltd. Shanghai, China).

### Serum Biochemical Indices

The contents in serum of total protein (TP), albumin (ALB), creatinine (CRE), UA, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, glucose, total cholesterol (TC), triglyceride (TG), calcium, and phosphorus were determined with kits in an automatic biochemistry analyzer (all from BIOSINO Biotechnology Co., Ltd., Beijing, China).

### Statistical Analysis

Replicate (each replicate containing 12 cages, 2 ducks in each cage) served as the experimental unit for analysis of performance and egg quality data; the average of 2 ducks in each replicate was the experimental unit for other assessments. The normality of the data and homogeneity of variances were first verified by the Explore procedure using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL). The effects of dietary CP levels were analyzed by one-way ANOVA procedure, and then means were compared using Tukey's multiple range tests. Regression analysis was employed to test the linear (L) and quadratic (Q) effects using SPSS 16.0 for Windows (SPSS Inc.). Data are expressed as means and pooled SEM.

## RESULTS

### Laying Performance

The effects of different dietary CP levels on laying performance of laying ducks are shown in Table 2. Dietary CP levels did not affect the egg production, egg mass, and average daily feed intake during 1 to 3 wk and 4 to 6 wk ( $P > 0.05$ ) but affected them from 7 to 12 wk and 1 to 12 wk ( $P < 0.05$ ), and the responses were L (7 to 12 wk, and 1 to 12 wk;  $P < 0.05$ ) and Q (1–12 wk;  $P < 0.05$ ) with decreasing CP levels. Dietary CP levels did not affect egg weight and feed conversion ratio (FCR), but egg weight decreased (L,  $P < 0.05$ ; 4 to 6 wk, 7 to 12 wk, 1 to 12 wk); FCR increased (L,  $P < 0.01$ ; Q,  $P < 0.05$ ; 1–12 wk) with decreasing CP levels. During the trial period of 7 to 12 wk, the egg production, egg mass, and feed intake were decreased in ducks fed with 13.5% CP diet in contrast to those fed the normal diet (CP 17.5%,  $P < 0.05$ ). For the whole feeding period, the duck layers fed 13.5% CP compared with those given the normal diet (CP 17.5%) had decreased egg production (72.3 vs. 79.4%;  $P < 0.05$ ), egg mass (48.8 vs. 55.2 g;  $P < 0.05$ ), and feed intake (136 vs. 144;  $P < 0.05$ ) and increased FCR (2.77 vs. 2.62 g:g). There were no obvious changes in egg production, egg mass, and FCR of the other 3 treatments relative to the normal diet.

**Table 2.** Effects of different dietary CP levels on the productive performance of laying ducks (50–61 wk).

Variables	CP levels (%) <sup>1</sup>					SEM	P-values		
	17.5	16.5	15.5	14.5	13.5		ANOVA	Linear	Quadratic
Egg production (%)									
1 to 3 wk	77.8	83.7	83.1	78.4	80.0	1.27	0.560	0.905	0.059
4 to 6 wk	78.8	82.1	83.2	81.3	78.9	1.09	0.648	0.923	0.218
7 to 12 wk	77.8 <sup>a</sup>	74.6 <sup>a</sup>	74.2 <sup>a</sup>	75.8 <sup>a</sup>	65.5 <sup>b</sup>	1.28	0.017	0.006	0.198
1 to 12 wk	79.4 <sup>a</sup>	80.0 <sup>a</sup>	78.9 <sup>a,b</sup>	78.0 <sup>a,b</sup>	72.3 <sup>b</sup>	0.94	0.044	0.013	0.010
Average egg weight (g)									
1 to 3 wk	69.3	68.8	68.1	68.7	68.2	0.242	0.530	0.180	0.336
4 to 6 wk	69.7	69.3	69.7	68.4	68.1	0.263	0.155	0.028	0.067
7 to 12 wk	71.8	71.5	70.3	69.4	69.4	0.389	0.135	0.013	0.738
1 to 12 wk	69.8	69.1	69.0	68.6	68.3	0.243	0.428	0.049	0.145
Egg mass (g)									
1 to 3 wk	53.7	57.2	58.1	53.7	53.1	0.861	0.218	0.434	0.124
4 to 6 wk	54.7	56.3	57.7	55.4	53.5	0.678	0.381	0.500	0.136
7 to 12 wk	54.2 <sup>a</sup>	51.3 <sup>a,b</sup>	49.0 <sup>a,b</sup>	52.0 <sup>a</sup>	44.4 <sup>b</sup>	0.998	0.013	0.004	0.559
1 to 12 wk	55.2 <sup>a</sup>	54.9 <sup>a</sup>	54.0 <sup>a</sup>	53.4 <sup>a,b</sup>	48.8 <sup>b</sup>	0.699	0.018	0.003	0.003
Average daily feed intake (g/per duck)									
1 to 3 wk	151	152	154	153	149	0.61	0.254	0.503	0.119
4 to 6 wk	145	145	143	144	141	0.76	0.457	0.082	0.204
7 to 12 wk	139 <sup>a</sup>	135 <sup>a</sup>	131 <sup>a,b</sup>	135 <sup>a</sup>	124 <sup>b</sup>	1.13	<0.001	<0.001	0.431
1 to 12 wk	144 <sup>a</sup>	141 <sup>a</sup>	140 <sup>a,b</sup>	142 <sup>a</sup>	136 <sup>b</sup>	0.75	0.024	0.006	0.022
FCR (g:g)									
1 to 3 wk	2.81	2.66	2.66	2.83	2.81	0.041	0.540	0.813	0.248
4 to 6 wk	2.67	2.59	2.49	2.59	2.64	0.027	0.358	0.632	0.114
7 to 12 wk	2.58	2.63	2.65	2.59	2.80	0.035	0.150	0.078	0.274
1 to 12 wk	2.62	2.58	2.60	2.65	2.77	0.026	0.070	0.039	0.036

<sup>a,b</sup>Means within a row with differing superscripts differ significantly ( $P < 0.05$ ). Abbreviation: FCR, feed conversion ratio.

<sup>1</sup>Mean of 6 replicates (24 ducks per replicate) per treatment.

## Egg Quality

Table 3 shows the effects of different dietary CP levels on egg quality in laying ducks during the trial period. There were no significant differences in eggshell breaking strength, shell thickness, albumen height, Haugh unit, shell ratio, albumen ratio, or yolk ratio among the different CP levels ( $P > 0.05$ ).

## Reproductive Organs and Ovarian Follicle Development

As shown in Table 4, dietary CP levels did not affect oviductal weight, but it decreased linearly with decreasing dietary CP levels ( $P < 0.05$ ). Ovarian weight, total and mean weight of POF, and total weight of SYF were affected by dietary CP levels ( $P < 0.05$ ), with L ( $P < 0.01$ ) and Q ( $P < 0.05$ ) decreases. The number of SYF was not affected by diets, but it decreased linearly and quadratically with decreasing CP levels ( $P < 0.05$ ). Other ovarian follicle variables were not influenced by dietary CP levels. Compared with the ducks fed with normal diet (CP, 17.5%), the ovarian weight, mean POF weight, and total SYL weight were decreased in ducks fed 14.5% CP ( $P < 0.05$ ); the total and mean POF weight were decreased in ducks fed 13.5% CP ( $P < 0.05$ ). For the development of reproductive organs and ovarian follicles, the laying ducks fed 16.5% CP had no difference in oviductal weight, total and mean weights of POF, and total weight of SYF compared with the normal diet, but these variables decreased in the other 3 treatments with about 10 g in ovary weight, 8 to 10 g in total weight of POF, 1.8 to 2 g in mean

weight of POF, and 0.6 to 1.1 g in total weight of SYF than the normal diet.

## Serum Hormones

The effects of different dietary CP levels on serum concentrations of hormones of laying ducks are shown in Table 5. Dietary CP levels did not affect serum E<sub>2</sub> content, through a L decrease ( $P < 0.05$ ) with decreasing CP levels was evident. The contents of LH, PRL, and P<sub>4</sub> were influenced ( $P < 0.05$ ) and decreased linearly ( $P < 0.01$ ) and quadratically ( $P < 0.01$ ) with decreasing CP levels. Dietary CP levels did not affect follicle-stimulating hormone content in serum. For the serum hormones, the contents of E<sub>2</sub> (41.6 vs. 54.5), LH (1.92 vs. 3.63;  $P < 0.05$ ), PRL (95.2 vs. 158;  $P < 0.05$ ), and P<sub>4</sub> (11.2 vs. 17.1;  $P < 0.05$ ) were much lower in ducks fed 13.5% CP than the normal diet, and these values were less obviously lower in the other 3 treatments.

## Serum Biochemical Indices

The effects of dietary CP levels on the serum biochemical indices of laying ducks are shown in Table 6. Dietary CP did not affect the TP and ALB content in serum but increases in serum TP (L,  $P < 0.05$ ) and ALB (L,  $P < 0.01$ ; Q,  $P < 0.05$ ) occurred with decreasing CP levels. The serum contents of CRE, TG, TC, and ALT activity were affected ( $P < 0.05$ ), and the CRE content decreased (L, Q,  $P < 0.01$ ), whereas increases occurred in TG (L, Q,  $P < 0.01$ ), TC (Q,  $P < 0.05$ ), and ALT activity (L, Q,  $P < 0.05$ ) with decreasing CP levels. The serum contents of ALB and P and AST activity were

**Table 3.** Effects of different dietary CP levels on the egg quality of laying ducks (50–61 wk).

Variables	CP level (%) <sup>1</sup>					SEM	P-values		
	17.5	16.5	15.5	14.5	13.5		ANOVA	Linear	Quadratic
Eggshell breaking strength (N)									
3 wk	44.1	44.8	44.3	43.7	41.8	0.628	0.628	0.190	0.265
6 wk	45.3	45.3	43.6	45.6	42.6	0.460	0.169	0.123	0.263
9 wk	39.7	41.9	40.1	41.7	41.1	0.680	0.827	0.621	0.817
12 wk	42.8	40.5	41.4	43.0	41.5	0.590	0.672	0.989	0.896
Eggshell thickness (mm)									
3 wk	0.339	0.340	0.335	0.329	0.326	0.003	0.393	0.050	0.136
6 wk	0.345	0.346	0.343	0.347	0.332	0.002	0.071	0.070	0.063
9 wk	0.344	0.336	0.345	0.337	0.344	0.002	0.564	0.909	0.762
12 wk	0.323	0.312	0.310	0.301	0.328	0.005	0.475	0.979	0.261
Albumen height (mm)									
3 wk	6.52	6.83	6.43	6.56	6.78	0.100	0.695	0.737	0.879
6 wk	6.82	6.67	6.71	6.69	6.37	0.085	0.556	0.144	0.295
9 wk	6.79	6.86	6.78	6.73	6.88	0.101	0.992	0.946	0.971
12 wk	7.00	7.43	7.13	7.29	7.41	0.080	0.364	0.225	0.472
Haugh unit									
3 wk	78.2	80.2	77.4	78.1	79.6	0.757	0.785	0.899	0.914
6 wk	79.4	78.6	78.2	77.9	76.0	0.590	0.484	0.074	0.189
9 wk	80.2	80.6	80.2	80.0	80.6	0.724	0.999	0.975	0.992
12 wk	81.5	85.1	82.7	83.4	83.9	0.526	0.269	0.401	0.550
Shell proportion (%)									
3 wk	9.64	9.57	9.58	9.35	9.31	0.067	0.435	0.065	0.177
6 wk	9.27	9.37	9.26	9.42	9.12	0.054	0.467	0.514	0.422
9 wk	9.16	9.12	9.12	9.10	9.23	0.053	0.958	0.778	0.773
12 wk	9.24	9.27	9.35	9.26	9.24	0.048	0.969	0.996	0.843
Albumen proportion (%)									
3 wk	56.6	57.2	56.8	57.5	57.5	0.214	0.621	0.163	0.385
6 wk	58.7	59.2	58.4	59.0	58.4	0.155	0.462	0.519	0.761
9 wk	58.7	59.6	59.0	59.5	59.3	0.193	0.606	0.408	0.569
12 wk	59.6	60.7	59.9	59.4	59.2	0.200	0.125	0.125	0.115
Yolk proportion (%)									
3 wk	34.2	33.3	33.6	33.2	33.2	0.218	0.503	0.138	0.270
6 wk	32.0	31.4	32.4	31.6	32.4	0.180	0.343	0.452	0.608
9 wk	32.1	31.3	31.9	31.4	31.5	0.178	0.581	0.353	0.581
12 wk	31.2	30.1	30.7	31.6	31.6	0.212	0.122	0.135	0.114

<sup>a,b</sup>Means within a row with differing superscripts differ significantly ( $P < 0.05$ ).

<sup>1</sup>Mean of 6 replicates (5 eggs per replicate) per treatment.

not affected, but these values linearly and quadratically increased ( $P < 0.05$ ) with decreasing dietary CP levels. Other biochemical indices in serum were not influenced by decreasing dietary CP levels. Compared with the ducks given the normal diet (CP 17.5%), the CRE content was decreased, and TG and TC contents were increased in ducks fed with 14.5% CP ( $P < 0.05$ ); the ALT content was increased in ducks fed with 13.5% CP ( $P < 0.05$ ).

## DISCUSSION

In the present study, reducing dietary CP levels with appropriate supplementation with amino acids decreased laying performance of laying ducks, gauged by egg production, mass, weight, and feed intake, and linearly increased FCR; these effects were especially evident in the ducks fed 13.5% CP showing the worst performance. Extreme reduction of dietary CP, despite being balanced with all critical amino acids, was detrimental for intestinal nitrogen metabolism and growth of pigs (Che et al., 2017). Similar effects were observed in laying hens, where 14% dietary CP supplemented with additional amino acids decreased egg production, mass and weight, and increased FCR (Poosuan et al., 2010), and egg production and mass decreased with

decreasing CP levels (10.5–16.5%, Toriki et al., 2015). Despite of the supplementation of amino acids in diets, the feed intake and egg weight were decreased in ducks fed low CP diets (especially 13.5% CP), which inferred the crystalline amino acids could not completely offset the decrease of CP in diet. There are different properties of amino acids in intact proteins and those provided as crystalline amino acids in terms of the digestion, absorption, and metabolism of nitrogen (Guay et al., 2006); these probably accounted for the decline of feed intake and egg weight in ducks fed here with low CP diets. Consistent with the poorer productive performance of the laying ducks, the reproductive organs, ovarian follicle development, and serum hormones decreased with decreasing CP levels. It was reported that the CP was an exogenous input affecting reproductive function at different levels of the hypothalamo-hypophyseal-gonadal axis, thereby affecting the secretion of hormones into the circulation (Meza-Herrera et al., 2007). Serum hormone levels are sensitive indicators of laying performance (Rozenboim et al., 2007) and affect development of the reproductive tract (Hu et al., 2019) and maturation of follicles (Liu and Zhang, 2008). In this respect, dietary CP levels in laying ducks had impact on the serum contents of hormones and development of reproductive organs

**Table 4.** Effects of different dietary CP levels on the reproductive organ and ovarian follicle development of laying ducks at the end of the trial (61 wk).

Variables	CP level (%) <sup>1</sup>					SEM	P-values		
	17.5	16.5	15.5	14.5	13.5		ANOVA	Linear	Quadratic
Initial Body weight (kg)	1.48	1.47	1.49	1.48	1.49	0.005	0.801	0.601	0.763
Final Body weight (kg)	1.48	1.47	1.48	1.46	1.51	0.007	0.128	0.240	0.123
Liver weight (g)	49.7	50.5	49.8	52.3	45.8	0.954	0.308	0.393	0.260
Oviduct length (cm)	53.2	58.2	57.1	53.2	58.3	0.849	0.103	0.400	0.668
Oviduct weight (g)	57.5	54.1	54.6	52.8	53.3	0.672	0.212	0.041	0.076
Ovarian weight (g)	51.7 <sup>a</sup>	52.3 <sup>a</sup>	42.9 <sup>a,b</sup>	39.7 <sup>b</sup>	41.8 <sup>a,b</sup>	1.561	0.012	0.002	0.006
Number of POF	4.83	5.08	5.25	4.83	4.67	0.122	0.604	0.507	0.312
Total POF weight (g)	42.0 <sup>a,b</sup>	43.5 <sup>a</sup>	36.2 <sup>a,b</sup>	33.1 <sup>a,b</sup>	31.4 <sup>b</sup>	1.665	0.048	0.005	0.021
Mean POF weight (g)	8.63 <sup>a</sup>	8.76 <sup>a</sup>	6.92 <sup>a</sup>	6.83 <sup>b</sup>	6.72 <sup>b</sup>	0.283	0.019	0.002	0.009
Number of SYF	11.6	10.4	9.58	6.67	7.92	0.643	0.104	0.012	0.039
Total SYF weight (g)	2.05 <sup>a,b</sup>	2.44 <sup>a</sup>	1.83 <sup>a,b</sup>	1.30 <sup>b</sup>	1.40 <sup>a,b</sup>	0.137	0.036	0.009	0.031
Mean SYF weight (g)	0.190	0.213	0.199	0.177	0.190	0.009	0.826	0.584	0.807
Number of LWF	14.5	12.9	11.6	13.8	13.3	0.810	0.854	0.788	0.700
Total LWF weight (g)	0.768	0.818	0.723	0.848	0.748	0.046	0.927	0.970	0.979
Mean LWF weight (g)	0.051	0.067	0.065	0.059	0.059	0.002	0.063	0.588	0.068

<sup>a,b</sup>Means within a row with differing superscripts differ significantly ( $P < 0.05$ ). Abbreviations: LWF, large white follicles; POF, preovulatory follicles; SYF, small yellow follicles.

<sup>1</sup>Mean of 6 replicates (2 ducks per replicate) per treatment.

and ovarian follicles and affected the productive performance.

Dietary crude protein and supplementation with amino acids are known to influence protein and lipid metabolism of animals (Duan et al., 2016; Jiang et al., 2017) and that was confirmed in the current study with laying ducks where serum contents of TP, ALB, TC, and TG increased with decreasing CP levels. In addition, the serum AST and ALT activities increased with decreasing CP levels. These results indicated that the status of lipid metabolism and liver function might be disturbed by reduced CP levels, as the excess accumulation of TG and TC leads to metabolic disorders in broilers (Alvarenga et al., 2011), and ALB and TP contents are reliable indicators of the function of the liver and, together with plasma AST and ALT activities, are closely correlated with the degree of hepatic lipidosis in animals (Attia and Al-Harathi, 2015). Among the treatments, the values of serum TP, ALB, TG, and TC content, AST and ALT activities were almost the highest in ducks fed 14.5% CP, and lower values of TP, ALB, TG, and TC contents were observed in those given 16.5 and 15.5% CP. The contents of serum TP, ALB, AST, and ALT activities were similar between 14.5% and 13.5% treatments. These data implied that the lipid metabolism and liver function and the health status of laying ducks were damaged when the dietary CP was lower than 15.5%.

Previous studies have reported that dietary CP level can be reduced by 3 to 4% with addition of amino acids in broilers (Corzo et al., 2005; Ospina-Rojas et al., 2012) and laying hens (Poosuwan et al., 2010; Toriki et al., 2015). Similarly, in the current study, after feeding for 12 wk, the laying performance of duck layers provided with dietary CP level  $\geq 14.5\%$ , with added amino acids, performed similarly to ducks fed the normal 17.5% CP diet, without supplemental amino acids. In contrast, compared with the normal diet (17.5% CP), the reproductive organ and ovarian follicle development of ducks fed 16.5% CP showed no difference, but these variables were decreased by the other 3 reduced CP levels (13.5, 14.5, 15.5%). It can be concluded, therefore, that dietary CP can be reduced to 14.5% for 12 wk, provided additional amino acids are supplemented, without negative effects on laying performance and egg quality; there may be a need for  $>14.5\%$  dietary CP for periods longer than 12 wk.

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**Table 5.** Effects of different dietary CP levels on the serum hormones of laying ducks at the end of the trial (61 wk).

Variables	CP level (%) <sup>1</sup>					SEM	P-values		
	17.5	16.5	15.5	14.5	13.5		ANOVA	Linear	Quadratic
Estradiol (ng/L)	54.5	48.6	50.5	47.9	41.6	1.84	0.272	0.039	0.116
Follicle-stimulating hormone (IU/L)	1.52	1.58	1.50	1.59	1.35	0.039	0.299	0.205	0.192
Luteinizing hormone (U/L)	3.63 <sup>a</sup>	2.76 <sup>a,b</sup>	2.69 <sup>a,b</sup>	2.46 <sup>a,b</sup>	1.92 <sup>b</sup>	0.160	0.008	$<0.001$	0.002
Prolactin (mIU/L)	158 <sup>a</sup>	119 <sup>b</sup>	103 <sup>b</sup>	91.1 <sup>b</sup>	95.2 <sup>b</sup>	5.20	$<0.001$	$<0.001$	$<0.001$
Progesterone (ng/mL)	17.1 <sup>a</sup>	15.6 <sup>a,b</sup>	12.3 <sup>b</sup>	13.9 <sup>a,b</sup>	11.2 <sup>b</sup>	0.61	0.005	0.001	0.003

<sup>a,b</sup>Means within a row with differing superscripts differ significantly ( $P < 0.05$ ).

<sup>1</sup>Mean of 6 replicates (2 ducks per replicate) per treatment.

**Table 6.** Effects of different dietary CP levels on the serum biochemical indices of laying ducks at the end of the trial (61 wk).

Variables	CP level (%) <sup>1</sup>					SEM	P-values		
	17.5	16.5	15.5	14.5	13.5		ANOVA	Linear	Quadratic
TP (g/L)	53.4	55.0	55.9	59.5	57.8	0.94	0.286	0.044	0.120
ALB (g/L)	16.8	17.3	17.4	19.3	19.3	0.38	0.090	0.007	0.028
UA (μmol/L)	259	268	246	249	279	5.60	0.329	0.614	0.329
CRE (μmol/L)	39.1 <sup>a,b</sup>	37.9 <sup>a,b,c</sup>	40.0 <sup>a</sup>	34.5 <sup>c</sup>	34.9 <sup>b,c</sup>	0.60	0.002	0.003	0.008
AST (U/L)	25.0	29.2	35.6	34.1	33.8	1.35	0.068	0.016	0.015
ALT (U/L)	29.5 <sup>b</sup>	35.9 <sup>a</sup>	34.2 <sup>a,b</sup>	35.5 <sup>a,b</sup>	36.5 <sup>a</sup>	0.78	0.020	0.011	0.017
TB (μmol/L)	5.22	5.77	4.85	7.04	5.35	0.314	0.214	0.500	0.702
GLU (mmol/L)	10.0	9.99	10.2	10.3	9.90	0.140	0.943	0.988	0.856
TG (mmol/L)	5.91 <sup>b</sup>	7.28 <sup>b</sup>	6.72 <sup>b</sup>	9.77 <sup>a</sup>	7.50 <sup>b</sup>	0.312	<0.001	0.008	0.009
TC (mmol/L)	2.59 <sup>b</sup>	3.36 <sup>a,b</sup>	3.16 <sup>a,b</sup>	3.54 <sup>a</sup>	2.83 <sup>a,b</sup>	0.111	0.029	0.406	0.019
Ca (mmol/mL)	7.62	7.83	7.69	7.78	7.61	0.152	0.990	0.943	0.922
P (mmol/mL)	2.33	2.42	2.24	2.68	3.07	0.107	0.086	0.019	0.022

<sup>a-c</sup>Means within a row with differing superscripts differ significantly ( $P < 0.05$ ). Abbreviations: ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Ca, calcium; CRE, creatinine; GLU, glucose; P, phosphorus; TB, total bilirubin; TC, total cholesterol; TG, triglycerides; TP, total protein; UA, uric acid.

<sup>1</sup>Mean of 6 replicates (2 ducks per replicate) per treatment.

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## DISCLOSURES

No conflict of interest.

## REFERENCES

- Alvarenga, R. R., M. G. Zangeronimo, L. J. Pereira, P. B. Rodrigues, and E. M. Gomide. 2011. Lipoprotein metabolism in poultry. *Worlds Poult. Sci. J.* 67:431–440.
- Attia, Y. A., and M. A. Al-Harthi. 2015. Nigella seed oil as an alternative to antibiotic growth promoters for broiler chickens. *Europ. Poult. Sci.* 79:1612–9199.
- Burley, H. K., P. H. Patterson, and M. A. Elliot. 2013. Effect of a reduced crude protein, amino acid-balanced diet on hen performance, production costs, and ammonia emissions in a commercial laying hen flock. *J. Appl. Poult. Res.* 22:217–228.
- Che, L. Q., X. Peng, L. Hu, C. Wu, Q. Xu, Z. F. Fang, Y. Lin, S. Y. Xu, J. Li, B. Feng, G. Tian, R. N. Zhang, H. Sun, D. Wu, and D. W. Chen. 2017. The addition of protein-bound amino acids in low-protein diets improves the metabolic and immunological characteristics in fifteen-to thirty-five-kg pigs. *J. Anim. Sci.* 95:1277–1287.
- Corzo, A., C. A. Fritts, M. T. Kidd, and B. J. Kerr. 2005. Response of broiler chicks to essential and non-essential amino acid supplementation of low crude protein diets. *Anim. Feed Sci. Tech.* 118:319–327.
- Duan, Y. H., Y. M. Duan, F. N. Li, Y. H. Li, Q. P. Guo, Y. J. Ji, B. Tan, T. J. Li, and Y. L. Yin. 2016. Effects of supplementation with branched-chain amino acids to low-protein diets on expression of genes related to lipid metabolism in skeletal muscle of growing pigs. *Amino Acids* 48:2131–2144.
- Guay, F., S. M. Donovan, and N. L. Trottier. 2006. Biochemical and morphological developments are partially impaired in intestinal mucosa from growing pigs fed reduced-protein diets supplemented with crystalline amino acids. *J. Anim. Sci.* 84:1749–1760.
- Hofmann, P., W. Siegert, Á. Kenéz, V. D. Naranjo, and M. Rodehutschord. 2019. Very low crude protein and varying glycine concentrations in the diet affect growth performance, characteristics of nitrogen excretion, and the blood metabolome of broiler chickens. *J. Nutr.* 149:1122–1132.
- Hu, S. Q., X. H. Liang, X. F. Ren, Y. Shi, H. Su, Y. H. Li, K. Du, J. Wang, X. B. Jia, S. Y. Chen, and S. J. Lai. 2019. Integrated analysis of mRNA and miRNA expression profiles in the ovary of *Oryctolagus cuniculus* in response to gonadotrophic stimulation. *Front. Endocrinol.* 10:744.
- 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.
- Liu, H. Y., and C. Q. Zhang. 2008. Effects of daidzein on messenger ribonucleic acid expression of gonadotropin receptors in chicken ovarian follicles. *Poult. Sci.* 87:541–545.
- Meza-Herrera, C. A., T. Ross, D. Hallford, D. Hawkins, and A. Gonzalez-Bulnes. 2007. Effects of body condition and protein supplementation on LH secretion and luteal function in sheep. *Reprod. Dom. Anim.* 42:461–465.
- Ospina-Rojas, I. C., A. E. Murakami, C. Eyng, R. V. Nunes, C. R. A. Duarte, and M. D. Vargas. 2012. Commercially available amino acid supplementation of low-protein diets for broiler chickens with different ratios of digestible glycine+serine:lysine. *Poult. Sci.* 91:3148–3155.
- Parenteau, I. A., M. Stevenson, and E. G. Kiarie. 2020. Egg production and quality responses to increasing isoleucine supplementation in Shaver white hens fed a low crude protein corn-soybean meal diet fortified with synthetic amino acids between 20 and 46 weeks of age. *Poult. Sci.* 99:1444–1453.
- Pingel, H. 2011. Waterfowl production for food security. *Lohmann Inf.* 46:32–42.
- Poosuwan, K., C. Bunchasak, and C. Kaewtapee. 2010. Long-term feeding effects of dietary protein levels on egg production, immunocompetence and plasma amino acids of laying hens in subtropical condition. *J. Anim. Physiol. N.* 94:186–195.
- Rozenboim, I., E. Tako, O. Gal-Garber, J. A. Proudman, and Z. Uni. 2007. The effect of heat stress on ovarian function of laying hens. *Poult. Sci.* 86:1760–1765.
- SPSS for Windows. 2009. Version 16.0. SPSS software. Chicago, IL.
- Summers, J. D. 1993. Reducing nitrogen excretion of the laying hen by feeding lower crude protein diets. *Poult. Sci.* 72:1473–1478.
- Torki, M., A. Mohebbifar, H. A. Ghasemi, and A. Zardast. 2015. Response of laying hens to feeding low-protein amino acid-supplemented diets under high ambient temperature: performance, egg quality, leukocyte profile, blood lipids, and excreta pH. *Int. J. Biometeorol.* 59:575–584.
- Zhang, Y. N., S. Wang, X. B. Huang, K. C. Li, W. Chen, D. Ruan, W. G. Xia, S. L. Wang, K. F. M. Abouelezz, and C. T. Zheng. 2020. Estimation of dietary manganese requirement for laying duck breeders: effects on productive and reproductive performance, egg quality, tibial characteristics, serum biochemical and antioxidant indices. *Poult. Sci.* 99:5752–5762.