Estimation of dietary zinc requirement for laying duck breeders: effects on productive and reproductive performance, egg quality, tibial characteristics, plasma biochemical and antioxidant indices, and zinc deposition

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ABSTRACT This study evaluated the effects of different dietary zinc (Zn) levels on productive and reproductive performance, egg quality, tibial characteristics, plasma biochemical and antioxidant indices, and zinc deposition in laying duck breeders. A total of 504 Longyan duck breeders aged 21 wk were randomly allocated to 6 treatments and fed a basal diet (Zn, 27.7 mg/kg) or that basal diet supplemented with Zn (as $ZnSO_4 \cdot H_2O$) at 10, 20, 40, 80, or 160 mg Zn per kg of feed for 20 wk. Each group had 6 replicates of 14 ducks each. Dietary Zn supplementation affected ($P <$ 0.05) the egg production, FCR, and shell thickness of laying duck breeders from 21 to 40 wk, and there was a quadratic ($P < 0.05$) effect between them. Dietary Zn supplementation affected ($P < 0.05$) and quadratically ($P < 0.001$) increased the breaking strength, density, and dry defatted weight of tibias. Alkaline phosphatase, calcium, phosphorus, total superoxide dismutase, glutathione peroxidase (GSH-Px), and malondialdehyde (MDA) activities or content in plasma were affected ($P < 0.05$), and quadratically ($P < 0.01$) changed by dietary Zn levels. Dietary Zn supplementation affected ($P < 0.01$) and increased the Zn deposition in egg yolk (linear, $P < 0.05$; quadratic, $P < 0.001$) and tibia (linear, $P < 0.05$). The dietary Zn requirements, in mg/kg for a basal diet containing 27.7 mg/kg Zn, for Longyan duck breeders from 21 to 40 wk of age were estimated to be 65.4 for optimizing egg production, 68.6 for FCR, 102 for hatchling BW, 94.7 for eggshell thickness, 77.2 for tibial breaking strength, 81.4 for tibial density, 78.9 for tibial dry defatted weight, 69.5 for plasma GSH-Px activity, 72.4 for plasma MDA content, and 94.6 for Zn content in tibia. Overall, dietary Zn supplementation, up to 160 mg/kg feed, affected the productive performance, eggshell thickness, tibial characteristics, plasma antioxidant status, and Zn deposition of layer duck breeders. Supplementing this basal diet (27.7 mg/kg Zn) with 70 to 80 mg/kg additional Zn was adequate for laying duck breeders during the laying period.

Key words: zinc, productive performance, tibial characteristics, laying duck breeder

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INTRODUCTION

Zinc (**Zn**) is an essential trace element for the growth and development of poultry, contributing to enzyme structure and function, bone development, feathering, antioxidant capacity. It is involved in diverse biological and metabolic processes affecting nutrition and metabolism (McCall et al., 2000; Park et al., 2004; Prasad, 2009). It is widely acknowledged that adequate Zn, from supplementation, is essential to optimize animal health and productivity in corn-soybean based diets for broilers (Liu et al., 2011), meat ducks (Wen et al., 2018), laying hens (Qin et al., 2017), and ducks (Chen et al., 2017). Dietary Zn deficiency affected organ systems of the body (Tuerk and Fazel, 2009), influenced the eating behavior and taste bud morphology of chicks (Gentle et al., 1981), and suppressed bone mineralization and growth (Nagata et al., 2011). Supplementing diets with Zn improved growth performance, intestinal microflora and morphology, digestive enzymes, bone development, and antioxidant status of poultry (Salim et al., 2012; Hu et al., 2013; Tang et al., 2014; Zhao et al., 2014; Yang et al., 2016), and Zn played a positive effect in heat-stressed hens (Sahin et al., 2009). In contrast, long-term or excessive exposure to zinc had adverse or even toxic effects on poultry, depressed growth,

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or productive performance, induced histological lesions of organs, and disrupted body homeostasis (Chen et al., 2018). In addition, the European Food Safety Authority (EFSA, 2014) has advanced a scientific opinion for potentially reducing the currently authorized maximum zinc content in complete feeds; accordingly, there is a need for optimizing the dietary level of Zn for animals. Only limited study and data exist on the effect of Zn in laying duck breeders, and the optimal level of Zn provided in common diets is unknown.

Decreased hatchability, an important factor for breeders, has recently become a major problem. McDaniel et al. (1979) reported the shell quality was a significant factor in declining hatchability as the hen ages, and hatchability was affected by eggshell thickness and mammillary layer thickness (Liao et al., 2013). In addition, previous studies have shown that dietary Zn supplementation of laying hens improved shell thickness and ultrastructure (Qin et al., 2017; Zhang et al., 2017). Moreover, dietary Zn deficiency in chickens decreased the fertility and hatchability (Zhu et al., 2017); however, Stahl et al. (1986) found no improvement from zinc supplementation. Supplementing diets of Japanese quail with Zn-Met improved reproductive performance and hatchability traits, whereas zinc oxide nanoparticles had detrimental effects (Khoobbak et al., 2018). It was speculated here that dietary Zn supplementation might improve the hatchability of laying duck breeders.

The present study has examined the effects of Zn supplementation of laying duck breeders on productive and reproductive performance, along with egg quality, tibial characteristics, plasma biochemical and antioxidant indices, and zinc deposition, as relevant indicators when estimating dietary zinc requirement.

MATERIALS AND METHODS

Experimental Design and Diets

The use of the ducks and the experimental protocol were approved by the Animal Care and Use Committee of the Animal Science Institute of Guangdong Academy of Agriculture Sciences (No. GAASIAS-2016–017). A total of 504 Longyan duck breeders (21 wk) were randomly divided into 6 groups which were fed a basal diet (Table 1, 27.7 mg Zn/kg) or the basal diet supplemented with 10, 20, 40, 80, and 160 mg Zn (added as $ZnSO_4 \cdot H_2O$) per kg feed for 20 wk. Each treatment had 6 replicates of 14 ducks, each housed singly in a cage (42 cm × 30 cm × 50 cm) with a nipple drinker and feeder (Guangzhou Huanan Poultry Equipment, Guangzhou, P.R. China). Fresh drinking water was available ad libitum, and 80 g of pelleted feed per duck was introduced twice daily at 07:00 and 15:00. The basal diet was composed mainly of corn and soybean meal and was formulated to supply adequate levels of all nutrients, except for Zn. The dietary composition and nutrient levels are listed in Table 1. The actual concentrations, by analysis, of total Zn in the 6 treatment diets are shown in Table 2.

Table 1. Dietary composition and nutrient levels of the basal diet.

Ingredients	%	Nutrients	%
Corn	53.2	AME (MJ/kg)	10.75
Soybean meal	29.3	Crude protein (CP)	18.02
Wheat bran	6.2	Calcium	3.65
Limestone	8.3	Methionine	0.45
Salt	0.3	Lysine	0.96
DL-Methionine	0.18	Total phosphorus	0.56
Dicalcium phosphate	1.5	Available phosphorus	0.40
50% choline chloride	0.10	Methionine + cysteine	0.67
Premix[1]	1.0	Zinc (mg/kg)	24.3 (27.7)[2]
Total	100.00		

[1]Provided per kilogram of diet: VA 12,500 IU; VD_3 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_{12} 5 mg; Mn 100 mg; I 0.5 mg; Fe 60 mg; Cu 8 mg; Se 0.2 mg; Co 0.26.

[2]The number in parentheses is analyzed value.

Table 2. The concentrations of Zn in 6 treatment diets (mg/kg).

Dietary Zn supplementation[1]	Calculated	Analyzed
0 (basal diet)	24.3	27.7
10	34.3	35.7
20	44.3	47.1
40	64.3	62.4
80	104.3	106.1
160	184.3	183.4

[1]Added as zinc sulfate monohydrate.

Starting at 38 wk of age, each breeder was artificially inseminated twice weekly with 100 μL of pooled semen to evaluate reproductive performance (fertility, hatchability, and proportion of healthy ducklings). In total, 1,800 eggs (50 eggs from each replicate) were collected over 5 sequential days between 38 and 39 wk from the second day after the first artificial insemination. The eggs were weighed, labeled with number and date, and stored in a dark controlled temperature room (18°C; 75% to 80% relative humidity), and then incubated in the incubator (JXB2000; Dezhou Jingxiang Technology Co, Dezhou, P.R. China) for 28 D. Temperatures and humidity were as follows: 38.4°C and 45% (day 0 to 5); 38.0°C and 50% (day 6 to 10); 37.5°C with 50% (day 11 to 15); 37.1°C and 55% (day 16 to 20); 36.8°C and 60% (day 21 to 25); 36.5°C and 65% (day 26 to 28). The eggs were candled on days 6 and 18 to eliminate infertile eggs and dead embryos. Fertility and hatchability were determined along with hatchling weights of ducklings.

Sample Collection

Five eggs per replicate were collected at 5 wk intervals 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 for sampling. Blood samples were taken from the wing vein into heparinized tubes and centrifuged at 3,000 × g for 10 min at 4°C to harvest plasma. Plasma and a second sample (approx. 3 mL) of whole blood were kept at −20°C until analysis. The sampled ducks were then killed by cervical dislocation. Two tibias of each duck were dissected to measure their characteristics.

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 and Egg Force Reader (Israel Orka Food Technology Ltd., Ramat Hasharon, Israel). 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 (Orka).

Measurement of Tibial Characteristics

Two pairs of tibias were collected from each replicate for analyses. Both left and right tibias were cleaned of all adherent tissues, and then length was measured with a caliper with a minimum scale of 0.01 mm. Bone breaking strength of the left tibias was determined with a materials tester (Instron 4411, Instron Corporation, Grove City, PA) using software version 8.09, a standard 50-kg load cell, and a modified shear plate (8 cm in length and 1 mm in width), as described by Wang et al. (2014). The bone mineral density of the right tibia was measured at the Guangzhou Overseas Chinese Hospital with an Xray osteodensitometer (Lunar Prodigy, General Electric Company, Fairfield, CT). All right and left tibias were immersed in alcohol for 48 h, then diethyl ether for 48 h, then dried at 105°C for 1 h and weighted to obtain the dry defatted weight. Tibias were then ashed for 24 h, and content of Zn in bone ash was measured.

Zn Content of Egg Yolk, Tibia, and Blood

At the end of the trial, 5 eggs with weights similar to the average for each replicate were collected. The egg white was separated from the yolk, and each yolk was stored at −80°C for 48 h, then freeze-dried for 72 h using a freeze-drying equipment (FD-12, Beijing Huichengjia Scientific Instrument Factory Co., Ltd., Beijing, P.R. China). Dried yolk samples were weighed and ground carefully to pass a 40-mesh sieve and mixed. The Zn content in yolk, tibia, and whole blood was measured by inductively coupled plasma/mass spectrometry (Agilent 7700 series ICP/MS; Agilent Technologies Inc., Alpharetta, GA).

Plasma Biochemical and Antioxidant Indices Assay

Total superoxide dismutase (**T-SOD**) activity, total antioxidant capacity (**T-AOC**), glutathione peroxidase (**GSH-Px**), and malondialdehyde (**MDA**) content in plasma were analyzed using commercially available kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China), following the manufacturer's instructions. The contents in plasma of total protein, albumin, creatinine, alanine aminotransferase, aspartate aminotransferase, total bilirubin, urea, glucose, total cholesterol, triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, alkaline phosphatase (**ALP**), calcium (**Ca**), and phosphorus (**P**) were determined with kits in an automatic biochemistry analyzer (all from Shanghai Kehua Bio-Engineering Co., Ltd., Shanghai, China).

Statistical Analysis

Replicate served as the experimental unit. The normality of the data and homogeneity of variances were first verified. The effects of dietary Zn supplementation were analyzed by 1-way ANOVA procedure, and then regression analysis was employed to test the linear (L) and quadratic (Q) effects using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL). Quadratic regressions ($Y = aX^2 + bX + c$) were fitted to the responses of the dependent variables to Zn supplementation. The dietary concentration of Zn at which the response first reached 95% of the maximum was used to estimate the requirement (Ruan et al., 2017). Data were expressed as mean and pooled SEM.

RESULTS

Productive and Reproductive Performance

The effects of dietary Zn supplementation on productive and reproductive performance of laying duck breeders are shown in Table 3. Dietary Zn supplementation affected ($P < 0.05$) the egg production and FCR of laying duck breeders from 21 to 40 wk, and it quadratically ($P < 0.05$) increased egg production and decreased FCR. Dietary Zn addition did not affect average egg weight, fertility, hatchability, and hatchling BW; however, hatchling BW was quadratically increased ($P < 0.05$) with dietary supplemental Zn.

Egg Quality

Table 4 shows the effects of dietary Zn addition on egg quality in laying duck breeders during the trial period. Dietary supplementation with Zn affected ($P < 0.01$) the shell thickness after feeding for 5 and 15 wk and average thickness. The shell thickness was quadratically increased ($P < 0.05$) with increasing dietary Zn after feeding for 5, 10, and 15 wk, and the

Table 3. Effects of dietary zinc (Zn) supplementation on the productive (21 to 40 wk) and reproductive performance (36 to 37 wk) of duck breeders in the laying period.

	Zn supplemental level (mg/kg)[1]							*P*-value		
Variables	0	10	20	40	80	160	SEM	ANOVA	Linear	Quadratic
Productive performance										
Egg production (%)	81.2	85.7	88.8	90.0	84.9	82.6	0.83	0.006	0.323	0.046
Average egg weight (g)	64.4	64.9	65.2	64.9	65.0	64.4	0.22	0.882	0.650	0.595
FCR (g:g)	3.06	2.88	2.76	2.74	2.89	3.00	0.029	0.007	0.395	0.046
Reproductive performance										
Fertility (%)	88.3	88.9	90.0	83.3	87.2	88.7	1.31	0.781	0.977	0.693
Hatchability (%)	89.1	85.1	90.0	88.5	85.1	87.4	0.93	0.551	0.624	0.741
Hatchling BW (g)	38.4	38.7	39.7	39.9	40.2	39.1	0.26	0.317	0.491	0.049

[1]Mean of 6 replicates (14 ducks per replicate) per treatment.
FCR, feed conversion ratio; BW, body weight.

Table 4. Effects of dietary zinc (Zn) supplementation on the egg quality of duck breeders in the laying period (21 to 40 wk).

		Zn supplemental levels (mg/kg) [1]							*P*-value		
Variables	Time	0	10	20	40	80	160	SEM	ANOVA	Linear	Quadratic
Eggshell thickness (mm)	5 wk	0.385	0.397	0.408	0.401	0.402	0.403	0.0016	<0.001	0.064	0.018
	10 wk	0.397	0.399	0.408	0.409	0.411	0.407	0.0019	0.166	0.137	0.037
	15 wk	0.387	0.399	0.406	0.402	0.402	0.401	0.0016	0.004	0.191	0.049
	20 wk	0.384	0.393	0.394	0.392	0.396	0.395	0.0014	0.095	0.084	0.074
	Mean	0.388	0.397	0.404	0.401	0.403	0.402	0.0010	<0.001	0.011	<0.001
Eggshell breaking strength (N)	5 wk	45.3	48.7	48.8	47.0	44.6	48.9	0.55	0.057	0.617	0.332
	10 wk	43.8	42.8	44.2	43.4	41.7	41.8	0.40	0.353	0.063	0.163
	15 wk	43.1	43.6	42.3	43.4	41.6	42.8	0.36	0.612	0.514	0.492
	20 wk	37.9	42.0	38.7	38.6	39.7	38.1	0.42	0.047	0.354	0.568
	Mean	42.5	44.3	43.5	43.1	41.9	42.9	0.27	0.169	0.318	0.367
Eggshell ratio (%)	5 wk	10.0	10.2	10.5	10.1	10.3	10.2	0.055	0.250	0.674	0.755
	10 wk	9.66	9.47	9.74	9.92	10.0	9.63	0.061	0.102	0.650	0.028
	15 wk	9.58	9.78	9.71	9.46	9.42	9.59	0.055	0.411	0.438	0.271
	20 wk	9.11	9.54	9.31	9.32	9.41	9.32	0.057	0.417	0.793	0.693
	Mean	9.59	9.76	9.81	9.70	9.78	9.70	0.035	0.556	0.871	0.526
Albumen height (mm)	5 wk	6.92	6.59	6.47	6.33	6.61	6.84	0.086	0.361	0.559	0.217
	10 wk	6.27	6.20	6.27	6.33	6.19	6.51	0.088	0.928	0.374	0.594
	15 wk	6.37	6.54	6.56	6.49	6.15	6.62	0.088	0.700	0.824	0.553
	20 wk	6.21	5.79	6.01	6.11	6.05	5.98	0.086	0.839	0.895	0.980
	Mean	6.44	6.28	6.33	6.32	6.25	6.49	0.043	0.595	0.432	0.222
Yolk color	5 wk	4.83	5.00	4.78	4.94	4.78	5.17	0.061	0.419	0.169	0.209
	10 wk	4.83	5.17	5.33	5.33	5.33	5.50	0.092	0.414	0.095	0.175
	15 wk	4.40	4.46	4.38	4.50	4.19	4.38	0.055	0.718	0.533	0.629
	20 wk	4.47	4.30	4.18	4.53	4.42	4.53	0.052	0.297	0.222	0.478
	Mean	4.63	4.73	4.67	4.83	4.68	4.89	0.037	0.271	0.066	0.182
Haugh unit	5 wk	83.0	80.5	79.9	78.5	80.6	79.9	0.70	0.596	0.529	0.538
	10 wk	76.6	76.3	76.8	77.3	75.8	76.5	0.64	0.993	0.860	0.975
	15 wk	77.2	77.9	79.8	78.7	77.0	79.2	0.53	0.623	0.645	0.849
	20 wk	76.9	73.9	75.9	75.9	74.8	74.9	0.58	0.769	0.589	0.834
	Mean	78.5	77.2	78.1	77.6	77.1	77.6	0.305	0.791	0.612	0.599

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

average thickness of the total trial period. Egg quality, including shell breaking strength, shell ratio, albumen height, yolk color, and Haugh unit were not affected by dietary Zn inclusion.

Tibial Characteristics

The effects of dietary Zn level on the tibial characteristics of duck breeders in the laying period are shown in Table 5. The breaking strength, density, and dry defatted weight of tibias were influenced ($P < 0.01$) by dietary Zn supplemental levels, and these variables were quadratically increased ($P < 0.001$) with increasing Zn. However, tibial length was not influenced by dietary Zn supplementation.

Plasma Biochemical and Antioxidant Indices

The effects of dietary Zn level on the plasma biochemical and antioxidant indices of duck breeders are shown in Table 6. The ALP activity, and Ca and P content in plasma were affected ($P < 0.05$) by dietary Zn supplementation. The ALP activity and P content were both linearly ($P < 0.01$) and quadratically

Table 5. Effects of dietary zinc (Zn) supplementation on the tibial characteristics of duck breeders in the laying period (21 to 40 wk).

	Zn supplemental levels (mg/kg) [1]							*P*-value		
Variables	0	10	20	40	80	160	SEM	ANOVA	Linear	Quadratic
Breaking strength (N)	127	141	146	158	150	136	3.0	<0.001	0.911	<0.001
Density (g/cm^2)	0.243	0.289	0.298	0.323	0.322	0.276	0.0068	<0.001	0.562	<0.001
Dry defatted weight (g)	2.47	2.73	2.86	2.94	2.98	2.65	0.069	0.003	0.707	<0.001
Length (mm)	91.9	92.3	93.2	95.0	93.5	94.0	0.395	0.249	0.174	0.166

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

Table 6. Effects of dietary zinc (Zn) supplementation on the plasma biochemical and antioxidant indices of duck breeders (40 wk of age) in the laying period.

	Zn supplemental level (mg/kg)[1]							*P*-value		
Variables	0	10	20	40	80	160	SEM	ANOVA	Linear	Quadratic
TP (g/L)	63.1	60.0	60.3	63.1	62.7	56.6	1.11	0.517	0.176	0.226
ALB (g/L)	20.0	17.7	18.7	19.5	20.0	16.9	0.42	0.132	0.156	0.100
UA (μmol/L)	307	278	336	311	273	325	13.1	0.724	0.781	0.773
CRE (μmol/L)	3.77	4.03	4.63	3.81	3.70	3.06	0.271	0.740	0.194	0.410
AST (U/L)	117	144	152	132	110	130	5.9	0.230	0.283	0.526
ALT (U/L)	31.8	33.8	37.5	34.4	30.6	36.1	1.15	0.547	0.729	0.772
TB (μmol/L)	16.7	16.2	15.1	15.6	11.2	11.7	0.74	0.114	0.009	0.019
GLU (mmol/L)	11.4	11.2	10.0	10.5	10.3	9.79	0.183	0.058	0.018	0.045
TG (mmol/L)	11.8	10.5	11.9	13.0	10.6	11.2	0.68	0.921	0.798	0.966
TC (mmol/L)	3.46	3.51	3.33	3.01	3.28	3.29	0.122	0.905	0.684	0.701
HDL-C (mmol/L)	2.02	2.22	2.13	1.98	1.82	2.14	0.054	0.327	0.819	0.203
LDL-C (mmol/L)	0.72	0.75	0.79	0.67	0.70	0.68	0.025	0.748	0.311	0.573
ALP (U/L)	133	166	174	174	149	274	13.9	0.049	0.005	0.009
Ca (mmol/mL)	6.91	6.65	6.16	6.63	3.43	2.82	0.307	<0.001	<0.001	<0.001
P (mmol/mL)	3.20	3.56	2.63	3.10	5.88	5.90	0.266	<0.001	<0.001	<0.001
Antioxidant indices										
T-AOC (U/mL)	8.39	9.85	11.1	11.3	10.18	9.58	0.339	0.104	0.828	0.459
T-SOD (U/mL)	197	196	246	244	200	209	2.5	<0.001	0.142	<0.001
GSH-Px (U/mL)	95.6	104	120	130	116	92.3	2.6	<0.001	0.046	<0.001
MDA (nmol/mL)	10.1	8.80	7.85	6.23	7.97	8.82	0.265	<0.001	0.778	0.002

TP, total protein; ALB, albumin; UA, uric acid; CRE, creatinine; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TB, total bilirubin; GLU, glucose; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ALP, alkaline phosphatase; Ca, calcium; P, phosphorus; T-AOC, total antioxidant capacity; T-SOD, total superoxide dismutase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde.

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

Table 7. Effects of dietary zinc (Zn) supplementation on the Zn deposition in egg yolk, tibia, and blood of duck breeders (40 wk).

	Zn supplemental levels (mg/kg)[1]							*P*-value		
Variables	0	10	20	40	80	160	SEM	ANOVA	Linear	Quadratic
Zn content in yolk (mg/kg)	31.7	33.6	33.0	33.3	35.9	34.7	0.34	0.005	0.016	0.065
Zn content in tibia (mg/kg)	316	314	353	366	379	361	6.2	0.002	0.012	<0.001
Zn content in blood (mg/kg)	6.26	6.76	6.76	7.04	6.59	6.60	0.09	0.199	0.991	0.405

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

($P < 0.01$) increased, but Ca content was linearly ($P < 0.001$) and quadratically ($P < 0.001$) decreased by dietary Zn levels. Other biochemical indices in plasma were not influenced by dietary Zn supplementation.

Dietary Zn supplementation did not affect the T-AOC content in plasma but did influence ($P < 0.05$) T-SOD and GSH-Px activities and MDA content in plasma; T-SOD activity was quadratically ($P < 0.001$) increased by dietary Zn levels, and there were linear ($P < 0.05$) and quadratic ($P < 0.001$) effects of Zn supplementation on GSH-Px activity. In addition, MDA content in plasma was quadratically ($P < 0.01$) decreased in response to dietary Zn supplementation levels.

Zn Deposition in Egg Yolk, Tibia, and Blood

As shown in Table 7, dietary Zn supplementation affected ($P < 0.01$) the Zn deposition in egg yolk and tibia; there was a linear effect ($P < 0.05$) of supplemental Zn on egg yolk content of Zn. The tibial Zn content was increased linearly ($P < 0.05$) and quadratically

Table 8. Estimations of the dietary zinc (Zn) requirements based on quadratic regressions of egg production, FCR, hatchling BW, eggshell thickness, tibial breaking strength, density, dry defatted weight, and Zn content in tibial on dietary Zn supplemental levels.

Items	Regression equation[1]	R^2	*P*-value	Zn requirement (mg/kg)[2]
Egg production (%)	$Y = -0.0008X^2+0.1101X+84.26$	0.422	0.046	65.4
FCR (g:g)	$Y = 3.116 \times 10^{-5}X^2-0.0045X+2.944$	0.471	0.046	68.6
Hatchling BW (g)	$Y = -0.0002X^2+0.0431X+38.52$	0.891	0.049	102
Eggshell thickness (mm)	$Y = -1.321 \times 10^{-6}X^2+2.634 \times 10^{-4}X+0.393$	0.587	<0.001	94.7
Tibial breaking strength (N)	$Y = -0.0036X^2+0.585X+133.2$	0.744	<0.001	77.2
Tibial density (g/cm^2)	$Y = -1.051 \times 10^{-5}X^2+0.0018X+0.260$	0.841	<0.001	81.4
Tibial dry defatted weight (g)	$Y = -6.862 \times 10^{-5}X^2+0.0114X+2.574$	0.864	<0.001	78.9
Plasma GSH-Px activity (U/mL)	$Y = -0.0046X^2+0.6729X+101.1$	0.750	<0.001	69.5
Plasma MDA content (nmol/mL)	$Y = 0.0004X^2-0.061X+9.3545$	0.557	0.002	72.4
Zn content in tibia (mg/kg)	$Y = -0.0074X^2+1.4735X+314.2$	0.888	<0.001	94.6

[1]Y is the dependent variable and X are the dietary Zn supplemental levels (mg/kg).
[2]Dietary Zn requirement = X giving 95% of the maximal response (mg/kg).

($P < 0.001$) with Zn level of supplementation. The Zn content in blood was not influenced by dietary Zn supplementation.

Estimations of the Dietary Zn Requirements

The results of dietary Zn requirements of laying duck breeders as estimated by the quadratic regression analysis are shown in Table 8. The dietary Zn requirements, in mg/kg for a basal diet containing 27.7 mg/kg Zn, for Longyan duck breeders from 21 to 40 wk of age were estimated to be 65.4 for optimizing egg production, 68.6 for FCR, 102 for hatchling BW, 94.7 for eggshell thickness, 77.2 for tibial breaking strength, 81.4 for tibial density, 78.9 for tibial dry defatted weight, 69.5 for plasma GSH-Px activity, 72.4 for plasma MDA content, and 94.6 for Zn content in tibia.

DISCUSSION

The current study with laying duck breeders demonstrated that dietary Zn addition quadratically increased egg production and decreased the FCR similar to the findings of Chen et al. (2017) where egg production and daily egg mass increased quadratically with increasing supplemental levels of Zn (15 to 90 mg/kg) during the early and peak laying period of ducks. However, several studies failed to demonstrate any effects of dietary Zn supplementation on laying performance of aged hens (Zhang et al., 2017; Min et al., 2018) and broiler breeders (Stahl et al., 1986; Kidd et al., 1992; Liao et al., 2018). These studies indicated that supplemental Zn might have a positive effect on performance when it was provided as early as possible to young poultry rather than to mature animals. Moreover, when the layers were fed 77.21 mg/kg Zn from 18 to 19 wk, dietary Zn supplementation (30 to 120 mg/kg) did not affect the laying performance of hens during week 20 to 40 (Qin et al., 2017). In this respect, early manipulation of Zn status, by either dietary deficiency or supplementation, may affect the performance of poultry during the laying period. The present study has found that ducks fed the diet supplemented with around 70 mg/kg Zn (Table 8, total Zn 92.4 mg/kg) could attain the best performance, which was consistent with the enhanced antioxidant status and bone growth of the ducks. In rats, Zn deficiency reduced production or secretion of growth hormone and impaired anabolic effects of growth hormone (Root et al., 1979). For the duck breeders used here, the positive effect of Zn on antioxidant capacity and possibly growth hormone might underlie the response in laying performance.

There was no effect of added dietary Zn on fertility and hatchability of laying duck breeders studied here. Similarly, Stahl et al. (1986) reported no effects on fertility, and hatchability of chicken layers was observed when their basal diet (28 mg/kg Zn) was supplemented with Zn (10 to 40 mg/kg, as $ZnCO_3$). However, the current study showed that hatchling BW was quadratically increased with dietary Zn supplementation, which possibly resulted from the positive effect of Zn in egg yolk. As reported by Zhu et al. (2017), maternal dietary supplementation with Zn increased Zn content in yolk and progeny BW. Dietary Zn methionine or Zn oxide supplementation (80 mg/kg) of a basal diet containing 72 mg/kg of Zn did not affect the reproductive performance of mature broiler breeders (Kidd et al., 1992). In Japanese quail, however, Zn methionine improved fertility, hatchability, and hatchling weight, whereas zinc oxide had no effect, and zinc oxide nanoparticles reduced hatchability (Khoobbakht et al., 2018). In normal and heat-stressed broiler layers, Zhu et al. (2017) found nonsignificant improvement in hatchability by inorganic Zn at 110 mg/kg but increased by organic Zn. These studies indicate that the different results of Zn supplementation on reproductive performance might arise from differences in Zn forms, the extent of Zn deficiency of the basal diet, or the stress status of birds. Supplementation of Zn-deficient basal diets with Zn methionine might have a positive effect on reproductive performance but needs further study.

Consistent with several earlier studies with hens, dietary supplementation of breeder ducks here with Zn increased eggshell thickness. Supplementation of laying hens, from 20 to 40 wk of age, with Zn (0 to 120 mg/kg) linearly increased shell thickness (Qin et al., 2017).

Zhang et al. (2017) found dietary Zn supplementation, up to 140 mg/kg feed, linearly and quadratically increased eggshell thickness. Egg quality including shell thickness was not affected by Zn supplementation (15 to 90 mg/kg) of a basal diet with 37.4 mg/kg Zn in laying ducks (Chen et al., 2017). The higher Zn content of the basal diet (37.4 vs. 27.7 mg/kg) and lower supplemental levels (max, 90 vs. 160 mg/kg) may account for the different results to those obtained here. Likewise, Chen et al. (2017) found no effect of Zn supplementation on tibial characteristics contrasting with the significant effects of supplemental Zn on breaking strength, density, and weight of tibias found here (Table 4). Recent studies with aged layer chickens implicate an effect on the Zn-containing enzyme carbonic anhydrase in the shell gland to increase Ca deposition into forming eggshell (Zhang et al., 2017; Min et al., 2018). The present study with breeder ducks, along with the above chicken studies, indicates that dietary supplementation with Zn can increase eggshell thickness.

Zinc deficiency results in reduced rates of bone formation that can be corrected by supplementation with adequate amounts of zinc. Abnormal bone development was one of the primary symptoms associated with zinc deficiency in birds (Vohra and Kratzer, 1968). Dietary Zn levels had a direct significant effect on bone strength in broiler chickens (Klenholz et al., 1964). In the present study with duck breeders, dietary Zn supplementation quadratically increased the breaking strength, density, and dry defatted weight of the tibia, indicating the dose-response effect of Zn on bone formation. Scrimgeour et al. (2007) have described the influence of dietary Zn on bone integrity, density, and mechanical properties in rats. In chicken embryos, the effect of Zn on bone formation resulted from a zinc-induced increase in bone cell proliferation (Chen et al., 1999), and Zn deficiency directly inhibited the effect of growth hormone on growth of long bones in hypophyesectomized rats (Cha and Rojhanl, 1997). It can be speculated that increased tibial strength, density, and Zn content in Zn-supplemented duck breeders might result from similar mechanisms; diets supplemented with around 80 mg/kg Zn achieved the best tibial characteristics (Table 8).

Alkaline phosphatase is an important Zn-containing enzyme, and its activity in serum showed a significant quadratic response to dietary supplemental Zn levels (30 to 120 mg/kg) in laying hens (Qin et al., 2017). The current study with duck breeders also showed ALP activity in plasma to be linearly and quadratically increased with Zn supplementation, especially apparent in birds supplemented with 160 mg/kg. Plasma Ca concentration was linearly and quadratically decreased, and that of P increased in ducks with the addition of Zn in diets, especially with 80 and 160 mg/kg Zn (Table 6), indicating that high dietary Zn affected Ca and P metabolism. Intake of high levels of minerals can interact with other minerals (Sirirat et al., 2012), possibly by competition for binders. Added dietary calcium may cause or accentuate poor utilization of zinc from soy products (Forbes et al., 1979), and excessive calcium decreased zinc absorption because of competition (Ao and Pierce, 2013). Plasma Ca and P are tightly regulated, and their metabolism is closely related in broilers (Proszkowiec-Weglarz and Angel, 2013). In the present study, dietary supplementation with Zn could affect the Ca and P metabolism in duck breeders, with obvious effects at supplemental levels of 80 and 160 mg/kg.

Dietary Zn supplementation is known to have a positive effect on the antioxidant status of animals, which functions through the protection of sulfhydryl groups against oxidation and the inhibition of the production of reactive oxygen species by transition metals (Bray and Bettger, 1990). The antioxidant capacity in plasma or liver was improved with Zn addition to diets of broilers (Hu et al., 2013), broiler breeders (Liao et al., 2018), laying hens (Qin et al., 2017), and ducks (Chen et al., 2017). As expected, dietary Zn supplementation of laying duck breeders in the present study quadratically increased the T-SOD and GSH-Px activities, and decreased MDA content in plasma; around 70 mg/kg of additional Zn was optimal in improving antioxidant status (Table 8).

Dietary Zn supplementation levels of duck breeders here linearly increased Zn deposition in egg yolk and tibias. Similarly, tibial zinc content of broilers increased linearly with dietary concentration of Zn (Mohanna and Nys, 1999; Star et al., 2012). Broiler breeders fed a diet with organic Zn (110 mg/kg) increased Zn content in the liver (Liao et al., 2018), and dietary Zn supplementation increased Zn deposition in tibiotarsus, liver, and eggs of laying hens (Abedini et al., 2018). Increased Zn accumulation in hens was a result of increased abundance of zinc transporter gene transcripts in tissues (Li et al., 2015), which probably also mediated the Zn deposition responses shown here with duck breeders.

For variables of egg production, FCR, eggshell thickness, hatchling BW, tibial characteristics, plasma antioxidant status measured here, we may conclude that using a basal diet with 27.7 mg/kg Zn, an additional 70 to 80 mg/kg Zn was adequate for laying duck breeders during the laying period. It is a little higher than the recommended levels of chicken layer breeders (60 mg/kg, Ministry of Agriculture of China, NY/T 33–2004. Wen et al., 2004), which possibly a result of increased body weight and egg weight compared with the chicken breeders. In addition, 60 mg/kg of Zn was recommended for meat duck breeders (Ministry of Agriculture of China, NY/T 2122–2012. Hou et al., 2012). The increased egg production of laying duck breeders compared to the meat duck breeders may account for the higher requirement of Zn. As there is no feeding standard of laying duck breeders, the present results provide a scientific basis for application to the duck industry.

In conclusion, dietary Zn supplementation of laying duck breeders with up to 160 mg/kg feed increased productive performance, eggshell thickness, tibial characteristics, plasma antioxidant status, and Zn deposition.

For most variables examined here using a basal diet with 27.7 mg/kg Zn, an additional 70 to 80 mg/kg Zn was adequate for laying duck breeders during the laying period.

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