

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

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ABSTRACT This study was aimed at estimating the dietary manganese (Mn) requirement for laying duck breeders. A total of 504 Longyan duck breeders (body weight: 1.20 ± 0.02 kg) aged 17 wk were randomly allocated to 6 treatments. The birds were fed with a basal diet (Mn, 17.5 mg/kg) or diets supplemented with 20, 40, 80, 120, or 160 mg/kg of Mn (as $\text{MnSO}_4 \cdot \text{H}_2\text{O}$) for 18 wk. Each treatment had 6 replicates of 14 ducks each. As a result of this study, dietary Mn supplementation did not affect the productive performance of laying duck breeders in the early laying period (17–18 wk), but affected egg production, egg mass, and feed conversion ratio (FCR) from 19 to 34 wk ($P < 0.05$), and there was a linear and quadratic effect of supplement level ($P < 0.05$). The proportion of preovulatory ovarian follicles increased ($P < 0.01$) linearly and quadratically, and atretic follicles (weight and percentage) decreased ($P < 0.05$) quadratically with dietary Mn supplementation. The density and breaking strength of tibias increased (quadratic; $P < 0.05$), the calcium content of tibias decreased (linear, quadratic; $P < 0.01$), and Mn content increased (linear, quadratic; $P < 0.001$) with increase in Mn. The addition of Mn had a quadratic

effect on serum contents of estradiol, prolactin, progesterone, luteinizing hormone, and follicle-stimulating hormone ($P < 0.001$). Dietary Mn supplementation decreased serum contents of total protein (linear, $P < 0.05$), glucose (quadratic, $P < 0.05$), total bilirubin, triglycerides, total cholesterol, low-density lipoprotein cholesterol, and calcium (linear, quadratic; $P < 0.05$). The serum total antioxidant capacity and total and Mn-containing superoxide dismutase activities increased (linear, quadratic; $P < 0.001$), and malondialdehyde content decreased (linear, quadratic; $P < 0.001$) in response to Mn supplemental levels. The dietary Mn requirements, in milligram per kilogram for a basal diet containing 17.5 mg/kg of Mn, for Longyan duck breeders from 19 to 34 wk of age were estimated to be 84.2 for optimizing egg production, 85.8 for egg mass, and 95.0 for FCR. Overall, dietary Mn supplementation, up to 160 mg/kg of feed, affected productive performance, tibial characteristics, and serum biochemical and antioxidant status of layer duck breeders. Supplementing this basal diet (17.5 mg/kg of Mn) with 85 to 95 mg/kg of additional Mn was adequate for laying duck breeders during the laying period.

Key words: laying duck breeder, manganese, productive performance, serum biochemical and antioxidant index, tibial characteristic

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INTRODUCTION

Manganese (Mn) is an essential trace element required for a multitude of enzymatic reactions and normal biological activities such as the regulation of reproduction and carbohydrate metabolism; the formation of connective tissues, bone marrow, and lipids; and the maintenance of neurological tissues (Park and Park, 2010). It is generally accepted that maize–soybean meal–based diets for

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poultry need to be supplemented with Mn because of relatively low availability of Mn (Ji et al., 2006; Liao et al., 2019). Previous studies have reported that dietary Mn deficiency led to perosis in chicks (Liu et al., 2015; Wang et al., 2015), suppressed the growth of broilers (Li et al., 2011a; Lu et al., 2016), and decreased egg production and shell quality of hens (Cui et al., 2019a). In addition, supplementing diets with Mn at a concentration of 10 to 40 mg/kg increased shell thickness, weight, and hatchability of fertile eggs, decreased dead embryos and abnormal chicks (Attia et al., 2010); Mn supplementation at a concentration of 50 to 100 mg/kg decreased leg abnormalities in broilers (Ghosh et al., 2016); Mn addition at a concentration of 120 mg/kg could alleviate the negative effect of high temperature on egg production, egg quality, and antioxidant status performance of broiler breeders (Zhu et al., 2015a,b; Zhu et al., 2016); Mn addition at a concentration of 20 to 160 mg/kg in corn-soybean-based diets increased egg production (Cui et al., 2019a), shell quality (Xiao et al., 2014, 2015; Zhang et al., 2017a,b), and internal egg quality (Li et al., 2018) in laying hens; Mn addition at a concentration of 40 to 100 mg/kg could enhance intestinal barrier and splenic inflammatory response to fight against *Salmonella* infection in broilers (Zhang et al., 2020a); and dietary Mn at a high concentration of 400 mg/kg could improve the immune responses of broilers after oral *Salmonella typhimurium* inoculation (Pan et al., 2018). Conversely, excessive dietary Mn had adverse or even toxic effects on poultry, and Mn supplementation at a concentration of ≥ 400 mg/kg causes local inflammation of kidney tissues in laying hens (Cui et al., 2019a). In addition, the European Food Safety Authority (EFSA, 2016) has reported that no more than a concentration of 150 mg/kg of Mn in complete feed is safe in poultry diets. Thus, there is a need for optimizing the dietary level of Mn for animals.

The Mn requirement in the diet varies with species and age. It was reported that Mn requirement for laying hens appeared to be at least 113 mg/kg (33 mg from basal diet + 80 mg of supplement) (Zhang et al., 2017a), and 60 mg/kg of Mn hydroxychloride was adequate for 45-wk-old White Leghorn layers, using yolk and shell Mn content as variables (Jasek et al., 2020). Around 130 mg of Mn per kilogram of diet is required for broilers fed with a conventional basal maize-soybean meal diet from hatching to 21 D of age (Li et al., 2011a), and 100 mg of Mn per kilogram of diet is required for broilers from 22 to 42 D of age (Lu et al., 2016). Adding 90 mg of Mn per kilogram to a basal diet (19.1 mg/kg) was required to improve the activities of Mn-containing superoxide dismutase (Mn-SOD) and total superoxide dismutase (T-SOD) in laying ducks (Fouad et al., 2016). The effect of Mn and the optimal level of Mn provided in common diets of laying duck breeders remains unknown.

The present study, therefore, has examined the effects of Mn supplementation on productive and reproductive performance, egg quality, reproductive organ and ovarian follicle development, tibial characteristics, and serum biochemical and antioxidant indices in laying

duck breeders and has used productive performance as relevant indicators to estimate dietary Mn requirement.

MATERIALS AND METHODS

Experimental Design and Diets

The use of 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 (17 wk of age, body weight: 1.20 ± 0.02 kg) were randomly divided into 6 groups. The birds were fed with a basal diet (17.5 mg of Mn per kilogram) or diets supplemented with 20, 40, 80, 120, and 160 mg/kg of Mn (added as $\text{MnSO}_4 \cdot \text{H}_2\text{O}$) for 18 wk. This duration consisted of the early (17–18 wk) and peak (19–34 wk) laying periods. Each treatment had 6 replicates of 14 ducks, housed singly in cages (42 cm \times 30 cm \times 50 cm) with a nipple drinker and feeder (Guangzhou Huanan Poultry Equipment, Guangzhou, PRC). Fresh drinking water was available *ad libitum*, and 80 g of pelleted feed per duck was provided 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 Mn. The dietary composition and analyzed nutrient levels (except for apparent metabolism energy and available phosphorus [P]) are listed in Table 1. The crude protein in the diet was analyzed using a Kjeltec 8400 Analyzer Unit (FOSS Analytical AB, Hoganas, Sweden). The amino acids in diet were analyzed after hydrolysis using an amino acid analyzer (HITACHI L-8900; Hitachi, Ltd., Tokyo, Japan).

The actual concentrations, by analysis, of total Mn in the 6 treatment diets are shown in Table 2.

Starting at 28 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 28 and 29 wk, starting on the second day after the first artificial insemination. The eggs were weighed, labeled with a number and date, stored in a dark temperature-controlled room (18°C; 75–80% relative humidity), and then incubated (JXB2000; Dezhou Jingxiang Technology Co., Dezhou, PRC) for 28 D. Temperatures and humidity were as follows: 38.4°C and 45% (day 0–5); 38.0°C and 50% (day 6–10); 37.5°C with 50% (day 11–15); 37.1°C and 55% (day 16–20); 36.8°C and 60% (day 21–25); and then 36.5°C and 65% (day 26–28) (Ruan et al., 2018). The eggs were candled on day 6 and 18 to eliminate infertile eggs and dead embryos. After 28 D, the healthy hatched ducklings were counted and recorded, and eggs that failed to hatch were counted. Fertility was calculated as fertile eggs as a proportion of set eggs. Hatchability was calculated on the basis of fertile eggs. Healthy ducklings (clean and dry, free of deformities, and with bright eyes) were determined macroscopically (Xia et al., 2019). The hatchling body weight was measured on a replicate basis.

Table 1. Composition and nutrient levels of the basal diet.

Ingredients	%	Nutrients	%
Corn (CP, 7.8%)	53.4	Apparent metabolism energy (AME, MJ/kg)	10.75
Soybean meal (CP, 43.6%)	29.3	Crude protein (CP)	17.90 ²
Wheat bran (CP, 15.7%)	6.0	Calcium	3.64 ²
Limestone	8.3	Methionine	0.45 ²
Salt	0.3	Lysine	0.90 ²
DL-Methionine	0.2	Total phosphorus	0.66 ²
Dicalcium phosphate	1.5	Available phosphorus	0.40
Vitamin–mineral premix ¹	1.0	Methionine + cysteine	0.65 ²
Total	100	Manganese (mg/kg)	17.5 ²

¹Provided per kilogram of diet: vitamin A, 12 500 IU; vitamin D₃, 4 125 IU; vitamin E, 15 IU; vitamin K, 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; vitamin B₁₂, 5 mg; Zn, 90 mg; I, 0.5 mg; Fe, 60 mg; Cu, 8 mg; Se, 0.2 mg; Co, 0.26 mg; choline chloride, 500 mg.

²Analyzed values.

Sample Collection

Five eggs per replicate were collected at 4-wk intervals during the treatment period for determining egg quality; measurements were performed 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. Between 4:00 and 5:00 pm, approximately 3 mL of blood was collected from a wing vein of each duck using non-coated evacuated tubes. The tubes were then incubated in a 37°C water bath, tilting at a 45° angle for 3 h and then centrifuged at 3,000 × *g* for 10 min to harvest serum. The serum samples were stored at –20°C for subsequent analysis (Cui et al., 2019b). The sampled ducks were then killed by cervical dislocation. Two tibias of each duck were dissected to measure their characteristics.

In addition, the weight and length of the oviduct were measured, and the ovary was collected and weighed. The preovulatory follicles (POF, > 10 mm in diameter), including the largest POF, the second largest POF, the third largest POF, small yellow follicles (6–10 mm in diameter), and large white follicles (2–5 mm in diameter), were dissected, counted, weighed, and recorded. The atretic follicles were weighed. The weight proportions of POF, small yellow follicles, large white follicles, and atretic follicles were calculated as percentages of ovarian weight (Cui et al., 2019b).

Productive Performance and Egg Quality Measurement

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

period: early period (17–18 wk of age, average daily egg production by all ducks ranged from 50–80%) and peak laying period (19–36 wk of age, average daily egg production by all ducks >80%).

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

Measurement of Tibial Characteristics

Two pairs of tibias were collected from each replicate for analysis. Both left and right tibias were cleaned of all adherent tissues and weighed as fresh weight, and then, length was measured using a caliper, and midpoint circumference was measured using a flexible rule, both with a minimum scale of 0.01 mm. Bone breaking strength of the left tibias was determined at mid-diaphysis via a 3-point bending test using a testing machine (TMS-Pro; Food Technology Ltd., Sterling, VA) equipped with an interchangeable load cell (model ILC-S; range of forces from 0–1,000 N), as described by Cui et al. (2019c). Bone mineral density and content of the right tibia were measured at the Guangzhou Overseas Chinese Hospital using an X-ray osteodensitometer (Lunar Prodigy; General Electric Company, Fairfield, CT). All right tibias were immersed in alcohol for 48 h, then immersed in diethyl ether for 48 h, then dried at 105°C for 1 h, and weighed to obtain the dry defatted weight. Tibias were then ashed for 24 h, and content of ash, Mn, Ca, and P in bone ash was measured.

Measurement of Calcium, P, and Mn Content

Approximately 0.2 g of feed or tibial powder was dissolved in 3 mL of nitric acid and 3 mL of H₂O₂ and then set aside for 2 h. The samples were digested using a

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

Dietary Mn supplementation ¹	Calculated	Analyzed
0 (basal diet)	16.1	17.5
20	36.1	35.7
40	56.1	57.1
80	96.1	97.4
120	136.1	135.5
160	176.1	180.4

¹Added as manganese sulfate monohydrate.

Table 3. Effects of dietary manganese (Mn) supplementation on productive performance of duck breeders in the laying period (17–34 wk).

Variables	Mn supplemental level (mg/kg) ¹						SEM	P-value		
	0	20	40	80	120	160		ANOVA	Linear	Quadratic
Early laying period (17–18 wk)										
Egg production (%)	66.1	71.0	74.4	64.8	72.4	70.0	1.393	0.327	0.736	0.396
Average egg weight (g)	53.4	52.7	54.0	52.0	53.6	53.6	0.263	0.277	0.736	0.908
Egg mass (g)	35.7	37.3	40.1	33.7	38.7	37.6	0.730	0.148	0.686	0.576
FCR (g:g)	3.87	3.58	3.60	4.30	3.73	3.67	0.064	0.004	0.714	0.935
Peak laying period (19–34 wk)										
Egg production (%)										
1 to 4 wk	86.4	86.8	89.5	88.4	90.2	90.2	0.659	0.390	0.046	0.118
5 to 8 wk	89.2	90.1	93.5	93.6	92.1	92.0	0.706	0.400	0.281	0.144
9 to 12 wk	83.3	84.8	85.1	88.3	89.4	84.8	0.715	0.094	0.133	0.020
13 to 16 wk	78.8	87.2	85.9	89.9	85.6	84.6	0.955	0.017	0.293	0.011
1 to 16 wk	84.2	86.8	88.3	89.4	89.2	87.8	0.591	0.015	0.047	0.001
Average egg weight (g)										
1 to 4 wk	59.4	59.0	59.5	59.4	59.0	59.3	1.210	0.984	0.807	0.971
5 to 8 wk	63.1	63.1	63.7	63.4	63.5	63.4	0.197	0.961	0.618	0.770
9 to 12 wk	65.5	65.1	65.6	65.3	65.5	65.9	0.168	0.873	0.371	0.538
13 to 16 wk	66.1	64.3	66.2	66.2	66.7	66.8	0.251	0.046	0.038	0.119
1 to 16 wk	63.4	62.6	63.6	63.6	63.6	63.7	0.184	0.515	0.248	0.513
Egg mass (g)										
1 to 4 wk	51.3	51.2	53.2	52.4	53.3	53.4	0.394	0.362	0.058	0.143
5 to 8 wk	56.3	56.3	59.5	59.3	58.5	58.3	0.456	0.141	0.151	0.060
9 to 12 wk	54.6	54.0	55.8	57.7	58.6	55.9	0.480	0.037	0.034	0.010
13 to 16 wk	52.0	56.1	56.8	59.5	57.0	56.5	0.592	0.007	0.049	0.001
1 to 16 wk	53.4	54.3	56.2	56.8	56.7	55.9	0.386	0.038	0.020	0.003
FCR (g:g) ²										
1 to 4 wk	3.07	3.07	2.95	3.00	2.96	2.94	0.022	0.334	0.055	0.138
5 to 8 wk	2.94	2.94	2.77	2.78	2.83	2.83	0.022	0.124	0.144	0.057
9 to 12 wk	3.03	3.06	2.96	2.87	2.82	2.96	0.025	0.035	0.032	0.010
13 to 16 wk	3.18	2.95	2.91	2.78	2.90	2.93	0.031	0.006	0.049	0.001
1 to 16 wk	3.05	3.00	2.90	2.87	2.88	2.92	0.020	0.033	0.018	0.003

¹Mean of 6 replicates (14 ducks per replicate) per treatment.

²FCR: feed conversion ratio.

microwave digestion instrument (MDS-10; Shanghai Xinyi Instrument Technology Co., Ltd., Shanghai, China). The contents of Mn and calcium (**Ca**) were analyzed by flame atomic absorption spectrophotometry (Zeenit700P; Analytik Jena, Jena, Germany), and the content of P was measured spectrophotometrically (UV-2700; Shimadzu, Kyoto, Japan) (Zhang et al., 2017a).

Serum Hormone Analysis

The concentrations of estradiol (**E₂**), prolactin (**PRL**), follicle-stimulating hormone (**FSH**), luteinizing hormone (**LH**), and progesterone (**P₄**) were measured in serum after thawing at 4°C for 2 h using ELISA kits for ducks

(Nanjing Jiancheng Bioengineering Institute, Nanjing, PRC; Cui et al., 2019b).

Analysis of Serum Biochemical and Antioxidant Indices

Total superoxide dismutase activity, Mn-SOD activity, total antioxidant capacity (**T-AOC**), and malondialdehyde (**MDA**) content in serum were analyzed using commercially available kits (Nanjing Jiancheng Bioengineering Institute) (Zhang et al., 2020b). The contents of total protein (**TP**), albumin, creatinine, total bilirubin (**TB**), uric acid, glucose (**GLU**), total cholesterol (**TC**), triglyceride (**TG**), high-density lipoprotein cholesterol,

Table 4. Effects of dietary manganese (Mn) supplementation on reproductive performance of duck breeders in the laying period (28–29 wk).

Variables	Mn supplemental level (mg/kg) ¹						SEM	P-value		
	0	20	40	80	120	160		ANOVA	Linear	Quadratic
Average egg weight (g)	65.3	64.8	65.6	65.5	64.7	64.8	0.167	0.451	0.314	0.479
1-D hatchling BW (g) ²	39.4	40.3	41.2	41.4	39.6	39.4	0.343	0.340	0.523	0.134
Fertility of set eggs (%)	94.6	93.3	90.8	89.7	90.7	92.7	0.909	0.632	0.428	0.177
Hatchability of fertile eggs (%)	84.1	86.3	90.6	86.1	88.3	89.3	0.700	0.071	0.092	0.198
Healthy duckling (%)	95.6	97.1	94.5	95.6	97.5	97.5	0.599	0.634	0.266	0.452

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

²BW: body weight.

Table 5. Effects of dietary manganese (Mn) supplementation on egg quality of duck breeders in the laying period (19–34 wk).

Variables	Mn supplemental levels (mg/kg) ¹						SEM	P-value		
	0	20	40	80	120	160		ANOVA	Linear	Quadratic
Albumen height (mm)										
4 wk	6.89	7.00	6.62	6.79	6.68	6.66	0.087	0.810	0.327	0.589
8 wk	6.55	6.31	6.47	6.06	6.70	6.28	0.074	0.171	0.773	0.862
12 wk	6.76	6.32	6.27	6.39	6.15	6.21	0.081	0.157	0.046	0.074
16 wk	6.44	6.53	6.52	6.72	6.64	6.59	0.063	0.848	0.351	0.415
Yolk color										
4 wk	4.33	4.28	4.17	4.06	4.11	4.17	0.050	0.652	0.220	0.192
8 wk	4.56	4.50	4.39	4.17	4.50	4.33	0.057	0.388	0.329	0.348
12 wk	4.32	4.39	4.43	4.50	4.54	4.29	0.052	0.719	0.841	0.277
16 wk	4.43	4.47	4.32	4.23	4.27	4.37	0.043	0.603	0.314	0.218
Haugh unit										
4 wk	82.2	82.7	80.0	81.3	80.6	81.1	0.586	0.819	0.468	0.613
8 wk	77.7	78.1	78.9	76.4	80.4	76.7	0.520	0.252	0.896	0.794
12 wk	80.2	76.8	76.4	77.0	73.4	75.6	0.667	0.271	0.060	0.113
16 wk	77.0	78.0	77.4	78.1	77.9	77.7	0.442	0.986	0.750	0.838
Eggshell thickness (mm)										
4 wk	0.392	0.407	0.418	0.410	0.410	0.411	0.003	0.212	0.215	0.142
8 wk	0.380	0.385	0.389	0.388	0.384	0.385	0.001	0.143	0.523	0.116
12 wk	0.332	0.334	0.334	0.356	0.353	0.353	0.002	0.001	<0.001	<0.001
16 wk	0.330	0.338	0.341	0.341	0.345	0.339	0.002	0.127	0.088	0.023
Eggshell breaking strength (N)										
4 wk	45.6	45.1	42.8	45.8	44.5	45.5	0.452	0.417	0.721	0.683
8 wk	42.6	43.6	43.9	44.8	42.4	41.9	0.422	0.394	0.360	0.134
12 wk	42.5	42.8	42.2	43.9	42.1	43.5	0.422	0.784	0.576	0.857
16 wk	41.9	43.6	43.9	43.2	43.9	42.4	0.347	0.460	0.888	0.225
Eggshell proportion (%) ²										
4 wk	10.3	10.4	10.2	10.4	10.4	10.5	0.047	0.618	0.299	0.510
8 wk	9.75	10.0	9.87	9.74	9.76	9.74	0.035	0.160	0.150	0.340
12 wk	9.39	9.56	9.72	9.54	9.63	9.56	0.033	0.061	0.287	0.019
16 wk	9.36	9.50	9.48	9.39	9.50	9.46	0.035	0.795	0.651	0.841

¹Mean of 6 replicates (5 eggs per replicate) per treatment.²Eggshell proportion (%) = 100 × eggshell weight/egg weight.

low-density lipoprotein cholesterol (LDL-C), Ca, and P in serum were determined using kits in an automatic biochemistry analyzer (all from Shanghai Kehua Bio-Engineering Co., Ltd., Shanghai, China) (Zhang et al., 2020b).

Statistical Analysis

Replicate (each replicate containing 14 cages, one duck in each cage) served as the experimental unit for analysis of performance and egg quality data; the

Table 6. Effects of dietary manganese (Mn) supplementation on reproductive organ and ovarian follicle development of duck breeders at the end of the trial (34 wk).

Variables ²	Mn supplemental level (mg/kg) ¹						SEM	P-value		
	0	20	40	80	120	160		ANOVA	Linear	Quadratic
Oviduct weight (g)	49.5	45.9	44.6	45.6	46.3	53.2	0.81	0.011	0.097	0.001
Oviduct length (cm)	45.6	39.0	37.6	40.4	44.9	39.7	0.81	0.009	0.869	0.688
Ovary weight (g)	61.9	53.0	55.1	58.3	62.4	56.6	1.89	0.685	0.805	0.965
F1 weight (g)	5.17	5.08	5.50	5.17	5.33	5.33	0.097	0.858	0.599	0.852
F2 weight (g)	50.0	45.7	49.0	49.9	55.2	50.0	1.71	0.782	0.391	0.667
F3 weight (g)	9.58	8.90	8.86	9.73	10.3	9.40	0.233	0.515	0.341	0.605
Number of POF	20.3	18.7	19.7	20.5	20.9	20.9	0.438	0.720	0.220	0.473
Total POF weight (g)	13.6	14.5	14.9	15.1	15.7	15.2	0.553	0.926	0.338	0.509
Mean POF weight (g)	7.61	7.48	7.91	8.24	9.86	8.49	0.460	0.732	0.212	0.409
POF percentage (%)	78.75	87.2	88.7	87.9	87.9	88.1	0.774	<0.001	0.008	<0.001
Number of SYF	9.50	9.58	6.67	10.83	9.58	6.75	0.522	0.099	0.405	0.388
Total SYF weight (g)	1.78	2.24	1.36	1.87	2.00	1.27	0.106	0.050	0.222	0.312
Mean SYF weight (g)	0.19	0.23	0.20	0.18	0.22	0.21	0.008	0.317	0.961	0.961
SYF percentage (%)	3.07	3.65	2.53	3.19	2.81	2.29	0.160	0.156	0.076	0.176
Number of LWF	36.1	43.2	42.9	36.4	45.0	37.6	1.59	0.422	0.964	0.698
Total LWF weight (g)	1.05	1.25	1.40	1.17	1.41	1.36	0.058	0.411	0.171	0.330
Mean LWF weight (g)	28.5	29.4	33.6	32.0	30.6	35.0	0.852	0.221	0.068	0.192
LWF percentage (%)	1.70	2.40	2.56	2.00	2.29	2.47	0.102	0.119	0.248	0.466
Atretic follicles weight (g)	9.11	4.13	3.32	5.41	3.76	4.04	0.535	0.010	0.061	0.042
Atretic follicles percentage (%)	16.21	7.94	6.22	6.81	6.32	7.27	0.712	<0.001	0.003	<0.001

¹Mean of 6 replicates (2 ducks per replicate) per treatment.²POF: preovulatory follicles; SYF: small yellow follicles; LWF: large white follicles; F1: the largest POF; F2: the second largest POF; F3: the third largest POF.

Table 7. Effects of dietary manganese (Mn) supplementation on the tibial characteristics and ash, Ca, and P content in the tibia of duck breeders at the end of the trial (34 wk).

Variables	Mn supplemental level (mg/kg) ¹						SEM	P-value		
	0	20	40	80	120	160		ANOVA	Linear	Quadratic
Bone fresh weight (g)	5.67	5.77	5.92	5.85	5.98	5.68	0.057	0.558	0.796	0.219
Fat-free dry weight (g)	3.43	3.57	3.60	3.64	3.60	3.40	0.038	0.304	0.848	0.050
Length (mm)	94.9	94.9	95.1	95.5	97.1	95.4	0.353	0.428	0.181	0.267
Midpoint circumference (mm)	16.4	16.9	17.5	17.3	17.3	17.1	0.146	0.280	0.246	0.086
Bone mineral density (g/cm ²)	0.258	0.280	0.306	0.305	0.284	0.286	0.004	0.008	0.231	0.006
Bone mineral content (g)	1.46	1.48	1.62	1.58	1.53	1.53	0.025	0.216	0.329	0.088
Breaking strength (N)	97.1	98.5	101	101	102	90.2	1.21	0.038	0.389	0.014
Ash content (%)	65.7	65.2	65.6	64.6	64.9	64.9	0.169	0.336	0.109	0.167
Ca content (%) ²	23.7	23.7	23.8	23.1	23.2	23.1	0.080	0.005	<0.001	0.001
P content (%) ²	10.5	10.5	10.5	10.5	10.4	10.5	0.034	0.918	0.338	0.607
Mn content (%)	15.4	15.0	17.3	18.2	18.5	18.5	0.353	0.001	<0.001	<0.001

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

²Ca: calcium; P: phosphorus.

average of 2 ducks in each replicate was the experimental unit for other assessment. The normality of the data and homogeneity of variances were first verified by Explore procedure using SPSS 16.0 for Windows (version 16.0; SPSS Inc., Chicago, IL). The effects of dietary Mn supplementation were analyzed using the one-way ANOVA procedure, and then, regression analysis was used to test the linear and quadratic effects using SPSS 16.0 for Windows. Quadratic regressions ($Y = aX^2 + bX + c$) were fitted to the responses of the dependent variables to Mn supplementation. The dietary concentration of Mn at which the response first reached 95% of the maximum was used to estimate the requirement (Zhang et al., 2020b). Data are expressed as means and pooled SEM.

RESULTS

Productive and Reproductive Performance

The effects of dietary Mn supplementation on productive performance of laying duck breeders are shown in Table 3. In the early laying period (17–18 wk of age), with the exception of feed conversion ratio (FCR) being affected by supplemental Mn ($P < 0.05$, lowest efficiency with 80 mg of added Mn), dietary Mn did not affect productive performance. During the subsequent 16-week peak laying period (19–34 wk of age), dietary Mn supplementation affected (13–16 wk and 1–16 wk; $P < 0.05$) egg production in laying duck breeders, and the responses were linear (1–4 wk and 1–16 wk; $P < 0.05$) and quadratic (9–12 wk, 13–16 wk, and 1–16 wk; $P < 0.05$) with dietary

level. Dietary Mn supplementation affected average egg weight, and it linearly increased from 13 to 16 wk ($P < 0.05$), but not during other times. Dietary Mn supplementation affected (9–12 wk, 1–16 wk, and 13–16 wk; $P < 0.05$) egg mass and FCR; responses in egg mass were linear (9–12 wk, 13–16 wk, and 1–16 wk; $P < 0.05$) and quadratic (9–12 wk, 13–16 wk, and 1–16 wk; $P < 0.01$). In contrast, FCR of laying duck breeders decreased linearly (9–12 wk, 13–16 wk, and 1–16 wk; $P < 0.05$) and quadratically (9–12 wk, 13–16 wk, and 1–16 wk; $P < 0.01$) during the peak laying period from 19 to 34 wk.

Dietary Mn addition did not affect average egg weight, fertility, hatchability, hatchling body weight, and proportion of healthy ducklings (Table 4).

Egg Quality

Table 5 shows the effects of dietary Mn addition on egg quality in laying duck breeders during the trial period. Dietary supplementation with Mn did not affect egg index, albumen height, yolk color, Haugh unit, and eggshell breaking strength during the treatment period. The shell thickness was affected (week 12, $P = 0.001$) and increased with increase in dietary Mn after feeding for 12 (linear and quadratic; $P < 0.001$) and 16 (quadratic; $P < 0.05$) wk of the peak laying period. There was a tendency for eggshell proportion to be affected (12 wk, $P = 0.061$), and it was quadratically increased with dietary Mn inclusion at 12 wk ($P < 0.05$), but not at other times.

Table 8. Effects of dietary manganese (Mn) supplementation on serum hormones of duck breeders at the end of the trial (34 wk).

Variables	Mn supplemental level (mg/kg) ¹						SEM	P-value		
	0	20	40	80	120	160		ANOVA	Linear	Quadratic
Estradiol (ng/L)	436	529	501	522	470	421	7.7	<0.001	0.037	<0.001
Luteinizing hormone (ng/L)	455	559	556	566	549	494	8.0	<0.001	0.685	<0.001
Follicle-stimulating hormone (IU/L)	8.76	10.1	10.4	10.1	10.3	7.80	0.18	<0.001	0.061	<0.001
Prolactin (ng/L)	389	382	396	382	376	368	2.7	0.041	0.005	0.013
Progesterone (pmol/L)	1,731	1,780	1,798	1,887	2,007	2,254	35.7	<0.001	<0.001	<0.001

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

Table 9. Effects of dietary manganese (Mn) level on serum biochemical and antioxidant indices of duck breeders at the end of the trial (34 wk).

Variables ²	Mn supplemental level (mg/kg) ¹						SEM	P-value		
	0	20	40	80	120	160		ANOVA	Linear	Quadratic
TP (g/L)	59.4	63.7	55.2	57.9	57.7	54.5	0.93	0.049	0.045	0.139
ALB (g/L)	20.4	19.6	19.4	19.5	19.5	19.4	0.29	0.941	0.448	0.626
UA (μmol/L)	251	240	255	201	229	266	8.2	0.238	0.965	0.136
CRE (μmol/L)	3.95	3.76	3.79	3.81	4.15	3.48	0.187	0.959	0.760	0.881
TB (μmol/L)	19.8	21.9	11.6	16.3	12.2	12.6	0.84	<0.001	0.001	0.001
GLU (mmol/L)	11.6	12.0	9.93	10.8	10.4	10.8	0.19	0.003	0.053	0.021
TG (mmol/L)	10.2	11.3	5.32	6.30	7.73	6.77	0.481	<0.001	0.016	0.005
TC (mmol/L)	2.51	2.94	1.89	2.15	2.20	1.91	0.088	0.001	0.009	0.027
HDL-C (mmol/L)	1.40	1.40	1.17	1.17	1.25	1.25	0.037	0.221	0.177	0.088
LDL-C (mmol/L)	0.50	0.51	0.33	0.38	0.35	0.41	0.018	0.003	0.036	0.005
Ca (mmol/mL)	5.37	5.73	5.04	5.27	5.03	4.95	0.073	0.011	0.008	0.030
P (mmol/mL)	3.00	4.08	3.30	2.98	3.14	3.43	0.114	0.040	0.655	0.765
T-AOC (U/mL)	5.76	5.50	7.36	6.95	10.0	8.83	0.344	<0.001	<0.001	<0.001
T-SOD (U/mL)	46.5	52.9	58.5	69.6	78.2	79.3	2.210	<0.001	<0.001	<0.001
Mn-SOD (U/mL)	5.45	17.7	19.7	26.1	28.5	38.1	1.84	<0.001	<0.001	<0.001
MDA (nmol/mL)	10.0	10.0	8.90	8.71	7.85	6.37	0.282	<0.001	<0.001	<0.001

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

²TP: total protein; ALB: albumin; UA: uric acid; CRE: creatinine; TB: total bilirubin; GLU: glucose; TG: triglycerides; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; Ca: calcium; P: phosphorus; T-AOC: total antioxidant capacity; T-SOD: total superoxide dismutase; Mn-SOD: Mn-containing superoxide dismutase; MDA: malondialdehyde.

Reproductive Organ and Ovarian Follicle Development

As shown in Table 6, weight and length of the oviduct were affected by dietary Mn levels, with weight decreasing quadratically ($P < 0.05$). The proportion of POF was affected ($P < 0.001$) and increased linearly and quadratically with increase in Mn levels ($P < 0.01$). The weight and percentage of atretic follicles were affected ($P < 0.05$) and decreased quadratically with dietary Mn supplementation ($P < 0.05$). Other ovarian follicle variables were not influenced by dietary Mn levels.

Tibial Characteristics

Effects of dietary Mn level on the tibial characteristics of duck breeders in the laying period are shown in Table 7. The density and breaking strength of tibias were affected ($P < 0.05$) and increased quadratically ($P < 0.05$) with dietary Mn supplemental levels. Tibial contents of Ca and Mn were influenced ($P < 0.05$) by dietary Mn levels, Ca content decreased (linear and quadratic; $P < 0.01$), and Mn content increased (linear and quadratic; $P < 0.001$) with increase in Mn supplemental levels. Tibial fresh and fat-free dry weight, length, midpoint circumference, and contents of mineral, ash, and P were not influenced by dietary Mn supplementation.

Serum Hormones

The effects of dietary Mn level on serum concentrations of hormones of duck breeders are shown in Table 8. The contents of E₂, LH, FSH, PRL, and P₄ were all influenced by dietary Mn supplementation ($P < 0.05$). Additional Mn had linear and quadratic effects on serum E₂, PRL, and P₄ contents (linear, $P < 0.05$; quadratic, $P < 0.001$) and had quadratic effects on serum LH and FSH content ($P < 0.001$).

Serum Biochemical and Antioxidant Indices

The effects of dietary Mn level on the serum biochemical and antioxidant indices of duck breeders are shown in Table 9. The serum contents of TP, TB, GLU, TG, TC, LDL-C, Ca, and P were affected ($P < 0.05$) by the supplementation with Mn. The TP content linearly decreased ($P < 0.05$), the GLU content quadratically decreased ($P < 0.05$), and the contents of TB, TG, TC, LDL-C, and Ca decreased both linearly and quadratically ($P < 0.05$) with dietary Mn levels. Other biochemical indices in plasma were not influenced by dietary Mn supplementation.

Dietary Mn supplementation affected T-AOC and MDA content and T-SOD and Mn-SOD activities in serum ($P < 0.001$); T-AOC content, T-SOD activities,

Table 10. Estimation of the dietary manganese (Mn) requirements based on quadratic regressions of egg production, egg mass, and FCR on dietary Mn supplemental levels.

Variables	Time (wk)	Regression equation ¹	R ²	P-value	Mn requirement (mg/kg) ²
Egg production (%)	1 to 16	$Y = -0.0006X^2 + 0.1063X + 84.573$	0.973	0.001	84.2
Egg mass (g/d)	1 to 16	$Y = -0.0004X^2 + 0.0722X + 53.371$	0.953	0.003	85.8
FCR (g:g) ³	1 to 16	$Y = 1.90 \times 10^{-5}X^2 - 0.0038X + 3.05$	0.948	0.003	95.0

¹Y is the dependent variable, and X is the dietary Mn supplemental levels (mg/kg).

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

³FCR: feed conversion ratio.

and Mn-SOD activities increased linearly and quadratically ($P < 0.001$) with dietary Mn levels, whereas MDA content decreased linearly and quadratically ($P < 0.001$) in response to dietary Mn supplementation levels.

Estimations of the Dietary Mn Requirements

The results of dietary Mn requirements for laying duck breeders, as estimated from the quadratic regression analyses of productive traits, are shown in Table 10. Additional Mn requirements, in milligram per kilogram for a basal diet containing 17.5 mg/kg of Mn, for Longyan duck breeders from 19 to 34 wk of age were estimated to be 84.2 for optimizing egg production, 85.8 for egg mass, and 95.0 for FCR.

DISCUSSION

In the present study, dietary Mn supplementation was found to linearly and quadratically improve productive performance, including increased egg production and mass and decreased FCR. Ducks supplemented with 80 mg/kg of Mn had the best performance, with no further improvement from 120 and 160 mg/kg of supplementation. Similarly, Cui et al. (2019a) observed that dietary supplementation with amino acid-complexed Mn linearly and quadratically increased egg mass in laying hens aged 23 to 46 wk, with the addition of 40 and 80 mg/kg of Mn increasing egg production and decreasing FCR and no benefit being observed with a concentration of more than 120 mg/kg. Several studies on laying hens, however, failed to demonstrate any benefit effect of Mn supplementation on laying performance for a period of 8 to 12 wk (Yildiz et al., 2010; Xiao et al., 2014, 2015; Zhang et al., 2017a). This difference may result from an insufficient treatment duration or the different ages of poultry. It was reported that Mn^{2+} could cross the blood-brain barrier (Crossgrove et al., 2003), and young rats were more sensitive to Mn than old rats (Erikson et al., 2004). The present study also found that dietary Mn supplementation reduced follicular atresia while increasing the development of POF, which may account for the increased egg production of duck breeders. Related to this, supplementation with Mn increased the serum concentrations of E_2 , FSH, LH, and P_4 . These hormones are affected by the hypothalamic-pituitary-gonadal axis (Ahmed et al., 2014; Bedecarrats, 2015), which influence development of reproductive organs and ovarian follicles (Nicks et al., 2010) and affect changes in egg production (Ahmed et al., 2014). It can be concluded that dietary Mn supplementation, as used here in laying duck breeders, modulated hormones of the hypothalamic-pituitary-gonadal axis, influenced the development of reproductive organs and ovarian follicles and then affected egg production.

Serum hormone levels are sensitive indicators of laying performance (Rozenboim et al., 2007). For example, E_2 is a key regulator of the development of the reproductive tract (Hu et al., 2019), FSH serves as the main hormone

responsible for the development and maturation of small follicles (Liu and Zhang, 2008), and the primary target of LH is the granulosa layer of the larger POF (Liu and Zhang, 2008; Yin et al., 2018). Moreover, FSH and LH promote the secretion of P_4 and estrogen in granulosa and thecal cells of ovarian follicles (He et al., 2013; Rutigliano et al., 2014). In the present study, dietary Mn supplementation decreased follicular atresia and increased the development of POF in the duck breeders, likely owing to increased serum levels of hormones. This interpretation is consistent with genotypic differences in egg production of broiler breeder hens being closely related to follicular differentiation, regulated by LH, FSH, P_4 , and E_2 hormones in plasma (Onagbesan et al., 2006).

Manganese could elevate serum levels of LH, FSH, and E_2 in female rates (Michelle et al., 2005), which is similar to the effects of dietary Mn supplementation found here in duck breeders on serum contents of E_2 , FSH, LH, and P_4 . These changes possibly stem from the effect of Mn on soluble guanylyl cyclase to activate the protein kinase G pathway controlling the release of gonadotropin-releasing hormone I (GnRH-I; Lee et al., 2007), regulating secretion of FSH and LH (Thompson and Kaiser, 2014). Low-level supplementation with Mn increased LH secretion in adult male rats (Prestifilippo et al., 2007), and addition of Mn to broiler breeder hens' diet affected expression of *GnRH-I* and *FSH* (Xie et al., 2014). In the present study, high-level supplementation with Mn (160 mg/kg) decreased serum contents of E_2 , FSH, and PRL, possibly because of the known dual effects of Mn on PRL secretion (Kim et al., 2009). Toxic high doses of Mn had a negative effect on dopamine synthesis (Guilarte, 2010), and dopamine is a potent regulator of pituitary PRL (Fitzgerald and Dinan, 2008). In addition, PRL and dopamine could interactively regulate FSH and LH secretion (Gregory et al., 2004). In the case of high-level supplementation of Mn in duck breeders, the observed decrease in reproductive hormones might indicate toxicity.

There are several reports of dietary Mn supplementation improving eggshell quality in laying hens (Xiao et al., 2014, 2015; Zhang et al., 2017a,b; Cui et al., 2019a). Interestingly, in the duck breeders studied here, there was no benefit on eggshell quality from Mn supplementation, except for increased shell thickness after feeding for 12 wk. Supplementation of laying ducks with 15 to 90 mg/kg of Mn similarly had no effect on eggshell quality (Fouad et al., 2016). The different effects of Mn on shell quality in laying hens and ducks may result from the different characteristics of hen and duck eggs; duck eggs are bigger and heavier, and shells are thicker and tougher than hen eggs. The mammillary knobs in the shell ultrastructure of duck egg are more compact and shorter than those in hen eggs, and dietary Mn supplementation modulated shell quality in laying hens mainly by effects on the mammillary knobs of the eggshell ultrastructure (Zhang et al., 2018).

Manganese is essential for development of normal bones and prevention of perosis in the chick (Wang et al., 2015; Spears, 2019). Dietary Mn addition here

quadratically increased bone mineral density and breaking strength, which suggests Mn promoted mineral deposition and increased mechanical strength of bone, whereas high levels of Mn (160 mg/kg) did the reverse. The effects of Mn on hormone-stimulated bone resorption are known to be biphasic (Wang et al., 2013), and trace elements had beneficial, as well as detrimental, effects on bone homeostasis (Zofkova et al., 2017). The highest level of Mn supplementation decreased Ca content and increased Mn content in tibias, another of many known antagonisms between Ca and Mn. For example, cellular Mn²⁺ transport into mitochondria is via the Ca²⁺ uniporter (Kamer et al., 2018), Mn deficiency increased serum Ca content in chicks (Wang et al., 2013), and intestinal transport of Mn was modulated by Ca (Ji et al., 2006). Dietary Ca and P levels in broiler chicks influence Mn deposition in bone (Singh et al., 2013), and bone strength of aged laying hens was affected by levels of Ca, P, and Mn (Jiang et al., 2013; Min et al., 2019).

In the present study, supplementation of duck breeders with Mn quadratically decreased the serum contents of TG, TC, and LDL-C, consistent with Mn improving lipid metabolism, the status of which is reflected in these compounds; excess accumulation of TG and TC leads to metabolic disorders in broilers (Alvarenga et al., 2011). Manganese has been shown to influence lipid metabolism in a previous study (Legleiter et al., 2005). In the duck breeders here, dietary Mn addition decreased serum contents of TP, TB, and GLU, which is consistent with its promotion of liver function. In addition, it improved serum antioxidant status by increasing T-AOC content, T-SOD activities, and Mn-SOD activities and decreasing MDA content. Several studies have reported the antioxidant activity of Mn in broilers (Li et al., 2008), laying hens (Cui et al., 2019a), and ducks (Fouad et al., 2016). Manganese is a critical component of Mn-SOD, and it modulates gene expression and activity of Mn-SOD in chickens (Li et al., 2011b; Gao et al., 2011), even being used as a biomarker to estimate Mn requirement and bioavailability of Mn sources in broilers (Luo et al., 2007; Lu et al., 2016). Overall, dietary Mn supplementation improved antioxidant status and the health status of laying duck breeders.

For variables of egg production, egg mass, and FCR measured here, it can be concluded that for a basal diet with 17.5 mg/kg of Mn, an additional 85 to 95 mg/kg of Mn was adequate for laying duck breeders during the laying period. This is a little higher than the recommended levels of chicken layer breeders (60 mg/kg, Wen et al., 2004), which is possibly a result of increased body weight and egg weight compared with chicken breeders. The current NRC (1994) guidelines recommend 20 and 60 mg/kg of Mn for laying hens and broilers, respectively. However, a recent review found that the dietary Mn requirement for laying hens and broilers appeared to be 90 mg/kg (Olgun, 2016). Similarly, 100 mg/kg of Mn was recommended for meat duck breeders (Hou et al., 2012). The increased

egg production of laying duck breeders compared with the meat duck breeders may account for a little higher requirement of Mn. As there is no existing feeding standard for laying duck breeders, the present results provide the needed information. For most variables examined here with a basal diet containing 17.5 mg/kg of Mn, an additional 85 to 95 mg/kg of Mn was adequate for laying duck breeders during the laying period.

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