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Research Article

Effects of Dietary Manganese Supplementation on Laying Performance, Egg Quality and Antioxidant Status in Laying Ducks

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Abstract

Objective: Manganese (Mn) is a crucial trace element for poultry nutrition because it has multiple physiological functions. Thus, the main goal of this study was to evaluate the effects of dietary Mn supplementation on laying performance, egg quality and antioxidant status in Shanma laying ducks. **Methodology:** Five hundred and four Shanma laying ducks, at 17 weeks of age, were randomly assigned to 7 treatments, with 6 replicates per treatment and 12 ducks per replicate. Birds were fed the same basal diet, which was supplemented with 0.0 (control), 15, 30, 45, 60, 75 or 90 mg Mn/kg in the form of Mn-sulfate. **Results:** Results showed that dietary Mn supplementation did not affect egg production, egg weight, feed conversion ratio, egg mass, egg quality, tibia characteristics, total antioxidant capacity, copper-zinc superoxide dismutase or lipid peroxidation (malondialdehyde), but supplementing 90 mg Mn/kg diet significantly ($p < 0.05$) improved the activities of total superoxide dismutase, Mn-containing superoxide dismutase and increased Mn content in egg yolk compared with the control group. **Conclusion:** These results indicate that corn-soybean meal diet containing sufficient amount of Mn for laying performance and egg quality in Shanma laying ducks under the conditions of current experiment, but adding 90 mg Mn/kg basal diet is required to improve the activities of Mn-containing superoxide dismutase and total superoxide dismutase and elevate Mn content in their egg yolk.

Key words: Manganese, laying ducks, laying performance, egg quality, manganese-containing superoxide dismutase

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Manganese (Mn) is an essential trace element in poultry nutrition due to its role in normal bone and eggshell formation, enzyme function and nutrients (carbohydrate and lipid) metabolism^{1,2}. The Mn activates numerous enzyme such as hydrolases, transferases, kinases, lipoprotein lipase, hormone sensitive lipase and mitochondrial superoxide dismutase³⁻⁵. The Mn involves in body fat partition and enhances meat shelf-life in chickens⁶. The Mn-deficient diet increases incidence of perosis and decreases tibia length in broiler chickens^{7,8}, while in laying hens elevates the production of eggs with thinner shells, with translucent areas and abnormalities in eggshell ultrastructure, particularly in the mammillary layer^{9,10}. The Mn regulates hormones participate in bone metabolism and ovarian follicular development and egg production¹¹⁻¹³. In aged laying hens, dietary Mn supplementation improved eggshell thickness and breaking strength by enhancing uronic and glycosaminoglycan deposition in egg shell membrane^{9,10}. In broiler chickens, laying hens and broiler breeders, influence of Mn on productive performance, meat quality, egg quality, fertility and hatchability has been identified^{4,8,9,10,13}, but in laying ducks no information is available about its effects on laying performance and egg quality. Therefore, the main goal of the current experiment was to determine Mn requirements of laying ducks based on laying performance and egg quality.

MATERIALS AND METHODS

Animals and diets: This study was approved by the Animal Care and Use Committee of Institute of Animal Science, Guangdong Academy of Agriculture Science. Five hundred and four, 17 weeks of age, Shanma female ducks (*Anas platyrhynchos*, a typical breed of laying ducks in South China) were randomly divided in the same room, with incandescent lighting of 10 lx, providing 15 L: 9D into 7 treatment groups, each of which included 6 replicates of 12 ducks for 19 weeks study period. Birds were housed in controlled cages (27.8×40×55 cm), separately. All ducks were given the same basal diet for 4 weeks to deplete their bodies of store Mn. The concentration of Mn in the basal diets (Table 1) was 19.1 mg kg⁻¹. Basal diet was formulated based on corn soya bean meal to meet or exceed all nutrient requirements of egg laying ducks as described by Ruan *et al.*¹⁴ with the exception of Mn. Then graded levels of Mn (Mn sulfate) were added at 0 (Control), 15, 30, 45, 60, 75 and 90 mg kg⁻¹ basal diet. All birds were given in average 160 g

feed daily divided to two times at 0700 and 1500 h without their leaving refusals, while water was allowed *ad libitum*.

Performance and egg quality: Feed consumption and egg production were recorded daily. The average daily egg production of all ducks ranged from 85-100% for the peak-laying period. Eggs were collected and individually weighed and graded daily according to¹⁵. Feed Conversion Ratio (FCR) was calculated as grams of feed per gram of egg mass and then presented as the averages for the complete 19 weeks study period. Four eggs were collected at random from each replicate each month and the average of the 24 eggs per replicate was used to determine egg quality. Egg shape was calculated as following; egg width*100/egg length after we measured egg width and egg length with a digital caliper. Haugh unit and yolk color were measured using Egg Analyzer (model EA-01, ORKA Food Technology Ltd., Ramat Hasharon, Israel). Eggshells were washed under running water, dried and weighed. Albumen height was determined by digital caliper. Eggshell thickness was measured after removing shell membrane with a digital micrometer (model IT-014UT, Mitutoyo, Kawasaki, Japan). Eggshell thickness was a mean value of measurements at 3 locations on the eggs (air cell, equator and sharp end).

Blood sample collection: At the end of the experiment, two ducks from each replicate were randomly selected for blood

Table 1: Composition of the basal diet and nutrient levels

Ingredients	Value (%)
Maize	54.48
Soybean meal	24.27
Wheat by-product	10.1
Limestone	8.47
Calcium hydrogen phosphate	1.207
DL-methionine	0.139
L-lysine-HCl	0.034
Sodium chloride	0.3
Premix*	1.0
Total	100.0
Nutritional value	
AME (kcal kg ⁻¹)	2500
Crude protein	17.0
Calcium	3.6
Total P	0.61
Available phosphorus	0.35
Total lysine	0.86
Total methionine	0.40
Mn (mg kg ⁻¹)	19.1**

*Supplied per kilogram of diet: Retinyl palmitate 12000 IU, cholecalciferol 2000 IU, DL- α -tocopheryl acetate 38 mg, menadione sodium bisulphite 1.0 mg, thiamin mononitrate 3.0 mg, ribo avin 9.6 mg, pyridoxine hydrochloride 6.0 mg, cobalamin 0.03 mg, chloride choline 500 mg, nicotinic acid 25 mg, calcium-D-pantothenate 28.5 mg, folic acid 0.6 mg, biotin 0.15 mg, Fe 50 mg, Cu 10 mg, Zn 90 mg, I 0.5 mg, Se 0.4 mg **Measured values

sampling. Ten milliliters blood was collected from the wing vein using heparinized tubes. Blood was centrifuged at (1200×g) at 4°C for 10 min to harvest plasma, then plasma was stored at -80°C until assay.

Tibia characteristics: The frozen tibias were thawed inside the plastic bags at room temperature for 2 h. The left tibias were ashed for 24 h and the content of tibial ash was determined on a dry defatted weight basis. Right tibias were cleaned of all tissue and bone mineral density of the right tibia was measured at the Guangzhou Overseas Chinese Hospital with a X-ray osteodensitometer (Lunar Prodigy, General Electric Company, Fairfield, USA) and the breaking strength of the middle of the bone was determined with a materials tester (Instron 4411, Instron Corporation, Grove City, PA).

Antioxidant capacity measurements: Total antioxidant capacity (T-AOC), total superoxide dismutase (T-SOD), copper-zinc superoxide dismutase (Cu-Zn-SOD), Mn-containing superoxide dismutase (Mn-SOD) and concentration of malondialdehyde (MDA) in plasma were determined with commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Mn content in egg yolk: The albumen and yolk were separated, pooled within each replicate and freeze-dried. Aliquots of all dried samples were weighed into digestion tubes and subjected to Mn determination by flame atomic absorption spectrophotometry.

Statistical analysis: Data were analyzed as a single factor design using PROC GLM procedure of SAS 8.1 software¹⁶. The linear and quadratic effects of Mn among treatments were analyzed using a contrast statement. Significant differences among treatments were tested using Duncan's multiple-range test at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Laying performance: Shanma laying ducks were used here because this breed makes up approximately 60% of the modern laying ducks in South China. No differences in egg production, average egg weight, daily egg mass, FCR, broken egg rate, or abnormal egg rate among Mn treatments ($p > 0.05$, Table 2) were found in this study. In agreement with earlier study by Sazzad *et al.*¹⁷ who reported that increasing Mn level in different commercial laying hens strains did not cause any significant influence on laying rate, egg weight, or FCR. Yildiz *et al.*¹⁸ observed that increasing Mn level from its different sources had no significant effect on laying performance of laying hens. Also, Venglovska *et al.*¹⁹ found that supplemental 120 mg Mn/kg feed did not appear any improvements in egg production, egg weight, egg mass or FCR in laying hens. Bai *et al.*²⁰ feeding laying hens Mn-deficient diet (24.4 mg kg^{-1} diet) or diet contained high level of Mn (300 mg kg^{-1} diet) did not affect laying performance compared with control (60 mg kg^{-1} diet). Xiao *et al.*^{9,10} reported that Mn levels (0, 25, 50, 100 or 200 mg kg^{-1} diet) or sources (Mn-sulfate or Mn-amino acid) had no effects on egg production, egg weight, egg mass and FCR in laying hens. Additionally, in broiler breeders, adding Mn from organic or inorganic source at level of 120 or 240 mg kg^{-1} diet did not alter their egg production, egg weight, egg mass or FCR compared with the control group¹³. Moreover, feeding broiler breeders diets supplemented with 120 mg Mn/kg did not change their laying performance under thermoneutral temperature or heat stress conditions²¹.

Egg quality: Egg shape index, Haugh units, albumen height, yolk color, yolk%, albumen%, eggshell weight, eggshell% or eggshell thickness did not change by adding Mn ($p > 0.05$, Table 3). In agreement with these results, Bai *et al.*²⁰, who reported that Haugh units, albumen height, yolk color,

Table 2: Effect of dietary manganese supplementation on performance of laying ducks

Items	Dietary manganese (mg kg^{-1})							SEM**	p-value	
	Control	15	30	45	60	75	90		Linear	Quadratic
Egg production (%)	88.60	88.70	89.20	88.80	89.40	90.20	87.90	2.10	0.80	0.50
Egg weight (g)	63.30	64.00	63.80	64.10	63.10	63.90	64.60	0.50	0.20	0.40
Egg mass (g)	55.80	56.40	57.00	56.50	56.60	57.70	55.90	1.50	0.50	0.30
FCR*	2.88	2.85	2.82	2.84	2.79	2.87	0.08	0.50	0.30	0.60
Broken rate (%)	1.90	1.80	1.70	1.90	2.10	2.20	1.40	0.40	0.70	0.50
Abnormal rate (%)	0.56	0.63	0.60	0.76	0.45	0.50	0.28	0.31	0.07	0.20

*Feed conversion ratio (g) of feed/g of egg mass and **Standard error of means (n = 12 birds/replicate, n = 6 replicates/treatment)

Table 3: Effect of dietary manganese supplementation on egg quality

Items	Dietary manganese (mg kg ⁻¹)							SEM*	p-value	
	Control	15	30	45	60	75	90		Linear	Quadratic
Shape index	74.80	74.20	74.50	73.70	74.30	74.80	74.90	0.01	0.4	0.5
Haugh unit	79.00	79.30	76.70	79.00	80.40	78.90	77.50	0.90	0.8	0.9
Yolk color	4.47	4.59	4.46	4.53	4.59	4.48	4.70	0.07	0.07	0.2
Albumen height (mm)	6.45	6.56	6.44	6.68	6.42	6.41	6.53	0.14	0.9	0.9
Albumen (%)	59.70	60.20	60.31	60.10	60.50	60.10	60.00	0.10	0.8	0.9
Yolk (%)	30.50	29.90	30.10	30.00	29.90	30.20	30.20	0.20	0.6	0.6
Eggshell weight (g)	6.18	6.28	6.22	6.33	6.22	6.23	6.26	0.06	0.8	0.8
Eggshell (%)	9.79	9.83	9.77	9.88	9.87	9.77	9.71	0.07	0.1	0.7
Eggshell thickness (mm)	0.352	0.353	0.356	0.357	0.353	0.352	0.348	0.003	0.1	0.1

*Standard error of means (n = 4 eggs/replicate, n = 6 replicates/treatment)

Table 4: Effect of dietary manganese supplementation on tibia characteristics

Items	Dietary manganese (mg kg ⁻¹)							SEM*	p-value	
	Control	15	30	45	60	75	90		Linear	Quadratic
Weight (g)	4.40	4.60	4.50	4.70	4.50	4.30	4.60	0.50	0.9	0.8
Length (cm)	9.30	9.55	9.49	9.53	9.42	9.43	9.44	0.30	0.9	0.5
Circumference (cm)	1.89	1.96	1.94	1.98	1.95	1.92	1.90	0.11	0.8	0.1
Breaking strength (N)	12.10	12.70	11.90	12.30	12.30	11.80	12.00	2.05	0.4	0.7
Density (g cm ⁻³)	0.23	0.24	0.22	0.21	0.22	0.24	0.24	0.02	0.7	0.6
Mineral content (g)	0.92	1.03	0.88	0.91	0.86	0.98	0.90	0.08	0.6	0.8

*Standard error of means (n = 2 ducks/replicate, n = 6 replicates/treatment)

egg shell thickness and eggshell percentage did not response to Mn deficient diet and dietary Mn supplementation in laying hens. Also, Zhu *et al.*²¹ found that dietary Mn supplementation from different sources did not affect Haugh units, yolk color and egg shell thickness in broiler breeder hens. By contrast, Xiao *et al.*^{9,10} observed that egg shape index did not change by changing Mn level or its source in aged laying hens, but egg shell thickness improved. Also, Venglovská *et al.*¹⁹ and Sazzad *et al.*¹⁷ noted that eggshell thickness significantly improved due to Mn supplementation. This discrepancy may have been due to age, species, experimental period, Mn content in the basal diet, Mn level and/or Mn source. In this experiment we fed laying ducks from 17-36 weeks of age basal diet contained 19.1 mg Mn/kg and Mn was added to the basal diet in the form of Mn sulfate, while Sazzad *et al.*¹⁷ supplemented Mn in the form of Mn oxide. Different Mn forms have different bioavailability and different effects²² and Xiao *et al.*^{9,10} fed aged laying hen diet supplemented with high level of Mn as Venglovská *et al.*¹⁹ compared with the results of this experiment. Moreover, thinner eggshell problem is common in aged laying hens compared with early or peak production period²³.

Tibia characteristic: In broiler chickens, Mn plays a crucial role in bone development^{11,24}. Thus, we determined tibia weight, length, circumference, breaking strength, density and mineral content. No differences in tibia weight, length,

circumference, breaking strength, density or mineral content among the treatments ($p > 0.05$, Table 4). These results in agree with Attia *et al.*²⁵, who found that inclusion Mn from different sources did not affect tibia mineral content in hens.

Antioxidant status and Mn content in egg yolk: No significant differences in plasma concentrations of T-AOC, Cu Zn-SOD or MDA among the treatments ($p > 0.05$), while concentrations of T-SOD, Mn-SOD and Mn content in egg yolk increased significantly with increasing Mn level ($p < 0.05$, Table 5). In poultry concentrations of Mn-SOD in different tissues have been used as a biomarker to determine Mn needs^{7,20}. So, in current experiment, we determined concentrations of Mn-SOD in the plasma as a biomarker to Mn for the first time. Lu *et al.*^{4,5} and Li *et al.*⁷ found that feeding broiler chickens diets supplemented with Mn upregulated the expression of Mn-SOD gene and improved the activity of Mn-SOD in different tissues (heart, breast and leg muscles). Also, Bai *et al.*²⁰ reported that increasing Mn level in laying hen diets upregulated the activity and the expression of the Mn-SOD gene in the heart. May increase the activity of Mn-SOD led to enhancements in T-SOD, which explain why concentrations of T-SOD increased as dietary Mn level increased. Although, concentrations of Mn-SOD and T-SOD enhanced, but MDA did not decline in this experiment, which has been also reported in broiler breeders study by Zhu *et al.*²¹. On other hand, in broiler chickens, dietary Mn

Table 5: Effect of dietary manganese supplementation on antioxidant indexes in plasma and manganese content in egg yolk

Items	Dietary manganese (mg kg ⁻¹)							p-value	
	Control	15	30	45	60	75	90	SEM*	Linear Quadratic
T-AOC (U mL ⁻¹)	7.5	6.9	8.8	8.8	7.7	8.3	7.4	0.6	0.7 0.3
Cu-Zn-SOD (U mL ⁻¹)	12.3	12.4	12.1	13.3	11.9	11.1	12.4	0.7	0.6 0.8
Mn-SOD (U mL ⁻¹)	36.7 ^b	44.1 ^{ab}	34.4 ^b	40.3 ^b	42.7 ^{ab}	42.2 ^{ab}	50.8 ^a	2.6	0.09 0.004
T-SOD (U mL ⁻¹)	48.3 ^b	55.1 ^{ab}	46.30 ^b	53.7 ^{ab}	54.6 ^{ab}	53.5 ^{ab}	63.3 ^a	3.1	0.2 0.008
MDA (nmol mL ⁻¹)	14.5	13.8	15.9	15.2	14.8	15.3	15.8	1.4	0.2 0.4
Mn content in egg yolk	1.22 ^b	1.17 ^b	1.20 ^b	1.41 ^{ab}	1.35 ^{ab}	1.39 ^{ab}	1.51 ^a	0.1	0.8 0.02
Yolk DM (mg kg ⁻¹)									

T-AOC: Total antioxidant capability, Cu-Zn-SOD: Copper-zinc-superoxide dismutase, Mn-SOD: Manganese-containing superoxide dismutase, T-SOD: Total superoxide dismutase, MDA: Malondialdehyde and *Standard error of means (n = 2 ducks/replicate, n = 6 replicates/treatment)

supplementation enhanced the activity of Mn-SOD and decreased MDA concentration^{4,5,7}. Regardless experimental period, experimental animal and its age, in this experiment, the highest level of Mn was 90 mg kg⁻¹ feed, while in Lu *et al.*^{4,5} experiments, the highest level of Mn was 400 and 200 mg kg⁻¹ feed, respectively. Increasing Mn level significantly increased Mn content in the egg yolk, which was in consistent with previous study in laying hens¹⁹, but in contrast with previous studies in broiler chickens⁵. In wild birds, Mn-content in their eggs range from 1.8-2.0 µg g⁻¹, which is higher than Mn-content in the egg yolk in the current experiment²⁶⁻²⁸. However, different tissues have different ability to deposit Mn^{7,26}, which explain why increasing Mn level in laying hens or ducks diet increased Mn content in their egg yolks, but this result did not appear in breast or leg muscles of broiler chickens when Mn level increased in their diets⁵.

CONCLUSION

Mn content in corn-soybean meal diet (19.1 mg kg⁻¹) is sufficient to satisfy laying performance and egg quality of Shanma laying ducks under the conditions of this experiment, but added 90 mg Mn/kg basal diet is required to improve the activities of Mn-SOD and T-SOD and elevate Mn content in their egg yolk. In broiler breeders²¹, in laying hens^{10,20} and in broiler chickens^{7,12} corn-soybean meal diet is able to satisfy Mn requirements based on laying performance, but adding Mn to corn-soybean meal diets is required to improve the activity of Mn-SOD^{7,12}.

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