Effect of in ovo manganese injection on the embryonic development, antioxidation, hatchability, and performances of offspring broilers under normal and high temperatures

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ABSTRACT Two experiments were carried out to study the effect of in ovo manganese (**Mn**) injection on the embryonic development, antioxidation, hatchability, and performances of offspring broilers under normal temperature (**NT**) and high temperature (**HT**). Experiment 1 was conducted to investigate the effect of in ovo Mn injection on the embryonic hatchability of Arbor Acres broiler breeders. On D 9 of incubation, a total of 684 fertilized eggs were randomly allocated to 6 treatments: the non-injected positive control (**niPC**) and treatments injected with 0 (the negative control, **iNC**), 6.25, 12.5, 25.0, or 50.0 μg Mn/egg as Mn sulfate. Experiment 2 was conducted to investigate the effect of in ovo Mn injection on the embryonic development, antioxidation and performance of offspring broilers under NT and HT. A total of 792 fertilized eggs were randomly allocated to 6 treatments in a 1 (niPC) + 1 (iNC) + 2 (injected Mn sources: Mn sulfate and Mn proteinate) × 2 (injected Mn levels: 12.5 and 25.0 μg/egg) factorial arrangement during the embryonic stage and D1 to 28 at NT. Then, 288 birds were allotted to 12 treatments in a 6 (the above embryonic treatments) × 2 (environmental temperatures: NT-22℃ vs HT-34℃) factorial arrangement from D 29 to 42. The results showed that Mn injection affected ($P < 0.03$) the hatchability and the maximum level of in ovo injected Mn was 25.0 μg Mn/egg. The Mn injection upregulated ($P < 0.05$) *Mn-containing superoxide dismutase* mRNA expression in the embryonic heart compared to the iNC. Hyperthermia decreased ($P < 0.05$) ADG and ADFI, breast muscle percentage, plsma alkaline phosphatase activity, and red color values of breast and thigh muscles, but increased ($P < 0.05$) F/G, plasma aspartate aminotransferase and lactic dehydrogenase activities, total cholesterol, uric acid and triiodothyronine contents, abdominal fat, light values of breast and thigh muscles of offspring broilers. The results suggest that in ovo Mn injection can enhance antioxidant ability in the chick embryonic heart.

Key words: in ovo Mn injection, chick embryo, *MnSOD*, offspring broiler

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INTRODUCTION

Prenatal nutrition is crucial for embryonic development and neonatal growth, and it has the potential to be a main determinant of life-long health. Because of the increased metabolic rate of the modern embryos, the embryonic nutrient reserves are insufficient and might be depleted in the prenatal period (Yair et al., 2013). Chicken systems have been recently recognized as an excellent model for studying the embryonic development of animals. In ovo injection technology in particular provides a practical way to safely introduce external nutrients into developing embryos (Foye et al., 2006; Kadam et al., 2008; Bello et al., 2014a). Feeding feed components to the embryo before hatching by in ovo administration may induce a positive effect on hatchability, growth performance and carcass characteristics (Keralapurath et al., 2010; Bello et al., 2014b).

Manganese (**Mn**) is an essential trace element for animals. It is involved in numerous biochemical reactions both as an integral part of metalloenzymes and as an enzyme

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activator. Either inorganic or organic Mn has been used as a feed additive to improve the growth performance and carcass characteristics as well as the meat quality in broiler chickens (Lu et al., 2006, 2007). Avian birds have a relatively high requirement but less absorption efficiency for Mn compared to mammals (Underwood, 1977). The Mn deficiency in hens can cause lower egg production (Leach and Gross, 1983) and poor eggshell quality (Li et al., 2004; Xiao et al., 2014), decrease hatchability and increase mortality of embryos (Zhu et al., 2015a,c). Therefore, Mn supplementation in diets or by in ovo injection could improve the hatching quality, productive performance, antioxidation, and carcass characteristics (Oliveira et al., 2015; Zhu et al., 2015a,b,c; Noetzold et al., 2020).

The Mn is also a crucial component of the metalloenzyme Mn-containing superoxide dismutase (**MnSOD**), the most dominant superoxide dismutase functioning as a free radical scavenger in the mitochondria. The MnSOD may alleviate oxidative stress induced by extreme environments such as HT in broilers and broiler breeders via detoxification of superoxide free radicals (Zhu et al., 2015a,b,c). Previous studies from our laboratory indicated that dietary supplementation of Mn could enhance the heart MnSOD activity and mRNA expression and reduce lipid peroxidation in broiler chicks via enhancing epigenetic activated antioxidant and anti-apoptotic abilities, and protect the embryonic development of chicks against maternal heat stress (Li et al., 2004, 2011a; Lu et al., 2007; Luo et al., 2007; Zhu et al., 2015a, 2017; Liao et al., 2019). However, the effect of in ovo Mn injection on the embryonic development of broiler breeder eggs and growth performance of offspring broilers under different thermal conditions has not been reported before. Therefore, we hypothesized that the in ovo Mn injection might have a positive effect on the embryonic development and progeny growth performance and related aspects under different thermal conditions. To test our hypothesis, the effects of in ovo Mn injection on the embryonic development, antioxidant abilities, hatchability, and subsequent growth performance, plasma biochemical traits, meat quality and carcass characteristics of offspring broilers at different environmental temperatures (**TEMP**) were investigated in the present study.

MATERIALS AND METHODS

All experimental procedures were approved by the Animal Management Committee (in charge of animal welfare) of the Institute of Animal Science, Chinese Academy of Agricultural Sciences (IAS-CAAS, Beijing, China) and performed in accordance with the guidelines. Ethical approval on animal survival was given by the Animal Ethics Committee of IAS-CAAS.

Experimental Design and Treatments

In experiment 1 (Exp. 1), there were a total of 6 treatments with 6 replicates per treatment in a completely randomized design. These treatments included 0 (the sterilized water-injected negative control, **iNC**), 6.25, 12.5, 25.0, or 50.0 μg of injected Mn/egg as reagent grade $MnSO_4 \bullet H_2O$ (iMn, Sinopharm Chemical Reagent Co., Shanghai, China), and the non-injected positive control (**niPC**), respectively. In experiment 2 (Exp. 2), during the embryonic stage, a completely randomized design involving a 2 (injected Mn source) × 2 (injected Mn level) factorial arrangement of treatments plus the niPC and iNC groups was used. The two injected Mn sources were iMn and oMn [feed grade organic Mn-proteinate chelate with a moderate chelation strength, quotient of formation ($\mathbf{Q_f}$) = 61.9, 10.2% Mn by analysis] as described by Zhu et al. (2015c), and the two injected Mn levels were 12.5 and 25.0 μg/egg. There were a total of 6 treatments with 6 replicates per treatment. During the progeny stage, the 6 treatments during D 1 to 28 were the same as during the embryonic stage, while during D 29 to 42, a completely randomized design involving a 6 (injected Mn) × 2 (TEMP) factorial arrangement of treatments was adopted. The 6 injected Mn treatments were the same as during the embryonic stage, and 2 TEMP were 22 °C (normal temperature, **NT**) vs 34°C (high temperature, **HT**). Therefore, there were a total of 12 treatments with 6 replicates per treatment.

Broiler Breeders, Diet, and Egg Collections

One hundred and ten 22-wk-old female Arbor Acres (**AA**) broiler breeders were purchased from a commercial company (Huadu Broiler Company, Beijing, China). Every 2 female broiler breeders were housed in a stainless steel cage coated with plastic (0.50 m wide by 0.50 m deep by 0.50 m long). Lighting and feeding management throughout the experiment followed the AA breeder management guidelines, and ad libitum access to tap water (containing an undetectable Mn) was provided (Zhu et al., 2015b). After a 7-wk adaptation period, all broiler breeders were fed a corn-soybean meal basal diet with no Mn addition (the Mn-deficient diet containing 12.2 mg Mn/kg by analysis, Table 1) to deplete the storage of Mn from 30 to 43 wk of age. The basal corn-soybean meal diet was formulated to meet or exceed the NRC (1994) and China's Feeding Standard of Chicken (2004) requirements for all other nutrients of laying broiler breeders except for Mn. The diet was in mash form. All eggs from the breeders during 41 to 43 wk of age were collected and stored at 13 ± 2°C for the following egg hatching. The average Mn value in the analyzed egg yolks was as low as 9.74 μg/egg. The collected eggs were checked and fertilized for Exp. 2. All of fertilized eggs used in Exp. 1 were purchased from Huadu Broiler Company, Beijing, China, and the determined average Mn content in the egg yolks was about 24 μg/egg, a normal level of Mn in the yolk.

Egg Hatching

All of fertilized eggs were placed on egg trays and incubated at 38.0°C with a relative humidity of 50%.

Table 1. The composition and nutrient levels of the Mn-unsupplemented diet for broiler breeders during a laying period of 30 to 43 wk of age (as-fed basis) (Exp. 2).

Item	Contents
Ingredients, %	
Corn	64.99
Soybean meal	23.50
Soybean oil	1.70
$CaHPO_4$ [1]	1.45
$CaCO_3$ [1]	7.48
NaCl [1]	0.30
DL-Methionine (99%)	0.18
Micronutrients [2]	0.40
Nutrient levels, %	
Metabolizable energy[3], Kcal/kg	2,801
CP[4]	15.60
Lys[3]	0.77
Met[3]	0.40
Met + Cys[3]	0.63
Ca[4]	3.21
Total P[3]	0.55
Nonphytate P[3]	0.33
Mn[4], mg/kg	12.20

[1]Reagent grade.
[2]Provided per kilogram of diet for laying period 30 to 43 wk of age: vitamin A (alltrans-retinol acetate), 15,000 IU; cholecalciferol, 4,500 IU; vitamin E (all-rac-α-tocopherol acetate), 36 IU; vitamin K (menadione sodium bisulfate), 3.9 mg; thiamin (thiamin mononitrate), 4.5 mg; riboflavin, 10.5 mg; vitamin B_6, 4.5 mg; vitamin B_{12}, 0.024 mg; calcium pantothenate, 18 mg; niacin, 39 mg; folic acid, 1.5 mg; biotin, 0.18 mg; choline (choline chloride), 1,000 mg; Cu ($CuSO_4 \cdot 5H_2O$), 10 mg; Fe ($FeSO_4 \cdot 7H_2O$), 50 mg; Zn ($ZnSO_4 \cdot 7H_2O$), 100 mg; I (KI), 2.0 mg; Se (Na_2SeO_3), 0.30 mg.
[3]Calculated values.
[4]Values determined by analysis; each value is based on triplicate determinations.

The incubation procedure and management were carried out as described by Sun et al. (2018a,b).

Preparation of Injected Mn Solutions and In Ovo Injection Procedure

Preparation of injected Mn solutions and the in ovo injection procedure were carried out as described by Sun et al. (2018a,b). Briefly, either iMn or oMn was dissolved in deionized water to make a stock solution containing 2,000 μg/mL, and the stock solution was subsequently diluted with deionized water to make injection solutions containing 62.5, 125, 250, and 500 μg Mn mL^{-1}, respectively. All solutions were filtered using a 0.22-μm acetate filter (MSI, Westborough, MA, USA). The injection solutions were analyzed to contain 61.0, 121, 238, and 472 μg Mn mL^{-1}, respectively.

In Exp. 1, a total of 684 fertilized eggs with similar weights were randomly allotted to 1 of 6 treatments with 6 replicates per treatment and 19 eggs per replicate according to the above experimental design and treatments. The eggs with a normal level of Mn in the yolk were either not injected or injected with 0.1 mL of the sterilized water containing 0, 6.25, 12.5, 25.0, or 50.0 μg Mn/egg as iMn on the embryonic D 9 of incubation. Dead embryos during hatching, hatched birds and chick hatch weights at the end of hatching were recorded, and the embryonic mortality and hatchability were calculated as described by Sun et al. (2018a,b).

In Exp. 2, a total of 792 fertilized eggs with similar weights were randomly allotted to 1 of 6 treatments with 6 replicates per treatment and 22 eggs per replicate according to the above experimental design and treatments during the embryonic stage. The eggs with a very low Mn content in the yolk were either not injected or injected with 0.1 mL of the sterilized water containing 0, 12.5, or 25.0 μg Mn/egg as either iMn or oMn, respectively on the embryonic D 9 of incubation. As in Exp. 1, dead embryos during hatching as well as hatched birds, healthy chicks, and chick hatch weights at the end of hatching were recorded, and the embryonic mortality, hatchability, healthy chick ratios were calculated as described by Sun et al. (2018a,b).

Offspring Broilers and Diets

During the progeny stage of Exp. 2, one-day-old offspring broilers from each replicate of the 6 embryonic treatments were fed the same Mn-adequate corn-soybean meal diet until D 28 at a normal temperature, and then eight 29-day-old offspring broilers (4 males and 4 females) from each replicate of the 6 embryonic treatments were allotted to 2 TEMP (22°C vs. 34°C) groups (2 males and 2 females per replicate cage) based on gender and body weight, and fed the same Mn-adequate corn-soybean meal diet until D 42. Other feeding managements were followed according to the AA guidelines for feeding and managements. The broiler's weights and feed intakes, and the number of dead broilers were recorded based on each replicate on D 1, 21, 28 or 42 in order to calculate the ADG, ADFI, F/G and mortality of broilers during D 1 to 21, 22 to 28 and 29 to 42.

The Mn-adequate corn-soybean meal diets (Table 2) were formulated to meet or exceed the NRC (1994) requirements of broilers for all nutrients at 1 to 21 and 22 to 42 D of age. The diets were fed to birds in mash form.

Sample Collections and Preparations

The samples from the 3 diets in Exp. 2 were collected after the diets formulated in our laboratory. The collected diet samples were ground into 200-mm sieve for analyses of CP, Ca, and Mn contents. The tap water sample was collected from the experimental chicken house also for Mn concentration determination.

The egg samples from broiler breeders in Exp. 1 were collected from Huadu Broiler Company, Beijing, China. At each weekend during 30 to 43 wk of age in Exp. 2, 16 eggs were randomly collected. The above egg samples were used for the analysis of Mn contents in egg yolks.

In Exp. 2, at 20 D of incubation, the heart samples of five live embryos from each replicate were collected and stored at −20°C for assays of MnSOD activity and malonaldehyde (**MDA**) content or in liquid nitrogen for the *MnSOD* mRNA expression assay. On D 42 of offspring broilers, after a 12 h fast, 2 birds (1 male and 1 female) from each replicate cage were chosen based on the cage

Table 2. The composition and nutrient levels of the basal diets for offspring broilers during D 1 to 21 and 22 to 42 (as-fed basis) (Exp. 2).

Item	Starter diet (D 1 to 21)	Grower diet (D 22 to 42)
Ingredients, %		
Corn	52.29	56.47
Soybean meal	38.95	34.98
Soybean oil	4.54	5.00
$CaHPO_4$[1]	2.13	1.65
Limestone[1]	1.08	1.19
Salt[1]	0.30	0.30
DL-Methionine	0.34	0.20
L-Lys sulphate[1]	0.04	0.00
Micronutrients[2]	0.33	0.21
Nutrient levels, %		
Metabolizable energy, [3]Kcal/kg	2,990	3,085
CP[4]	21.77	20.20
Lys[3]	1.19	1.06
Met[3]	0.63	0.48
Met + Cys[3]	0.93	0.76
Ca[4]	1.05	0.97
Total P[3]	0.76	0.65
Nonphytate P[3]	0.45	0.35
Mn[4], mg/kg	88.90	73.80

[1]Feed grade.

[2]Provided per kilogram of starter diet: VA, 15,000 IU; VD_3, 4,500 IU; VE, 24 IU; VK_3, 3 mg; VB_1, 3 mg; VB_2, 9.6 mg; VB_6, 3 mg; VB_{12}, 0.018 mg; pantothenic acid calcium, 15 mg; niacin, 39 mg; folic acid, 1.5 mg; biotin, 0.15 mg; choline, 700 mg; Cu ($CuSO_4{\cdot}5H_2O$), 8 mg; Fe ($FeSO_4{\cdot}7H_2O$), 60 mg; Mn ($MnSO_4{\cdot}H_2O$), 60 mg; Zn ($ZnSO_4{\cdot}7H_2O$), 60 mg; I (KI), 0.35 mg; Se (Na_2SeO_3), 0.15 mg; aureomycin, 50 mg. Provided per kilogram of grower diet: VA, 10,000 IU; VD_3, 3,000 IU; VE, 16 IU; VK_3, 2 mg; VB_1, 2 mg; VB_2, 6.4 mg; VB_6, 2 mg; VB_{12}, 0.012 mg; pantothenic acid calcium, 10 mg; niacin, 26 mg; folic acid, 1.0 mg; biotin, 0.1 mg; choline, 500 mg; Cu ($CuSO_4{\cdot}5H_2O$), 8 mg; Fe ($FeSO_4{\cdot}7H_2O$), 40 mg; Mn ($MnSO_4{\cdot}H_2O$), 50 mg; Zn ($ZnSO_4{\cdot}7H_2O$), 40 mg; I (KI), 0.35 mg; Se (Na_2SeO_3), 0.15 mg.

[3]Calculated values.

[4]Values determined by analysis; each value is based on triplicate determinations.

average BW of birds, weighed individually, and then blood samples of 5 mL were collected from the wing vein of each bird in 10 mL tubes without an anticoagulant and immediately centrifuged for 10 min at 3,000 × *g* at 4°C to harvest plasma samples. These plasma samples were stored at −20°C for analyses of related biochemical traits. The heart samples from 5 live embryos and the plasma samples from 2 birds in each replicate cage were pooled into one sample in the same equal ratio prior to the following analyses.

Measurements of Carcass Traits and Meat Quality

Carcass traits and meat quality were measured following the procedures described by Liu et al. (2011) and Wang et al. (2021). Briefly, the 2 broilers from each replicate cage were anesthetized and euthanized immediately by intravenous injection of sodium pentobarbital (40 mg/kg of BW) after the blood samples were collected. Heads and feet were removed from the broilers, and then the carcasses were eviscerated and weighed to determine the % of dressing and eviscerated yield. The % of abdominal fat, breast, and thigh muscles were calculated after the abdominal fat, breast, and thigh muscles were dissected and weighed. The pH values of the right-side breast and thigh muscles in each broiler were measured immediately after the broilers were euthanized using a Model pH-211 meter (Hanna Instruments Inc., Padova, Italy) equipped with a spear electrode. The meat color [light values (L*), red values (a*) and yellow values (b*)] was determined at 3 points of every meat using an automatic colorimeter (model WSC-S; Shanghai Precision and Scientific Instrument Co., Shanghai, China).

Determinations of CP, Ca, and Mn Contents

The CP and Ca contents in feed ingredients and diets were determined following the procedures published by the Association of Official Analytical Chemists (1990). The Mn concentrations in diets, tap water, and yolks of breeder eggs were determined by inductively coupled plasma spectrometer (Model IRIS Intrepid II; Thermal Jarrell Ash, Waltham, MA, USA) as described by Li et al. (2011b) and Zhu et al. (2015b).

Biochemical Analyses

Plasma triiodothyronine (**T3**) and thyroxin (**T4**) levels were determined by RIA using a commercial kit (Beijing North Institute of Biological Technology, Beijing, China). Plasma uric acid (**UA**), Total cholesterol (**TC**), and triglyceride (**TG**) contents and aspartate aminotransferase (**AST**), lactic dehydrogenase (**LDH**), creatine kinase (**CK**), and alkaline phosphatase (**ALP**) activities were measured using a HITACHI 7180 automatic biochemical analyzer (Hitachi Ltd., Tokyo, Japan) with the detection kits (Nanjing JianCheng Bioengineering Institute, Nanjing, China), respectively. The MnSOD activity in the embryonic heart was measured using the nitrite method described by Li et al. (2011a,b). The MDA level in the embryonic heart was determined using a commercial assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Quantitative Real-Time PCR Assays

The total RNA in the embryonic heart was isolated using TRIzol reagent (Life Technologies, Carlsbad, CA) according to the manufacturer's protocols. The cDNA synthesis was performed using PrimeScript RT Reagent Kit with cDNA Eraser (Qiagen, Chatsworth, CA) according to the manufacturer's instructions. The protocol for PCR was as follows: denaturation at 95°C for 2 min followed by 40 cycles of 95°C for 60 s, 60°C for 30 s, and 72°C for 30 s. The geometric means of *β-actin* and glyceraldehyde 3-phosphate dehydrogenase (***GAPDH***) were used as references to normalize the expression of the target gene. The internal references and calculation of the relative mRNA expression for *MnSOD* target gene were used as described previously (Li et al., 2021). The primer sequences are listed in Table 3.

Table 3. Primers used for the target and reference genes (Exp. 2).

Genes	Gene bank accession number	Products length, bp	Primer sequences(5'-3')
β-actin	NM205518.1	95	F: ACCTGAGCGCAAGTACTCTGTCT R: CATCGTACTCCTGCTTGCTGAT
GAPDH	NM204305.1	128	F: CTTTGGCATTGTGGAGGGTC R: 5'-ACGCTGGGATGATGTTCTGG
MnSOD	NM_204211.2	215	F: AGGAGGGGAGCCTAAAGGAGA R: 5'-CCAGCAATGGAATGAGACCTG

Abbreviations: F, forward; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; *MnSOD*, manganese-containing superoxide dismutase; R, reverse.

Statistical Analyses

Data from Exp. 1 were analyzed by one-way ANOVA using the GLM procedure of SAS 9.4 (SAS Institute, 2013). The model included only the injected Mn level. As for the data from the embryonic stage and the progeny stage (D 1 to 21 and 22 to 28) of Exp. 2, in order to evaluate the puncture injury of in ovo injection, the T test was used to compare the iNC with the niPC. To test the effect of Mn injections, the single degree of freedom contrast was used to compare all injected Mn treatments with the iNC. The data excluding the iNC and niPC were then analyzed as a 2 (injected Mn source) × 2 (injected Mn level) factorial arrangement by two-way ANOVA using the GLM procedure. The model included the main effects of injected Mn source, injected Mn level, and their interaction. However, the data from the progeny stage (D 29−42) of Exp. 2 were analyzed as a 6 (injected Mn) × 2 (TEMP) factorial arrangement by two-way ANOVA using the GLM procedure, and the model included injected Mn, TEMP and their interaction. The LSD method was used to test the differences between treatment means (Wang et al., 2021). Percentage data were transformed to arcsine for analyses. Each replicate served as an experimental unit. Significant differences were set at $P \leq 0.05$.

RESULTS

Embryonic Development and Hatchability (Exp. 1 and 2)

In Exp. 1, the in-ovo injected Mn affected ($P < 0.03$) the hatchability, but had no effect ($P > 0.06$) on the embryonic mortality and chick hatch weight (Table 4). Compared to the niPC, the iNC decreased ($P \leq 0.05$) the hatchability. Compared to the iNC, Mn injection had no effect ($P > 0.05$) on the hatchability, however, the 50.0 μg Mn/egg group decreased ($P \leq 0.05$) the hatchability compared to the niPC and the 25.0 μg Mn/egg group. There were no differences ($P > 0.05$) in the hatchability between the 6.25, 12.5, and 25.0 μg Mn/egg groups and between the niPC and the 25.0 ug Mn/egg group.

In Exp. 2, no differences ($P > 0.05$) were observed in the embryonic mortality, hatchability, healthy chick ratio, or hatch weight of chicks between the niPC and the iNC (Table 5). Compared to the iNC, Mn injection had no effect ($P > 0.05$) on any of the above indices. Injected Mn source, injected Mn level, and their interaction also had no effect ($P > 0.13$) on any of the above indices.

MnSOD Activity, mRNA Expression Level, and MDA Content in the Embryonic Heart (Exp. 2)

No differences ($P > 0.05$) were observed in MnSOD activity, mRNA expression, or MDA content in the heart of chick embryos between the niPC and the iNC (Table 6). Compared to the iNC, Mn injection enhanced ($P \leq 0.05$) the *MnSOD* mRNA expression level in the embryonic heart, but had no effect ($P > 0.05$) on its MnSOD activity and MDA content. Injected Mn source, injected Mn level, and their interaction also had no effect ($P \geq 0.07$) on the above indices.

Growth Performance of Offspring Broilers at 1 to 21 and 22 to 28 D of Age (Exp. 2)

No differences ($P > 0.05$) were observed in ADFI, ADG, F/G and mortality at 1 to 21 and 22 to 28 D of age between the niPC and the iNC or between the iNC and Mn injection groups (Table 7). Injected Mn source, injected Mn level, and their interaction also had no effect ($P \geq 0.14$) on all of the above indices.

Growth Performance of Offspring Broilers at 29 to 42 D of Age (Exp. 2)

Injected Mn and its interaction with TEMP had no effects ($P > 0.14$) on ADG, ADFI, F/G and mortality. However, TEMP significantly affected ($P < 0.0001$) ADG, ADFI and F/G, but not mortality ($P > 0.79$; Table 8). Hyperthermia decreased ($P < 0.0001$) ADG and ADFI, and increased F/G compared to the NT.

Carcass Traits of Offspring Broilers at 42 D of Age (Exp. 2)

Injected Mn affected ($P = 0.002$) the eviscerated yield %, but had no effect ($P > 0.33$) on the % of dressing, breast muscle, thigh muscle and abdominal fat (Table 9). The TEMP affected ($P < 0.05$) the % of dressing, breast muscle and abdominal fat but had no effect ($P > 0.06$) on the % of eviscerated yield and thigh muscle. There were no

Table 4. Effect of in ovo injection of Mn on the embryonic development and hatch performance of fertilized eggs[1] (Exp. 1).

Injected Mn level(μg/egg)	Embryonic mortality, %	Hatchability, %	Chick hatch weight, g/bird
niPC	3.70	96.3[a]	47.4
0.00 (iNC)	10.2	89.8[bc]	47.4
6.25	10.2	89.8[bc]	47.3
12.50	10.2	89.8[bc]	47.7
25.00	7.4	92.6[ab]	48.8
50.00	14.8	85.2[c]	47.9
SEM	2.90	2.10	0.40
P-value	0.07	0.03	0.11

Abbreviations: niPC, non-injected positive control; iNC, injected negative control.
[1]Data represent the means of 6 replicates (n = 6).
[a,b,c]Means within the same column lacking a common superscript differ ($P \leq 0.05$).

interactions ($P > 0.17$) between injected Mn and TEMP for the above indices. Compared to the NT, HT increased ($P < 0.05$) the % of dressing and abdominal fat, but decreased ($P < 0.01$) the % of breast muscle. Among the injected Mn groups, the % of eviscerated yield was higher ($P \leq 0.05$) for the groups with oMn 25.0 μg than the iMn 12.5, iMn 25.0, and oMn 12.5 μg (Mn/egg), whereas no differences ($P > 0.05$) were detected among the oMn 25.0 μg Mn/egg group, the niPC, and the iNC, as well as among the iMn 12.5, iMn 25.0, and oMn 12.5 μg Mn/egg groups.

Meat Quality of Offspring Broilers at 42 D of Age (Exp. 2)

The injected Mn and its interaction with TEMP had no effects ($P > 0.10$) on pH and meat color of breast and thigh muscles (Table 10). The TEMP affected ($P < 0.0001$) the L* and a* of breast and thigh muscles, but had no effect ($P > 0.07$) on their pH and b*. Compared to the NT, HT increased ($P < 0.0001$) the L* of breast and thigh muscles but decreased ($P < 0.0001$) their a*.

Plasma Biochemical Parameters of Offspring Broilers at 42 D of Age (Exp. 2)

The TEMP affected ($P < 0.04$) plasma AST, LDH, ALP, TC, UA, and T3, and had no effect ($P > 0.06$) on plasma CK, TG, and T4 (Table 11). Injected Mn and its interaction with TEMP had no effects ($P > 0.14$) on any of the above plasma indices. Compared to the NT, HT elevated ($P < 0.01$) plasma AST and LDH activities as well as plasma TC, UA, and T3 contents, but decreased ($P < 0.04$) plasma ALP activity.

DISCUSSION

The hypothesis that in ovo Mn injection might have a positive effect on the embryonic development and progeny growth performance and related aspects under different thermal conditions has been partly supported by the results of the present study. In the present study, in ovo Mn injection into the breeder eggs with a very low Mn content in the yolk significantly enhanced the *MnSOD* mRNA expression level in the embryonic heart, suggesting that Mn injection might enhance the

Table 5. Effect of in ovo injection of Mn on the embryonic development and hatch performance of fertilized eggs (Exp. 2).

Injected Mn source	Injected Mn level, μg/egg	Embryonic mortality, %	Hatchability,%	Healthy chick ratio, %	Chick hatch weight, g/bird
niPC[1]	0	12.9	87.1	87.1	47.8
iNC[1]	0	12.9	87.1	87.1	47.2
iMn[1]	12.5	7.60	92.4	92.4	46.9
	25.0	10.6	89.4	88.6	46.8
oMn[1]	12.5	10.6	90.9	87.1	46
	25.0	10.6	90.2	86.4	46.8
SEM		3.15	4.68	2.73	0.35
Injected Mn source[2]	iMn	17.3	90.9	90.5	46.9
	oMn	17.6	90.5	86.8	46.4
SEM		1.80	1.60	1.70	0.35
Injected Mn level[2] (μg/egg)	12.5	17.0	91.7	89.8	46.5
	25.0	17.9	89.8	87.5	46.8
SEM		1.76	1.60	1.70	0.35
P-value					
Injected Mn source		0.89	0.87	0.13	0.40
Injected Mn level		0.70	0.41	0.36	0.47
Mn source × Mn level		0.42	0.62	0.53	0.42

Abbreviations: niPC, non-injected positive control; iNC, injected negative control; iMn, the inorganic $MnSO_4 \cdot H_2O$; oMn, the organic Mn proteinate with a moderate chelation strength ($Q_f = 61.9$).
[1]Data represent the means of 6 replicates (n = 6).
[2]Data represent the means of 12 replicates (n = 12).

Table 6. Effect of in ovo injection of Mn on the MnSOD activity, mRNA expression level and MDA content in the heart of chick embryos at 20 D of incubation (Exp. 2).

Injected Mn source	Injected Mn level, μg/egg	MnSOD activity, U/mg protein	*MnSOD* mRNA, RQ[3]	MDA, nmol/mg protein
niPC[1]	0	17.0	1.00	3.52
iNC[1]	0	15.8	0.99*	3.90
iMn[1]	12.5	14.1	1.19	3.38
	25.0	15.1	1.21	3.05
oMn[1]	12.5	14.0	1.15	3.47
	25.0	15.9	1.38	2.57
SEM		0.49	0.03	0.26
Injected Mn source[2]	iMn	14.6	1.20	3.21
	oMn	14.9	1.27	3.02
SEM		0.73	0.04	0.42
Injected Mn level[2] (μg/egg)	12.5	14.1	1.17	3.42
	25.0	15.5	1.29	2.81
SEM		0.73	0.04	0.42
P-value				
Injected Mn source		0.74	0.30	0.75
Injected Mn level		0.20	0.07	0.32
Mn source × Mn level		0.69	0.11	0.63

Abbreviations: niPC, non-injected positive control; iNC, injected negative control; iMn, the inorganic $MnSO_4 \cdot H_2O$; oMn, the organic Mn proteinate with a moderate chelation strength ($Q_f = 61.9$); MDA, malonaldehyde.

[1]Data represent the means of 6 replicates (n = 6).

[2]Data represent the means of 12 replicates (n = 12).

[3]The mRNA expressions were calculated as the relative quantities (RQ) of *MnSOD* mRNA to the geometric mean of *β-actin* and *GAPDH* mRNA using the $2^{-\Delta\Delta Ct}$ method.

*Different from all of the Mn injection treatments ($P \leq 0.05$).

antioxidant ability in the chick embryonic heart, although no significant influence was detected in the subsequent performances of offspring broilers. In our previous studies, we found that maternal dietary Mn supplementation played an important role in chick embryonic development and antioxidation (Zhu et al., 2015b; Liao et al., 2019). Therefore, these results indicate that both maternal dietary Mn supplementation and in ovo Mn injection enhanced the chick embryonic antioxidation. These findings will update our knowledge in providing a more effective nutritional strategy for protecting chick embryos against oxidative damages induced by heat stress.

In our previous study, we have demonstrated that in ovo injection at the embryonic D 9 of incubation had a minimal effect on the embryonic viability (Sun et al., 2018a). However, there is a paucity of data on the optimal level of in ovo Mn injection of the chick embryos. The results of this study showed that in ovo injection of 50 μg Mn into a breeder egg with a normal Mn level in the yolk decreased the hatchability about 14.3%, indicating that the injection of a higher dose of Mn (50 μg)

Table 7. Effect of in ovo injection of Mn on the growth performance and mortality of offspring broilers at 1 to 21 and 22 to 28 D of age (Exp. 2).

		D 1 to 21				D 22 to 28			
Injected Mn source	Injected Mn level, μg/egg	ADG, g	ADFI, g	F/G, g/g	Mortality, %	ADG, g	ADFI, g	F/G, g/g	Mortality, %
niPC[1]	0	33.1	42.8	1.29	10.6	66.1	101	1.53	0.00
iNC[1]	0	32.6	42.9	1.32	18.6	65.4	108	1.65	0.00
iMn[1]	12.5	33.7	44.6	1.32	5.87	67.9	106	1.57	4.02
	25.0	33.3	43.4	1.30	13.4	66.1	105	1.60	0.00
oMn[1]	12.5	33.6	43.9	1.31	14.6	64.4	100	1.55	4.02
	25.0	33.1	43.5	1.31	16.4	69.1	102	1.49	4.51
SEM		0.64	0.74	0.02	4.76	1.37	2.98	0.05	2.19
Injected Mn source[2]	iMn	44.0	33.5	1.31	9.6	67.0	106	1.58	2.00
	oMn	43.7	33.3	1.31	15.5	66.8	101	1.52	4.30
SEM		0.57	0.47	0.01	3.44	0.72	1.52	0.02	1.20
Injected Mn level[2] (μg/egg)	12.5	44.2	33.6	0.47	10.2	66.2	103	1.56	4.00
	25.0	43.4	33.2	1.32	14.9	67.6	104	1.54	2.30
SEM		0.57	0.47	1.31	3.45	0.72	1.52	0.02	1.20
P-value									
Injected Mn source		0.69	0.83	0.93	0.24	0.14	0.86	0.19	0.36
Injected Mn level		0.35	0.51	0.78	0.35	0.80	0.31	0.70	0.48
Mn source × Mn level		0.64	0.95	0.41	0.57	0.52	0.26	0.33	0.36

Abbreviations: niPC, non-injected positive control; iNC, injected negative control; iMn, the inorganic $MnSO_4 \cdot H_2O$; oMn, the organic Mn proteinate with moderate chelation strength ($Q_f = 61.9$).

[1]Data represent the means of 6 replicates (n = 6).

[2]Data represent the means of 12 replicates (n = 12).

Table 8. Effects of in ovo injection of Mn and environmental temperature (TEMP) on growth performance of offspring broilers at 29 to 42 D of age (Exp. 2).

	NT (22 ± 1°C)[1]						HT (34 ± 1°C)[1]							Injected Mn[2]							TEMP[3]			*P*-value		
Item	niPC	iNC	iMn12.5[1]	iMn25.0[1]	oMn12.5[1]	oMn25.0[1]	niPC	iNC	iMn12.5[1]	iMn25.0[1]	oMn12.5[1]	oMn25.0[1]	SEM	niPC	iNC	iMn12.5[1]	iMn25.0[1]	oMn12.5[1]	oMn25.0[1]	SEM	NT	HT	SEM	Mn	TEMP	Mn ×TEMP
ADG, g	98.4	92.1	98.4	98.9	95.6	98.1	47.3	47.4	50.9	51.8	44.7	45.8	2.25	72.9	69.8	74.7	75.3	70.1	72	2.07	96.9[a]	48.0[b]	1.19	0.37	< 0.0001	0.78
ADFI, g	161	162	169	158	160	167	106	109	110	116	112	106	3.82	133	135	139	137	136	137	3.18	163[a]	110[b]	1.84	0.85	< 0.0001	0.32
F/G, g/g	1.64	1.76	1.71	1.60	1.68	1.70	2.28	2.33	2.22	2.27	2.51	2.34	0.01	1.96	2.05	1.97	1.93	2.09	2.02	0.05	1.68[b]	2.32[a]	0.03	0.30	< 0.0001	0.42
Mortality, %	5.88	0.00	0.00	9.02	0.00	0.00	8.03	0.00	9.89	0.00	0.00	0.00	0.00	6.95	0.00	4.95	4.51	0.00	0.00	2.37	2.48	2.99	1.37	0.14	0.79	0.17

Abbreviations: NT, normal temperature; HT, high temperature; niPC, non-injected positive control; iNC, injected negative control; iMn 12.5 and iMn 25.0, in ovo injection of the inorganic $MnSO_4 \cdot H_2O$ at the doses of 12.5 and 25.0 μg Mn/egg, respectively; oMn 12.5 and oMn 25.0, in ovo injection of the organic Mn proteinate with a moderate chelation strength ($Q_f = 61.9$) at the doses of 12.5 and 25.0 μg Mn/egg, respectively.

[1]Data represent the means of 6 replicates (n = 6).

[2]Data represent the means of 12 replicates (n = 12).

[3]Data represent the means of 36 replicates (n = 36).

[a,b]Means within a row lacking a common superscript differ ($P \leq 0.05$).

Table 9. Effects of in ovo injection of Mn and environmental temperature (TEMP) on carcass traits of offspring broilers at 42 D of age (Exp. 2).

	NT (22 ± 1°C)[1]						HT (34 ± 1°C)[1]							Injected Mn[2]							TEMP[3]			*P*-value		
Item	niPC	iNC	iMn 12.5[1]	iMn 25.0[1]	oMn12.5[1]	oMn 25.0[1]	niPC	iNC	iMn 12.5[1]	iMn 25.0[1]	oMn12.5[1]	oMn25.0[1]	SEM	niPC	iNC	iMn 12.5[1]	iMn 25.0[1]	oMn12.5[1]	oMn25.0[1]	SEM	NT	HT	SEM	Mn	TEMP	Mn ×TEMP
Dressing, %	92.4	91.9	91.6	91.8	91.7	92.2	92.9	92.9	92.7	92.8	92.8	93.1	0.23	92.7	92.4	92.1	92.3	92.3	92.6	0.20	91.9[b]	92.9[a]	0.12	0.47	<0.0001	0.91
Eviscerated yield, %	75.6	75.2	74.4	74.2	74.8	75.6	76.1	75.3	74.8	74.7	75.0	76.0	0.44	75.9[a]	75.2[a,b]	74.6[b]	74.5[b]	74.9[b]	75.8[a]	0.30	74.9	75.3	0.18	0.002	0.14	1.00
Breast muscle, %	26.7	26.7	28.8	26.9	26.6	29.0	24.6	25.8	24.7	25.4	25.2	25.4	0.59	25.6	26.2	26.7	26.2	25.9	27.2	0.50	27.5[a]	25.2[b]	0.30	0.34	<0.0001	0.18
Thigh muscle, %	21.3	20.9	21.0	21.1	21.2	21.4	21.9	22.1	21.4	22.4	21.0	21.8	0.60	21.6	21.5	21.2	21.8	21.1	21.6	0.41	21.2	21.8	0.23	0.87	0.07	0.75
Abdominal fat, %	1.48	1.72	1.71	1.86	1.56	1.72	1.8	1.57	1.96	1.96	2.04	1.77	0.15	1.64	1.65	1.84	1.91	1.8	1.74	0.10	1.67[b]	1.85[a]	0.06	0.41	0.04	0.34

Abbreviations: NT, normal temperature; HT, high temperature; niPC, non-injected positive control; iNC, injected negative control; iMn 12.5 and iMn 25.0 mean in ovo injection of the inorganic $MnSO_4 \cdot H_2O$ at the doses of 12.5 and 25.0 μg Mn/egg, respectively; oMn 12.5 and oMn 25.0, in ovo injection of the organic Mn proteinate with a moderate chelation strength ($Q_f = 61.9$) at the doses of 12.5 and 25.0 μg Mn/egg, respectively.

[1]Data represent the means of 6 replicates (n = 6).

[2]Data represent the means of 12 replicates (n = 12).

[3]Data represent the means of 36 replicates (n = 36).

[a,b]Means within a row lacking a common superscript differ ($P \leq 0.05$).

Table 10. Effects of in ovo injection of Mn and environmental temperature (TEMP) on meat quality of offspring broilers at 42 D of age (Exp. 2).

	NT (22 ± 1°C)[1]						HT (34 ± 1°C)[1]							Injected Mn[2]							TEMP[3]			*P*-value		
Item	niPC	iNC	iMn 12.5[1]	iMn 25.0[1]	oMn 12.5[1]	oMn25.0[1]	niPC	iNC	iMn 12.5[1]	iMn 25.0[1]	oMn 12.5[1]	oMn 25.0[1]	SEM	niPC	iNC	iMn 12.5[1]	iMn 25.0[1]	oMn 12.5[1]	oMn25.0[1]	SEM	NT	HT	SEM	Mn	TEMP	Mn ×TEMP
Breast muscle																										
pH	6.73	6.73	6.75	6.66	6.75	6.73	6.74	6.71	6.74	6.73	6.76	6.69	0.06	6.73	6.72	6.74	6.69	6.75	6.71	0.02	6.72	6.72	0.01	0.33	0.96	0.56
L*	52.1	50.8	51.1	52.8	51.9	51.0	54.6	54.0	54.5	54.3	53.3	54.3	0.79	53.3	52.4	52.8	53.6	52.6	52.6	0.58	51.6[b]	54.2[a]	0.33	0.70	<0.0001	0.70
a*	11.6	12.2	11.5	11.2	12.2	11.7	11.3	8.73	11.0	9.18	10.1	9.0	0.50	11.4	10.4	11.3	10.2	11.1	10.3	0.53	11.7[a]	9.89[b]	0.30	0.39	<0.0001	0.25
b*	8.34	8.56	8.61	8.42	9.39	8.08	8.39	6.9	9.22	7.53	7.81	8.36	0.53	8.37	7.73	8.92	7.98	8.6	8.22	0.36	8.57	8.04	0.21	0.22	0.07	0.11
Thigh muscle																										
pH	6.75	6.81	6.81	6.77	6.77	6.78	6.8	6.8	6.8	6.8	6.82	6.8	0.02	6.78	6.80	6.80	6.79	6.79	6.79	0.02	6.78	6.8	0.01	0.90	0.11	0.65
L*	52.6	51.1	51.5	52.6	53.2	51.5	55.2	54.7	54.0	55.4	54.7	55.2	0.66	53.9	52.9	52.8	54	54	53.3	0.53	52.1[b]	54.9[a]	0.31	0.41	<0.0001	0.66
a*	13.8	13.0	13.2	13.3	13.3	12.8	10.3	11.3	12.2	12.4	11.6	11.3	0.45	12.1	12.1	12.7	12.9	12.4	12.1	0.39	13.2[a]	11.5[b]	0.23	0.57	<0.0001	0.22
b*	10.7	10.3	10.2	10.3	10.5	9.57	9.33	9.77	10.2	10.3	9.49	9.68	0.32	10.00	10.1	10.2	10.3	10.0	9.62	0.32	10.3	9.79	0.18	0.78	0.08	0.47

Abbreviations: NT, normal temperature; HT, high temperature; niPC, non-injected positive control; iNC, injected negative control; iMn 12.5 and iMn 25.0, in ovo injection of the inorganic $MnSO_4 \cdot H_2O$ at the doses of 12.5 and 25.0 μg Mn/egg, respectively; oMn 12.5 and oMn 25.0, in ovo injection of the organic Mn proteinate with a moderate chelation strength (Q_f = 61.9) at the doses of 12.5 and 25.0 μg Mn/egg, respectively; L*, light values; a*, red values; b*, yellow values.

[1]Data represent the means of 6 replicates (n = 6).

[2]Data represent the means of 12 replicates (n = 12).

[3]Data represent the means of 36 replicates (n = 36).

[a,b]Means within a row lacking a common superscript differ ($P \leq 0.05$).

Table 11. Effects of in ovo injection of Mn and environmental temperature (TEMP) on plasma biochemical parameters of offspring broilers at 42 D of age (Exp. 2).

	NT (22 ± 1°C)[1]						HT (34 ± 1°C)[1]							Injected Mn[2]							TEMP[3]			*P*-value		
Item	niPC	iNC	iMn 12.5[1]	iMn 25.0[1]	oMn 12.5[1]	oMn 25.0[1]	niPC	iNC	iMn12.5[1]	iMn 25.0[1]	oMn12.5[1]	oMn25.0[1]	SEM	niPC	iNC	iMn12.5[1]	iMn 25.0[1]	oMn12.5[1]	oMn 25.0[1]	SEM	NT	HT	SEM	Mn	TEMP	Mn × TEMP
TC, mmol/L	0.72	0.81	0.51	0.80	0.98	1.20	1.93	1.42	1.27	1.22	1.55	1.67	0.10	1.32	1.11	0.89	1.01	1.26	1.44	0.20	0.84[b]	1.51[a]	0.12	0.43	< 0.0001	0.77
TG, mmol/L	0.29	0.36	0.19	0.25	0.29	0.44	0.33	0.38	0.38	0.35	0.36	0.38	0.01	0.31	0.37	0.29	0.30	0.33	0.41	0.04	0.31	0.36	0.02	0.14	0.06	0.20
AST, U/L	71.3	105	63.4	92.2	108	170	264	218	216	180	150	284	83.9	168	162	140	136	129	227	32.7	102[b]	219[a]	18.9	0.34	< 0.0001	0.68
LDH, U/L	309	504	324	423	426	737	866	665	645	600	522	802	186	587	584	484	511	473	769	101	454[b]	683[a]	59.2	0.34	0.01	0.54
ALP, U/L	1,580	1,586	622	1,063	1,906	1,302	774	762	781	531	753	814	121	1,177	1,174	701	797	1,330	1,058	335	1,343[a]	736[b]	194	0.75	0.03	0.82
CK, U/L	364	424	380	500	360	594	581	537	519	432	578	551	210	472	481	450	466	469	573	76.9	437	533	44.3	0.89	0.13	0.67
UA, μmol/L	71.7	89.0	53.3	75.4	104	117	322	170	172	165	185	222	26.4	197	130	113	120	145	169	29.7	85.0[b]	206[a]	17.1	0.36	< 0.0001	0.32
T3, ng/mL	1.29	1.27	1.30	1.34	1.36	1.30	1.89	2.23	1.84	1.85	1.97	1.54	0.12	1.59	1.75	1.57	1.60	1.66	1.42	0.11	1.31[b]	1.89[a]	0.06	0.40	< 0.0001	0.33
T4, ng/mL	44.2	41.2	44.3	47.7	44.9	49.9	60.0	45.5	47.3	45.9	46.2	48.3	1.44	47.6	43.3	45.8	46.8	45.5	49.1	1.45	45.3	47.4	0.84	0.12	0.09	0.28

Abbreviations: ALP, alkaline phosphatase; AST, aspartate aminotransferase; CK, creatine kinase; LDH, lactic dehydrogenase; NT, normal temperature; HT, high temperature; niPC, non-injected positive control; iNC, injected negative control; iMn 12.5 and iMn 25.0, in ovo injection of the inorganic $MnSO_4 \cdot H_2O$ at the doses of 12.5 and 25.0 μg Mn/egg, respectively; oMn 12.5 and oMn 25.0, in ovo injection of the organic Mn proteinate with a moderate chelation strength (Q_f = 61.9) at the doses of 12.5 and 25.0 μg Mn/egg, respectively; TC, total cholesterol; TG, triglyceride; UA, uric acid; T3, triiodothyronine; T4, thyroxin.

[1]Data represent the means of 6 replicates (n = 6).

[2]Data represent the means of 12 replicates (n = 12).

[3]Data represent the means of 36 replicates (n = 36).

[a,b]Means within a row lacking a common superscript differ ($P \leq 0.05$).

to the egg containing a normal Mn content is harmful to the embryonic development. The maximum level of in ovo Mn injection from this study was 25 μg Mn/egg.

The results from our previous studies also demonstrated that a maternal diet without Mn supplementation significantly decreased the egg hatchability and increased the embryonic mortality, even though the productive performance of the broiler breeders was not affected by the supplementation of Mn (Zhu et al., 2015b,c, 2017). These results imply that the Mn content in the corn-soybean meal basal diet might be sufficient for the maintenance of egg production, but not enough for the deposition in the eggs for normal embryonic development. Klimis-Tavantzis et al. (1983) reported that when the eggs contained a low amount of Mn, the embryos were unable to initiate or to complete the process of hatching and died during the incubation. However, in the current study, in ovo Mn injection did not influence the embryonic development, and no obvious abnormality or death was observed in hatched chicks of breeder eggs with a very low Mn content in the yolk, suggesting that the yolk Mn content of these eggs might be not low enough to have a response to the in ovo Mn injection. In addition, in ovo Mn injection in the present study did not affect the subsequent offspring broiler performances either, possibly because the sufficient Mn in the diets of offspring broilers has covered the effect of Mn injection during the embryonic period. Therefore, further studies are needed using the breeder eggs containing a much lower Mn in the yolk and Mn-deficient diets of offspring broilers.

Manganese, as a crucial component of MnSOD, might alleviate oxidative stress induced by environmental stressors such as HT in broilers via detoxification of superoxide free radicals (Zhu et al., 2015b). The MnSOD activity is a sensitive criterion for assessing Mn status and requirements for rats (Paynter, 1980) and chicks (Luo et al., 1991). The increases in the activities of SOD enzymes might protect embryos against peroxidative damage induced by heat stress via scavenging superoxide radicals, as suggested by Yamashita et al. (1997) and Zhu et al. (2015b). Dietary Mn deficiency has been reported to decrease the activities of MnSOD in tissues of broilers (Luo et al., 1992). Further studies from our laboratory have demonstrated that dietary supplementation with Mn increased heart MnSOD activity and mRNA expression (Li et al., 2004, 2011b) and reduced lipid peroxidation in broilers (Lu et al., 2007). In addition, Mn concentrations were significantly lower in the heart than in other soft tissues of broilers, such as the liver and pancreas. However, MnSOD activity in the heart was significantly higher and very sensitive to supplemental Mn levels in a corn-soybean meal diet (Luo et al., 1991, 2007). The results from our previous studies (Luo et al., 1991, 2007) have also shown that broiler chicks that received a diet containing 17 mg Mn/kg developed Mn-responsive abnormalities in heart mitochondrial ultrastructure, accompanied by a reduction in MnSOD activity, indicating that the heart is the most sensitive organ for MnSOD. Moreover, heart *MnSOD* mRNA level was a more sensitive criterion than its MnSOD activity in reflecting the Mn status of broilers (Li et al., 2004, 2011b). Consistent with our previous findings, in ovo Mn injection upregulated the mRNA expression of *MnSOD* in the embryonic heart, but did not change its MnSOD activity. Thus, there may be other unknown factors involved in regulating the expression of *MnSOD* protein and its post-translation modification. In addition, malonaldehyde is a soluble degraded product of lipids and widely used to reflect the extent of lipid oxidation (Raharjo and Sofos, 1993). Lu et al. (2006) found that Mn supplementation could reduce MDA content in leg muscle by increasing *MnSOD* activity in the mitochondria of leg muscle cells. However, in the current study, in ovo Mn injection had no effect on MDA content in the chick embryonic heart, partially due to its unchanged *MnSOD* activity.

Sands and Smith (1999) reported that the addition of 240 mg Mn/kg as Mn proteinate decreased abdominal fat deposition of broilers as compared with an unsupplemented control (containing 19 mg Mn/kg in the starter diet and 28 mg Mn/kg in the grower diet) in a thermoneutral environment. The results from our previous studies indicated that the addition of Mn to broiler diets decreased the content of abdominal fat by decreasing LPL activity, and improved carcass quality through reducing MDA content in leg muscle with increasing MnSOD activity, and thus might prolong the shelf life of leg muscle (Lu et al., 2006, 2007). To our knowledge, the effects of in ovo Mn injection and heat stress on carcass traits and meat quality of offspring broilers have not been investigated before. The current study indicated that in ovo Mn injection had little effect on carcass traits and meat quality in the later phase of offspring broilers at different TEMP, possibly due to the sufficient Mn level in the diets of offspring broilers.

Heat stress is of great concern in all types of poultry operations, as birds are very susceptible to HT owing to their lack of sweat glands and fast metabolic rates (Goel, 2021). Hyperthermia induces blood compositional changes, including metabolic (Deaton et al., 1969; Borges et al., 2003; Torki et al., 2014) and endocrine alterations and oxidative damages (Quinteiro-Filho et al., 2010). In the present study, we observed that long-term heat exposure caused significant changes of some plasma biochemical parameters of offspring broilers as evidenced by decreased ALP activity, increased LDH and AST activities, and higher TC, UA, and T3 concentrations in plasma. These changes in plasma composition investigated in the current study may reflect physiological alterations in offspring broiler chickens during heat stress. However, no information is available about the effect of in ovo Mn injection on the above aspects of long-term heat stressed offspring broilers. In the current study, no effects of Mn injection and its interaction with TEMP on the above plasma parameters were observed, possibly due to the sufficient Mn supply in the diets of offspring broilers.

In summary, the results from the present study indicated that the maximum level of in ovo Mn injection was 25 μg Mn/egg. In ovo Mn injection enhanced

MnSOD mRNA expression in the embryonic heart, but had no effect on the embryonic development and subsequent performances of offspring broilers under NT and HT. Hyperthermia increased plasma AST and LDH activities, and decreased carcass traits and meat quality. It was concluded that in ovo Mn injection improved the antioxidative ability in the chick embryonic heart, but had no effect on other performances of embryos and performances of offspring boilers under NT and HT.

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DISCLOSURES

All authors declare that there is no conflict of interest.

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