Heat stress is of great concern in all types of poultry operations. High environmental temperature can cause a significant reduction in egg production and eggshell thickness of commercial laying hens (Emery et al., 1984; Star et al., 2008b) and broiler breeders. Effects of environmental temperature and dietary manganese on egg production performance, egg quality, and some plasma biochemical traits of broiler breeders


ABSTRACT: An experiment was conducted to investigate the effects of environmental temperature and dietary Mn on egg production performance, egg quality, and some plasma biochemical traits of broiler breeders. A completely randomized factorial design involved 2 environmental temperatures (a normal temperature, 21 ± 1°C, and a high temperature, 32 ± 1°C) × 3 dietary Mn treatments (a Mn-unsupplemented corn–soybean meal basal diet or the basal diet supplemented with 120 mg of Mn/kg of diet as either MnSO4·H2O or manganese proteinate). There were 6 treatments with 6 replicates (4 birds per replicate). High temperature decreased egg weight (P < 0.0001), laying rate (P < 0.0001), egg yield (P < 0.0001), feed intake (P < 0.0001), egg:feed ratio (P < 0.0001), eggshell strength (P < 0.05) and thickness (P < 0.0001), plasma triiodothyronine level (P < 0.05), and alkaline phosphatase activity (P < 0.04) whereas it increased rectal temperature (P < 0.0001); plasma malondialdehyde level (P < 0.02); and activities (P < 0.002) of lactic dehydrogenase, aspartate aminotransferase, and creatine kinase. Broiler breeders fed the diets supplemented with Mn regardless of source had greater (P < 0.05) eggshell strength and lower (P ≤ 0.05) plasma triiodothyronine level and protein carbonyl content than those fed the control diet. The broiler breeders fed the diet supplemented with the organic Mn had greater (P < 0.01) eggshell thickness than those fed the control diet. There were interactions (P < 0.05) between environmental temperature and dietary Mn in laying rate, egg yield, feed intake, and egg:feed ratio. Under normal temperature, dietary Mn did not affect the above 4 parameters; however, under high temperature, broiler breeders fed the diet supplemented with the organic Mn showed greater (P < 0.03) improvements in these 4 parameters than those fed the control diet. The results from this study indicated that high temperature significantly impaired egg production performance and eggshell quality and induced lipid peroxidation and tissue damage whereas dietary supplementation of either organic or inorganic Mn improved eggshell strength and thermotolerance and reduced protein oxidation and that the organic Mn could alleviate the negative effect of high temperature on egg production performance of broiler breeders at the period of 32 to 45 wk of age.

Key words: broiler breeders, high temperature, laying performance, manganese, plasma biochemical traits

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INTRODUCTION

Heat stress is of great concern in all types of poultry operations. High environmental temperature can cause a significant reduction in egg production and eggshell thickness of commercial laying hens (Emery et al., 1984; Star et al., 2008b) and broiler breeders.
Dietary Mn deficiency results in lower egg production (Leach and Gross, 1983) and poor eggshell quality (Luo et al., 2003; Xiao et al., 2014) of commercial laying hens under thermoneutral conditions, but inconsistent results of laying performance have been reported in other studies (Cox and Balloun, 1969; Saz-zad et al., 1994). Such discrepancies might be due to variations in breed and age of the hens, dietary composition, or experimental conditions. Notably, the effects of dietary supplementation of Mn, especially different Mn sources, on laying performance and other aspects of broiler breeders under different thermal conditions have not been investigated.

The results from our previous studies have demonstrated that dietary Mn can increase heart manganese superoxide dismutase (MnSOD) activities and reduce lipid peroxidation in broilers and commercial laying hens (Luo et al., 1992, 2003; Lu et al., 2007). Organic Mn with moderate chelation strength was the most effective in the upregulation of heart MnSOD expression in broilers (Li et al., 2004, 2011a,b; Luo et al., 2007). Our data imply that Mn, as a functional component of MnSOD, might alleviate oxidative stress induced by extreme environmental stress such as high temperature in broilers and broiler breeders via detoxification of superoxide free radicals. Therefore, the objective of the current study was to investigate the effects of environmental temperature and dietary Mn on egg production performance, egg quality, and plasma biochemical traits of broiler breeders to determine whether dietary supplemetations with Mn, especially organic Mn with moderate chelation strength, can reduce the detrimental effect of high temperature on laying broiler breeders.

**MATERIALS AND METHODS**

**Experimental Design and Treatments**

To examine the effects of environmental temperature (TEMP) and dietary Mn on laying performance, plasma metabolites and antioxidant status, a completely randomized design involving 2 temperatures × 3 dietary Mn treatments was used in this experiment. The 2 TEMP were a normal temperature of 21 ± 1°C (NT) and a high temperature of 32 ± 1°C (HT). The 3 dietary Mn treatments were the corn–soybean meal basal diet (CON) without Mn supplementation or the basal diet supplemented with 120 mg of Mn/kg of diet as either manganese sulfate (iMn) or manganese proteinate with a moderate chelation strength (quotient of formation \([Q_f] = 61.9, 10.2\% \text{ Mn; oMn}\)). Therefore, there were a total of 6 treatments (NT–CON, NT–iMn, NT–oMn, HT–CON, HT–iMn, and HT–oMn) in this experiment.

**Animals and Diets**

All experimental procedures were approved by the Office of Beijing Veterinarians. One hundred forty-four 18-wk-old female Arbor Acres broiler breeders were purchased from a commercial company (Huadu Broiler Company, Beijing, China) and randomly allotted to 6 treatments with 6 replicates of 4 birds per replicate based on BW. Four birds in each replicate were kept in 2 neighboring galvanized steel cages (0.50 m wide by 0.50 m deep by 0.50 m height) with 2 birds per cage. Lighting and feeding management throughout the experiment followed the Arbor Acres breeder management guidelines, and ad libitum access to water was provided. The photoperiodic lighting was programmed with 8 h of light and 16 h of dark per day from 18 to 21 wk and then gradually increased to 15 h to promote sexual maturity from 22 to 29 wk. After the adaptation period, all broiler breeders were fed with the corn–soybean meal basal diet (Table 1) with no Mn addition to deplete the storage of Mn from 30 to 31 wk. During the depletion, broiler breeders were adjusted appropriately to maintain similar laying rates in each treatment at the beginning of the formal experiment. After the depletion, the room temperature for the broiler breeders of NT–CON, NT–iMn, and NT–oMn was maintained at 21 ± 1°C whereas the room temperature for broiler breeders of HT–CON, HT–iMn, and HT–oMn was increased stepwise from 21 to 32°C over 2 d for these birds to acclimatize to the experimental chronic heat challenge and then maintained at 32 ± 1°C for the rest time of the experiment. Relative humidity was kept at 40 ± 5% for the 2 rooms during the experimental period of 14 wk (32–45 wk of age), which was divided into 3 periods (32–35, 36–40, and 41–45 wk of age).

Both BW and rectal temperature (RT) of broiler breeders were measured at 0830 h on the start of experiment and the last day of each period to check whether these broiler breeders were maintained in the standard BW range and heat exposure stage, respectively. The RT was monitored using a thermo-code electric gauge (JM222; JinMing, Tianjin, China) with an accuracy.
Manganese-induced thermotolerance for breeders

were recorded. The feed intake of broiler breeders in
with feed restriction in HT on the previous day.
formulated to meet or exceed the nutrient require

-4.0 · d−1

Table 1.

Table 2.

Ingredient, %

Table 1. Composition of the basal diet for laying broiler breeders

Table 2. Analyzed Mn concentrations in experimental diets

Item

Item

Ground yellow corn

CON2

CON2

Soybean meal (CP 43%)

iMn2

iMn2

Soybean oil

iMn2

iMn2

CaCO3

oMn2

oMn2

Soybean meal (CP 43%)

23.44

14.3

133

132

2 CON = corn–soybean meal basal diet; iMn = basal diet supplemented with 120 mg of Mn/kg of diet as MnSO4·H2O; oMn = basal diet supplemented with 120 mg Mn/kg as manganese proteinate with a moderate chelation strength of 61.9 quotient of formation (10.2% Mn).

1The Mn concentrations are on an as-is basis.

3Values were based on triplicate determinations.

of 0.1°C. Eggs were collected daily at 1430 h and the number of eggs and egg weight in each replicate cage were recorded. The feed intake of broiler breeders in each replicate cage was recorded each day. Broiler breeders in HT had lower feed intake than those in NT during the first period (32–35 wk of age; 125.5 vs. 136.1 g·bird−1·d−1 for HT vs. NT, respectively). After the first stage, to eliminate the potential effect of reduced feed intake under HT, broiler breeders in NT were pair-fed the same amount of feed consumed by broiler breeders with feed restriction in HT on the previous day.

The basal corn–soybean meal diet for the Mn-depleting and the experimental stages (Table 1) was formulated to meet or exceed the nutrient requirements for laying broiler breeders (NRC, 1994), except for Mn, which was added to the basal diet according to the experimental design. Manganese sulfate (MnSO4·H2O) was reagent grade (Beijing Chemical Company, Beijing, China) and contained 32.2% Mn on a basis of analysis (purity > 99%). Manganese proteinate was provided by a commercial company (Hebei Amino Acid Company, Hebei, China) and contained 10.2% Mn on a basis of analysis (purity > 90%). The chelation strength (Qf value) of the manganese proteinate was analyzed to be 61.9, which is categorized as a moderate chelation strength based on the classification of Holwerda et al. (1995). Manganese proteinate contained AA (% of product) with aspartic acid (6.77), serine (2.05), glutamic acid (4.49), threonine (0.57), glycine (12.36), arginine (1.56), alanine (3.76), proline (7.80), valine (1.04), phenylalanine (1.28), isoleucine (0.46), leucine (1.23), lysine (6.75), and methionine (0.34) on a basis of analysis. A single batch of basal diet was mixed and then divided into 3 aliquots according to the experimental treatments. Lysine and methionine levels in the control diet or diet supplemented with inorganic Mn were balanced by adding synthetic lysine HCl and DL-methionine based on supplemental amounts of lysine and methionine from a manganese proteinate source. The content of Mn in tap water was undetectable. The analyzed Mn concentrations in diets were presented in Table 2.

Sample Collections and Preparations

The feed ingredients and diet samples from all the treatments were collected and analyzed for CP, Ca, P, Cu, Fe, Mn, and Zn before the initiation of the experiment to confirm CP, Ca, P, Cu, Fe, Mn, and Zn contents in diets. The manganese proteinate was sampled for analyses of Mn, AA, and Qf. The control basal diet was also sampled for analyses of lysine, methionine, methionine + cysteine, and threonine contents. In each replicate cage, 2 eggs based on the average egg weight were collected on the last 3 consecutive days of each period for the measurements of egg quality. Blood samples
were collected via a bronchial vein from 2 fasted broiler breeders with average BW in each replicate at 0830 h on the last day of each period. Plasma samples were obtained by centrifuging blood samples at 3,000 × g for 20 min at 4°C and stored at −20°C for further analyses. All samples from 2 birds in each replicate were pooled into 1 sample in equal volume before analysis.

Sample Analyses

The concentrations of Mn in Mn sources, water, and diets and Ca in feed ingredients or diet samples were measured using an inductively coupled plasma emission spectroscope (model IRIS Intrepid II; Thermal Jarrell Ash, Waltham, MA) after wet digestions using HNO_3 and HClO_4 as described by Luo et al. (2007). Concentrations of CP and P in feed ingredients and diet samples were determined using Association of Official Analytical Chemists (1990) methods. Amino acid contents in manganese proteinate and lysine, methionine, methionine + cysteine, and threonine contents in the control basal diet were analyzed using an AA analyzer (model L-8500A; Hitachi Ltd., Chyou-daku, Japan), and the Q_f value of manganese proteinate was determined by polarography as described by Holwerda et al. (1995) and Li et al. (2004).

The color of the yolk and albumen height from the collected eggs were determined using an egg multitester (model EMT-5200; Touhoku Rhythm Ltd., Tokyo, Japan). Haugh unit score was calculated based on egg weight and albumen height (Haugh, 1937). Eggshell strength was measured with a force reader (model F0241; Robotmation Ltd., Tokyo, Japan). Eggshell thickness was measured without inner and outer shell membranes at the top, middle, and bottom of each egg using a peacock dial pipe gauge (model P-1; Ozaki MFG Ltd., Tokyo, Japan) and the average value of the 3 points was considered the eggshell thickness of an egg.

Plasma malondialdehyde (MDA) content was determined using the thiobarbituric acid colorimetric method as described by Mak et al. (1983). Plasma total superoxide dismutase (TSOD) activity was measured using the nitrite method as described by Li et al. (2004). Plasma protein carbonyl content (PCC) was measured using the 2,4-dinitrophenylhydrazine method as described by Oliver et al. (1987). Plasma triiodothyronine (T_3) and thyroxin (T_4) levels were determined by RIA using a commercial kit (Beijing North Institute of Biological Technology, Beijing, China). Plasma glucose (GLU), uric acid (UA), cholesterol (CHO), 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.

Statistical Analyses

Data were analyzed by 3-way ANOVA using the PROC GLM procedure of the SAS (SAS Inst. Inc., Cary, NC), and the model included TEMP, dietary Mn, period, and their interactions. The replicate served as the experimental unit. No interactions (P > 0.05) between period and TEMP or dietary Mn were observed for egg production, egg quality, and plasma parameters; therefore, only the main effects of TEMP, dietary Mn, and their interaction on the above indices were presented. Because the significant interactions (P < 0.05) between period and TEMP were observed for BW and RT, the effect of TEMP on these 2 indices was reported for each period. Differences among means were tested by the LSD method, and statistical significance was set at P ≤ 0.05.

RESULTS

Body Weight and Rectal Temperature

Dietary Mn and the interaction between TEMP and dietary Mn did not affect BW and RT, but there were interactions (P < 0.05) between TEMP and period for BW and RT. Changes in BW and RT of broiler breeders with age in response to TEMP are shown in Fig. 1. The BW was maintained in the standard weight range (3,550 ± 70 g) of laying broiler breeders throughout the experimental period. There was no difference in BW at the beginning of the study (31 wk of age). After heat exposure, broiler breeders at 35 wk of age had lower (P < 0.05) BW in HT than in NT. However, in the paired-feeding period of 36 to 45 wk of age, the BW of broiler breeders was not influenced by TEMP. The RT detected at 31 wk of age was maintained at 40.5 ± 0.2°C in both HT and NT before heat exposure. After heat exposure, broiler breeders at 35, 40, and 45 wk of age had approximately 1°C greater (P < 0.0001) RT values in HT than in NT.

Egg Production Performance

Egg weight, laying rate, egg yield, feed intake, and egg:feed ratio were affected (P < 0.0001) by TEMP but not by dietary Mn except for feed intake (P < 0.05; Table 3). There were interactions (P < 0.05) between TEMP and dietary Mn in laying rate, egg yield, feed intake, and egg:feed ratio except for egg weight
**Manganese-induced thermotolerance for breeders**

**Egg Quality**

Both TEMP and dietary Mn affected \( P < 0.05 \) eggshell strength and thickness but had no effect on the Haugh unit (Table 4). Yolk color was affected \( P < 0.0001 \) by TEMP but not \( P > 0.21 \) by dietary Mn (Table 4). There were no interactions between TEMP and dietary Mn in all of the abovementioned 4 indices (Table 4). Compared with NT, HT decreased eggshell strength \( P < 0.05 \) and thickness \( P < 0.0001 \) but increased \( P < 0.0001 \) yolk color. Broiler breeders in either the iMn or the oMn had greater \( P < 0.05 \) eggshell strength than those in the CON with no difference between the 2 Mn sources. Eggshell thickness of broiler breeders was greater \( P < 0.01 \) in the oMn than in the CON, and no difference was observed between the CON and the iMn.

**Plasma Biochemical Parameters**

The results on plasma GLU, UA, CHO, TG, AST, LDH, CK, ALP, T3, and T4 levels of broiler breeders are given in Table 5. The TEMP affected plasma UA \( P < 0.03 \), CHO \( P < 0.002 \), AST \( P < 0.0004 \), LDH \( P < 0.002 \), CK \( P < 0.0001 \), ALP \( P < 0.04 \), and T3 \( P < 0.05 \) but had no effect on plasma GLU, TG, and T4 levels. Dietary Mn influenced plasma levels of T3 \( P = 0.04 \) and T4 \( P = 0.05 \) but had no effect on all other plasma biochemical indices. No interactions between TEMP and dietary Mn were observed in all of these plasma biochemical indices. Compared with NT, HT increased \( P < 0.05 \) plasma concentrations of UA and CHO and decreased \( P < 0.05 \) plasma T3 level and ALP activity, whereas plasma activities of LDH, AST, and CK were increased \( P < 0.002 \) by 1.5- to 2.0-fold under HT. Broiler breeders had lower \( P \leq 0.05 \) plasma levels of T3 and T4 in either the iMn or the oMn than in the CON with no differences between the 2 Mn sources.

Plasma TSOD activity, MDA level, and PCC measured in broiler breeders are shown in Table 6. The TEMP affected \( P < 0.02 \) plasma MDA level but had no effect on plasma TSOD activity and PCC. Dietary Mn had an effect \( P < 0.05 \) on plasma PCC but did not influence the other 2 plasma indices. No interactions between TEMP and dietary Mn were observed in all of these 3 plasma indices. Compared with NT, HT increased \( P < 0.02 \) plasma MDA level by 18.6%. Broiler breeders had lower \( P < 0.05 \) plasma PCC level in either the iMn or the oMn than in the CON with no difference between the 2 Mn sources.

**DISCUSSION**

In the current study, the RT was significantly increased by approximately 1°C when broiler breeders were exposed to HT, indicating that all of the laying broiler breeders under HT were in a heat stress status throughout the experimental period. However, no difference in BW between NT and HT was observed in broiler breeders at 36 to 45 wk of age, probably due to the similar feed intake of broiler breeders between NT and HT in the paired-feeding period. Prolonged heat exposure severely depresses egg production performance and egg quality of commercial laying hens (Emery et
As expected, egg production performance and eggshell quality were significantly decreased in broiler breeders exposed to the constant 32°C in the current study, which agreed with the previous findings in broiler breeders (McDaniel et al., 1995; Samara et al., 1996; Tang, 2013). The HT might alter some plasma endocrine and biochemical indices involved in various aspects of reproductive performance and nutrient metabolism in commercial layers (Star et al., 2008a,b). However, similar studies have not been done with laying broiler breeders in response to heat challenges. In the present study, a significant increase in plasma activities of AST and CK was observed in broiler breeders exposed to HT, which was in agreement with the previous results of commercial laying hens (Melesse et al., 2011) and broilers (Mitchell and Sandercock, 1995), implying that possible metabolic disorders and tissue damage might be induced by HT. For example, Mitchell and Sandercock (1995) reported that elevated CK activity in plasma of broilers reflected possible muscle membrane damage and then the leakage of intracellular enzymes into the blood. In addition, broiler breeders under HT responded with a significant reduction in plasma T3 concentration, which agreed to the earlier findings described in commercial laying hens (Maak et al., 2003; Star et al., 2008a). These results imply that the heat-stressed broiler breeders might maintain

### Table 3. Effects of environmental temperature (TEMP) and dietary Mn on egg production performance of broiler breeders at 32 to 45 wk of age

<table>
<thead>
<tr>
<th>Item</th>
<th>NT1,2,3</th>
<th>HT1,3</th>
<th>TEMP4</th>
<th>Dietary Mn5</th>
<th>P-value6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>iMn</td>
<td>oMn</td>
<td>SEM</td>
<td>NT</td>
</tr>
<tr>
<td>Egg weight, g</td>
<td>CON</td>
<td>64.1</td>
<td>64.9</td>
<td>64.0</td>
<td>60.1</td>
</tr>
<tr>
<td>Laying rate, %</td>
<td>CON</td>
<td>76.8a</td>
<td>73.1ab</td>
<td>73.7ab</td>
<td>61.9a</td>
</tr>
<tr>
<td>Egg yield, g·bird –1·d –1</td>
<td>CON</td>
<td>48.1a</td>
<td>46.0a</td>
<td>45.5a</td>
<td>35.8a</td>
</tr>
<tr>
<td>Feed intake, g·bird –1·d –1</td>
<td>CON</td>
<td>135a</td>
<td>135a</td>
<td>135a</td>
<td>126a</td>
</tr>
<tr>
<td>Egg feed ratio</td>
<td>CON</td>
<td>0.356a</td>
<td>0.341ab</td>
<td>0.337ab</td>
<td>0.283a</td>
</tr>
</tbody>
</table>

a–c Means within a row lacking a common superscript differ (P < 0.05).

1 NT = normal temperature of 21 ± 1°C; HT = high temperature of 32 ± 1°C.

2 CON = corn–soybean meal basal diet; iMn = basal diet supplemented with 120 mg of Mn/kg of diet as MnSO₄·H₂O; oMn = the basal diet supplemented with 120 mg Mn/kg as manganese proteinate with a moderate chelation strength of 61.9 quotient of formation (10.2% Mn).

3 Values represented the means of 6 replicates (n = 6).

4 Values represented the means of 18 replicates (n = 18).

5 Values represented the means of 12 replicates (n = 12).

6 Probability values for main effects.

### Table 4. Effects of environmental temperature (TEMP) and dietary Mn on egg quality of broiler breeders at 32 to 45 wk of age

<table>
<thead>
<tr>
<th>Item</th>
<th>NT1,2,3</th>
<th>HT1,3</th>
<th>TEMP4</th>
<th>Dietary Mn5</th>
<th>P-value6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>iMn</td>
<td>oMn</td>
<td>SEM</td>
<td>NT</td>
</tr>
<tr>
<td>Eggshell strength, N</td>
<td>CON</td>
<td>36.7</td>
<td>39.4</td>
<td>39.5</td>
<td>35.6</td>
</tr>
<tr>
<td>Eggshell thickness, cm</td>
<td>CON</td>
<td>0.363</td>
<td>0.374</td>
<td>0.379</td>
<td>0.351</td>
</tr>
<tr>
<td>Haugh unit</td>
<td>CON</td>
<td>75.3</td>
<td>75.0</td>
<td>72.1</td>
<td>71.4</td>
</tr>
<tr>
<td>Yolk color</td>
<td>CON</td>
<td>6.29</td>
<td>6.47</td>
<td>6.26</td>
<td>6.80</td>
</tr>
</tbody>
</table>

a,b Means within a row lacking a common superscript differ (P < 0.05) for either environmental temperature or dietary Mn.

1 NT = normal temperature of 21 ± 1°C; HT = high temperature of 32 ± 1°C.

2 CON = corn–soybean meal basal diet; iMn = basal diet supplemented with 120 mg of Mn/kg of diet as MnSO₄·H₂O; oMn = the basal diet supplemented with 120 mg Mn/kg as manganese proteinate with a moderate chelation strength of 61.9 quotient of formation (10.2% Mn).

3 Values represented the means of 6 replicates (n = 6).

4 Values represented the means of 18 replicates (n = 18).

5 Values represented the means of 12 replicates (n = 12).

6 Probability values for main effects.

7 Eggshell strength and thickness were determined by 6 eggs from each replicate.
Table 5. Effects of environmental temperature (TEMP) and dietary Mn on plasma glucose (GLU), uric acid (UA), cholesterol (CHO), triglyceride (TG), aspartate aminotransferase (AST), lactic dehydrogenase (LDH), creatine kinase (CK), and alkaline phosphatase (ALP) activity and triiodothyronine (T3), and thyroxin (T4) levels of broiler breeders at 32 to 45 wk of age

<table>
<thead>
<tr>
<th>Item</th>
<th>CON iMn oMn</th>
<th>CON iMn oMn</th>
<th>SEM</th>
<th>NT</th>
<th>HT</th>
<th>SEM</th>
<th>CON iMn oMn</th>
<th>SEM</th>
<th>TEMP Mn</th>
<th>P-value6</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLU, mmol/L</td>
<td>11.8</td>
<td>12.1</td>
<td>12.4</td>
<td>12.3</td>
<td>12.1</td>
<td>11.9</td>
<td>0.32</td>
<td>12.1</td>
<td>12.1</td>
<td>0.23</td>
</tr>
<tr>
<td>UA, μmol/L</td>
<td>240</td>
<td>208</td>
<td>196</td>
<td>250</td>
<td>274</td>
<td>220</td>
<td>20.0</td>
<td>215b</td>
<td>248a</td>
<td>14.9</td>
</tr>
<tr>
<td>CHO, mmol/L</td>
<td>3.75</td>
<td>3.68</td>
<td>3.50</td>
<td>4.24</td>
<td>4.55</td>
<td>4.15</td>
<td>0.22</td>
<td>3.64b</td>
<td>4.31a</td>
<td>0.13</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>12.7</td>
<td>14.0</td>
<td>14.5</td>
<td>15.3</td>
<td>15.0</td>
<td>15.2</td>
<td>1.09</td>
<td>13.7</td>
<td>15.2</td>
<td>0.61</td>
</tr>
<tr>
<td>AST, units/L</td>
<td>102</td>
<td>82</td>
<td>88</td>
<td>129</td>
<td>157</td>
<td>132</td>
<td>14.7</td>
<td>90b</td>
<td>139a</td>
<td>8.4</td>
</tr>
<tr>
<td>LDH, units/L</td>
<td>288</td>
<td>217</td>
<td>236</td>
<td>378</td>
<td>406</td>
<td>332</td>
<td>42.5</td>
<td>243b</td>
<td>373a</td>
<td>24.5</td>
</tr>
<tr>
<td>CK, units/L</td>
<td>2,963</td>
<td>1,809</td>
<td>1,982</td>
<td>4,477</td>
<td>4,986</td>
<td>4,041</td>
<td>508</td>
<td>2,252b</td>
<td>4,501a</td>
<td>293</td>
</tr>
<tr>
<td>ALP, units/L</td>
<td>80.0</td>
<td>76.7</td>
<td>102.6</td>
<td>62.3</td>
<td>58.8</td>
<td>69.3</td>
<td>12.8</td>
<td>86.4b</td>
<td>63.5a</td>
<td>7.4</td>
</tr>
<tr>
<td>T3, nmol/mL</td>
<td>1.01</td>
<td>0.88</td>
<td>0.71</td>
<td>0.85</td>
<td>0.59</td>
<td>0.67</td>
<td>0.10</td>
<td>0.87a</td>
<td>0.77b</td>
<td>0.06</td>
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<tr>
<td>T4, nmol/mL</td>
<td>45.0</td>
<td>44.3</td>
<td>41.2</td>
<td>45.8</td>
<td>35.9</td>
<td>39.8</td>
<td>2.85</td>
<td>43.4</td>
<td>40.7</td>
<td>1.64</td>
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</tbody>
</table>

a,bMeans within a row lacking a common superscript differ (P < 0.05).
1NT = normal temperature of 21 ± 1°C; HT = high temperature of 32 ± 1°C.
2CON = corn–soybean meal basal diet; iMn = basal diet supplemented with 120 mg of Mn/kg of diet as MnSO4·H2O; oMn = the basal diet supplemented with 120 mg Mn/kg as manganese proteinate with a moderate chelation strength of 61.9 quotient of formation (10.2% Mn).
3Values represented the means of 6 replicates (n = 6).
4Values represented the means of 18 replicates (n = 18).
5Values represented the means of 12 replicates (n = 12).
6Probability values for main effects.

Table 6. Effects of environmental temperature (TEMP) and dietary Mn on plasma total superoxide dismutase (TSOD) activity, malondialdehyde (MDA) level, and protein carbonyl content (PCC) of broiler breeders at 32 to 45 wk of age

<table>
<thead>
<tr>
<th>Item</th>
<th>CON iMn oMn</th>
<th>CON iMn oMn</th>
<th>SEM</th>
<th>NT</th>
<th>HT</th>
<th>SEM</th>
<th>CON iMn oMn</th>
<th>SEM</th>
<th>TEMP Mn</th>
<th>P-value6</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA, mmol/mL</td>
<td>6.16</td>
<td>6.48</td>
<td>6.60</td>
<td>8.28</td>
<td>7.34</td>
<td>7.35</td>
<td>0.47</td>
<td>6.46b</td>
<td>7.66a</td>
<td>0.27</td>
</tr>
<tr>
<td>TSOD,7 NU/mL</td>
<td>171</td>
<td>170</td>
<td>165</td>
<td>161</td>
<td>181</td>
<td>183</td>
<td>12.4</td>
<td>169</td>
<td>173</td>
<td>7.1</td>
</tr>
<tr>
<td>PCC,8 ng/mg protein</td>
<td>5.63</td>
<td>4.86</td>
<td>5.22</td>
<td>5.75</td>
<td>5.16</td>
<td>5.06</td>
<td>0.24</td>
<td>5.23</td>
<td>5.32</td>
<td>0.14</td>
</tr>
</tbody>
</table>

a,bMeans within a row lacking a common superscript differ (P < 0.05).
1NT = normal temperature of 21 ± 1°C; HT = high temperature of 32 ± 1°C.
2CON = corn–soybean meal basal diet; iMn = basal diet supplemented with 120 mg of Mn/kg of diet as MnSO4·H2O; oMn = the basal diet supplemented with 120 mg Mn/kg as manganese proteinate with a moderate chelation strength of 61.9 quotient of formation (10.2% Mn).
3Values represented the means of 6 replicates (n = 6).
4Values represented the means of 18 replicates (n = 18).
5Values represented the means of 12 replicates (n = 12).
6Probability values for main effects.
7The TSOD activity in plasma was expressed as nitrite units (NU) per milliliter, and 1 NU was defined as the amount of enzyme needed to obtain 50% inhibition of nitrite formation.
8The PCC level in plasma was expressed as nanomoles per milligrams of protein.
adequate, the corn–soybean meal basal diet (14.3 mg Mn/kg of diet) seemed to supply a sufficient Mn for maintenance of egg production in broiler breeders at 32 to 45 wk of age under NT, although the dietary Mn requirement level of broiler breeders was 120 mg Mn/kg of diet (NRC, 1994; Leeson and Summer, 2009). However, under HT, compared with the CON, dietary supplementation with the organic Mn improved feed intake, laying rate, egg yield, and egg:feed ratio of broiler breeders, indicating that dietary organic Mn supplementation could alleviate the negative effect of HT on egg production performance of broiler breeder effectively. It was likely that HT enhanced mineral excretion (Belay et al., 1992) along with the lower feed intake and mineral digestibility (Bonnet et al., 1997). Therefore, a greater Mn for maintaining egg production performance was required under HT. Smith et al. (1995) reported that manganese proteinate demonstrated greater bioavailability in broilers reared at an elevated temperature. A series of previous studies from our laboratory has also demonstrated that organic Mn sources with moderate chelation strengths had greater bioavailabilities or efficacies for broilers than the inorganic manganese sulfate source (Li et al., 2004, 2005, 2008, 2011b; Luo et al., 2007). Therefore, the anti-heat stress effect of the organic Mn on egg production performance of broiler breeders might be related to the greater feed intake and Mn bioavailability.

Luo et al. (2003) and Xiao et al. (2014) indicated that dietary Mn deficiency resulted in poor eggshell quality of commercial laying hens. In the current study, dietary Mn supplementation improved eggshell quality of laying broiler breeders regardless of temperatures. The improved eggshell quality may be partly explained by the role of Mn as cofactors of metalloenzymes responsible for carbonate and mucopolysaccharide synthesis required for eggshell formation as reported by Leach and Gross (1983) and Mabe et al. (2003). However, inconsistent results were reported in other studies (Karunajeewa and Tham, 1987; Hossain and Bertechini, 1998). These inconsistencies may have been due to the differences in diet type, dietary Mn treatment, experimental duration time, and other parameters. Until now, the effect of different Mn sources on egg production and egg quality of broiler breeders has not been investigated. In our current study, dietary supplementation with the organic Mn was more favorable for the improvement of eggshell thickness regardless of TEMP, possibly due to its greater Mn bioavailability as discussed above. However, the exact reasons remain unclear.

Hyperthermia could lead to accumulation of reactive oxygen species and induce oxidative damage in broilers (Lin et al., 2006) and broiler breeders (Xie et al., 2014). And MDA content and PCC are frequently used as biomarkers of lipid peroxidation and protein oxidation (Chevion et al., 2000), respectively. The results from this study showed that HT elevated plasma MDA level, implying that oxidative stress and subsequent lipid peroxidation might be induced by HT in broiler breeders. Similar results were observed in heat-stressed broilers (Lin et al., 2006) and commercial laying hens (Sahin et al., 2003). As a free radical scavenger, MnSOD functions as one of the more important Mn-containing enzymes in the body (Luo et al., 1992). As such, MnSOD may enhance antioxidant ability and reduce oxidative damage induced by heat stress. Lu et al. (2007) reported that dietary Mn increased the MnSOD activity in the leg muscle of broilers in association with its decrease of MDA content. Moreover, Li et al. (2004, 2005, 2011a,b) demonstrated that dietary Mn upregulated heart MnSOD gene expressions in broilers and that the organic Mn with the moderate chelation strength was the most effective. Dietary supplementation of Mn as either inorganic or organic decreased plasma PCC regardless of temperatures, which might reflect a reduction of protein oxidation related to the enhanced antioxidant ability. However, significant changes in plasma TSOD activities were not detected among dietary Mn treatments. Obviously, the correlations between dietary Mn and the activities of tissue TSOD, MnSOD, and copper–zinc superoxide dismutase in broiler breeders need to be further investigated. In addition, dietary Mn induced a significant decrease in plasma T₃ level in the current study, which further confirmed the previous results in rats (Buthieau and Autissier, 1983; Eder et al., 1996). Furthermore, the results from both Eder et al. (1996) and Van der Geyten et al. (1999) implied that the decreased plasma T₃ level due to Mn supplementation might be related to the decreased hepatic 5′-deiodinase enzyme activity, which is a key enzyme of thyroid hormone metabolism in converting T₄ into the biological active T₃. The decreased plasma T₃ level might be involved in the improvement of thermotolerance, because hyperthyroidism accelerated the basal metabolic rate and oxidative metabolism and then resulted in oxidative damage (Asayama et al., 1987; Pereira et al., 1994). Therefore, the decreased plasma T₃ level due to dietary Mn addition might be another reason for dietary Mn for reducing protein oxidation of broiler breeders in the present study.

In conclusion, high environmental temperature significantly impaired egg production performance and eggshell quality and induced lipid peroxidation and tissue damage, whereas dietary supplementation of either organic or inorganic Mn improved eggshell
strength and thermotolerance and reduced protein oxidation, and the organic Mn with the moderate chelation strength could alleviate the negative effect of high temperature on egg production performance of broiler breeders at the period of 32 to 45 wk of age.

**LITERATURE CITED**


