Effect of manganese supplementation on the carcass traits, meat quality, intramuscular fat, and tissue manganese accumulation of Pekin duck

Ting Yang,*1 Xiaoyan Wang,†1 Min Wen,‡ Hua Zhao,* Guangmang Liu,* Xiaoling Chen,* Gang Tian,* Jingyi Cai,* and Gang Jia*,2

*Institute of Animal Nutrition, Key Laboratory for Animal Disease-Resistance Nutrition of China, Ministry of Education, Sichuan Agricultural University, Chengdu, Sichuan 611130, China; †Zhejiang animal disease control center, Zhejiang animal husbandry and agricultural machinery development center, Hangzhou, Zhejiang 311109, China; and ‡Faculty of Agriculture, Forestry and Food Engineering, Yibin University, Yibin, Sichuan, 644000, China

ABSTRACT Manganese (Mn) is a trace element present in all tissues and is essential for animal growth and health; it also has an antioxidant capacity in tissues. The effect of Mn on meat quality and the mechanism of fat deposition of the breast muscle is still unclear. Therefore, the present study aimed to investigate the effect of Mn supplementation on the growth performance, meat quality, the activity and transcription of antioxidant enzymes, and fatty acid profile in the breast muscle, and the Mn deposition in tissues of Pekin ducks. A total of 896 one-day-old Pekin ducks were allocated into 7 groups, with 8 replicates, each replicate containing 16 ducks. The treatment diets consisted of basal diet supplemented with manganese sulfate at levels 30, 60, 90, 120, 150, 240 mg/kg (as Mn). Results showed that ducks fed diets supplemented with Mn had no effect on the growth performance but decrease in the feed-to-gain ratio of day 1–14 (P<0.01). Dietary Mn increased significantly the a* (redness) value of the duck breast meat at 24 h and intramuscular fat (P<0.05), and decreased drip loss and shear force of the breast meat (P<0.05). Manganese supplement significantly reduced the malondialdehyde content (P<0.05), and significantly increased the mRNA expressions of manganese superoxide dismutase, thioredoxin 2, peroxiredoxin 3, and catalase (P<0.05). About the fatty acid profile, dietary Mn increased (P<0.05) the proportions of the C20 family. Manganese accumulation in the heart, breast muscle, and tibia was increased with Mn supplementation (P<0.05), and Mn content of the heart conforms to the quadratic curve. Besides, Mn supplementation notably increased mRNA expression in genes involved in lipogenesis and deposition and decreased in genes associated with lipolytic in the breast muscle. These findings reveal that dietary Mn could improve meat quality and enhance antioxidant activity and intramuscular fat, which via regulated gene expression involved in lipogenesis and lipolytic.

Key words: manganese, meat quality, antioxidant, lipid deposition, meat duck

INTRODUCTION

Manganese (Mn) is a trace element present in all tissues and is essential for animal growth and health (Vásquez-Procopio et al., 2020). Manganese ions serve as cofactors to 3 mitochondrial enzymes: manganese superoxide dismutase (MnSOD), arginase, and glutamine synthase, and to glycosyltransferases residing in the Golgi apparatus; those enzymes are involved in several functions including lipid and carbohydrate metabolism, and several enzymes’ activation (Lindberg and Ernster, 1954; Baly et al., 1990). Besides, Mn is also required for brain physiology and biology via its role as a cofactor in numerous enzymatic processes, but excess or insufficient Mn associated with neurological disease (Horning et al., 2015). For laying hens, dietary Mn supplementation could improve eggshell quality in the long term by elevating expression of follicle-stimulating hormone and gonadotropin-releasing hormone-I genes (Xie et al., 2014). Manganese had no effect on growth performance of pigs and broilers (Sawyer et al., 2007;
Another character of Mn is redox activity which plays a key role in cellular adaptation to oxidative stress (Aguirre and Culotta, 2012); this is largely due to being as a cofactor of MnSOD, which protects mitochondria from oxidative damage (Borgstahl et al., 1992; Reddi et al., 2009). In a study on broilers, dietary Mn upregulated muscle MnSOD gene expression pretranslationally and increased MnSOD activity, then decreased the malondialdehyde content in the leg muscle (Lu et al., 2007). In addition, Mn supplementation increased catalase (CAT) activity in the liver, glutathione peroxidase in plasma, and total antioxidative capacity (TAOC) in muscle of pigs (Schwarz et al., 2017). Rats were intraperitoneally injected with MnCl2; results have shown the decreased level of lipid peroxidation in the liver, spleen, and adrenal glands (Chen et al., 2000). Regrettably, no study was forced on the effect of Mn supplementation on the antioxidant capacity of meat in Pekin ducks. In 2018, China’s poultry consumption was 1.928 million tons, making it the world’s largest poultry consumer, and ducks provide about 40% of those (data form National waterfowl industry technology research and Development Center). Thus, the quality of breast muscles has become a more important tissue that determines the commercial value of duck. Many kinds of nutrients could improve breast muscle quality of birds, such as energy, protein, fatty acids, or feed additives; of course, breeding management and genetics play a critically greater role (Mir et al., 2017). In our previous study about zinc, we found proper zinc can improve carcass quality and breast muscle quality of ducks (Wen et al., 2019). Both zinc and Mn have antioxidant properties in animal tissues; consequently, Mn may improve the meat quality of poultry and pig by improving antioxidant capacity (Apple et al., 2004; Lu et al., 2007; Sawyer et al., 2007). Previous studies have commonly reported that the fatty acid composition is the key factor in the nutritional and healthy value of breast meat (Ahmed et al., 2015; Schiavone et al., 2019). To the best of our knowledge, there was no literature on the effects of Mn on breast muscle quality and fatty acid profile of Pekin ducks.

As we know, meat quality is largely influenced by intramuscular fat (IMF) content, which affects sensory properties and nutritional values of meats such as tenderness and flavor (Ruiz et al., 2001). In poultry, the fatty acid in the body came from dietary Mn less than 10%; thus, the liver plays a key role in providing lipids destined to be used by all tissues, including the liver itself (O’Hea and Leveille, 1969). For the lipid deposition, about 80–85% of the fatty acids that accumulated in the adipose tissue are derived from plasma lipids (Hermier, 1997), then the rest fatty acids that were synthesized by the liver were stored or used as energy by other tissues, such as the heart and muscle. In a study of TG content of chicken pectoralis muscle, compare with lower TG content chicken, higher expression of absorbed fat, de novo synthesis of fatty acids (lipogenesis) and lower expression fat metabolism of fatty acids were observed in higher TG content chicken. (Liu et al., 2019). As described by Saze et al. (2009), de novo lipogenesis and intracellular transport of long-chain fatty acid in the breast muscle were higher in Pekin ducks, which has higher IMF content than Muscovy ducks. Therefore, the effect of dietary Mn on IMF and underlying mechanism on Pekin ducks need to be explored.

As mentioned above, the aim of this study was to investigate the effect of dietary Mn supplementation on the meat quality, antioxidant status, fatty acid composition in breast muscle of ducks, and the underlying mechanism of IMF content changes. The Mn deposition in tissues is also investigated. 

**MATERIALS AND METHODS**

**Animals and Diets**

The experimental procedures for animal trials were conducted in accordance with the Chinese guidelines for animal welfare and approved by the Animal Health and Care Committee of Sichuan Agricultural University (SICAU 2009-0135).

A total of 896 1-day-old male Pekin ducks were obtained from commercial hatchery. The ducks were randomly allocated into 7 groups with 8 replicates, each replicate containing 16 ducks. The ducks were reared in cages (2.0 × 1.0 m) in a temperature-controlled room and maintained on a 24 h constant light schedule and allowed ad libitum free access to feed and water; the temperature control process was in accordance with the feeding manual. The basal diet was formulated based on the NRC (1994) and meets or exceeds the requirement of meat ducks (Table 1). The treatment diets consisted of basal diet supplement with manganese sulfate (Shu Xing, Chengdu, China) at levels 0, 30, 60, 90, 120, 150, 240 mg/kg (as Mn), and diets were in the pellet form. The measured values of Mn contained in diets are displayed in Table 1.

**Sample Collection and Carcass Traits Evaluation**

At the end of the feeding trial, birds were starved overnight (8:00 pm–8:00 am) before sampling. Birds were slaughtered by cervical dislocation, then underwent carotid bloodletting immediately. The heart and tibia were removed and stored at –20°C for the Mn determination. The left breast muscle (pectoralis major) was collected and stored at –80°C and used to measure the antioxidant capacity and isolate total RNA. The intact right breast muscle (pectoralis major) was removed and stored at 4°C for future meat quality analysis, after measuring the meat color and pH value for 45 min.
extracted lipid was hydrolyzed, methylated, and lipid as described before (Folch et al., 1957). The breast muscle was heated in 75°C water until the core temperature reached 75°C for 24 h. Approximately, 0.5 g ashes of C for 24 h. Approximately, 0.5 g ashes were digested by nitric acid as described by Wang et al. (2012). A bovine liver standard powder (Na2-FeSO4·7H2O, 60 mg; Zn (ZnSO4·7H2O), 60 mg; Se (Na2SeO3), 0.3 mg; I (KI), 0.4 mg. dissolved in n-hexane and saturated NaCl mix solution for further analysis. By comparing the retention times of the peaks with the standards (Sigma), the fatty acids could be identified; the fatty acid profile is shown as the percentage of total fatty acid.

**Antioxidant Enzyme Activity and Lipid Peroxidation Assays**

For enzymatic analysis, the breast muscle samples (about 0.5 g) were homogenized in 4.5 mL of ice-cold physiological saline and centrifuged at 4,000 rpm for 10 min; the supernatant was collected for enzyme activities analysis. The content of malonaldehyde (MDA) and protein capacity, anti-superoxide anion and anti-hydroxy radical, the activity of MnSOD and TAOC were analyzed by commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) as per the manufacturer's instructions using Multiskan Spectrum (Thermo Fisher Scientific Inc., Madison, WI). The results were normalized by total protein, and shown as U mg⁻¹ protein or nmol mg⁻¹ protein.

**Manganese Content Analysis**

Manganese contents in the diets and tissues were determined by A ContrAA-700 high-resolution continuum source atomic absorption spectrometer (Analytik Jena, Germany) equipped with flame (HR-CS FAAS). Samples were prepared and analyzed as per the method described by Bai et al. (2014). Briefly, tibias were defatted at petroleum ether for 48 h, then ashed in a muffle furnace at 550°C for 14 h. Diets were ashed in muffle furnace at 550°C for 24 h. Approximately, 0.5 g ashes of titania and diet, heart and breast muscle, and 0.5 mL serum were digested by nitric acid as described by Wang et al. (2012). A bovine liver standard powder (National Institute of Standards and Technology, Beijing, China) was included in analyses to verify the determination accuracy.

**Meat Quality Analysis**

The meat color and pH values of the breast muscle were measured at 45 min and 24 h after the birds were slaughtered. For the meat color, the values of lightness (L*), redness (a*), and yellowness (b*) of each breast muscle were determined by a portable chroma meter (CR-300, Minolta, Japan) at 3 different points that was previously calibrated with standard buffer solutions. The drip loss was measured by the method previously described (Otto et al., 2006). Briefly, the left breast muscle was cut into 1 cm cubes (about 5 g) and weighed, then reweighed after 24 h. For determination of shear force of the breast muscle, the muscles were heated in 75°C water until the core temperature reached 75°C, after which they were removed at 24 h and then cooled to 4°C. The shear force of the samples was measured 5 times using a TA.XT2 Texture Analyzer (Stable Micro Systems, Reading, UK). The fresh tissue was lyophilized in a vacuum dryer (DOU-1100; Eyela, Japan Tokyo Chemistry). The determination of IMF of dry samples was carrying out on a dry basis by using the Soxhlet extraction system (Foss Soxtect Avanti 2055; Foss, Hoganas, Sweden) in accordance with the methods of AOAC (AOAC 1990).

**Analysis of the Fatty Acid Composition**

Determination of the fatty acid profile in the breast muscle was analyzed by GC-2010 plus gas chromatograph (Shimadzu, Japan) equipped with AOC-20i auto-injector (Shimadzu, Japan) and a chromatographic column SP2560 for fatty acid methyl esters (100 m 0.25 mm id 0.2 μm) as described by Xu et al. (2019). Briefly, about 100 mg dry sample was used to extract lipid as described before (Folch et al., 1957). The extracted lipid was hydrolyzed, methylated, and

| Ingredient                  | Starter (Day 1–14) | Grower (Day 15–35) |
|----------------------------|--------------------|--------------------|
| Corn                       | 59.06              | 68.5               |
| Wheat                      | 5                  | 3.02               |
| Soybean meal               | 28.2               | 24.3               |
| Rapeseed meal              | 0                  | 0.6                |
| Limestone                  | 0.93               | 0.90               |
| CaHPO4 (2H2O)              | 1.84               | 1.72               |
| NaCl                       | 0.34               | 0.33               |
| Choline chloride           | 0.15               | 0.15               |
| Premix 1                   | 0.23               | 0.23               |
| DL-methionine              | 0.22               | 0.12               |
| L-lysine HCl               | 0.29               | 0.10               |
| L-tryptophan               | 0.08               | 0.02               |
| L-threonine                | 0.06               | 0.01               |
| Total                      | 100.00             | 100.00             |

1Supplied per kilogram diet: vitamin A, 9,000 IU; vitamin D₃, 2,000 IU; vitamin E, 10 IU; vitamin B₁, 2 mg; vitamin B₂, 4.8 mg; pantothenic acid, 20 mg; vitamin B₆, 0.02 mg; folic acid, 1 mg; niacin 50 mg; Cu (CuSO₄·5H₂O), 8 mg; Fe (FeSO₄·7H₂O), 60 mg; Zn (ZnSO₄·7H₂O), 60 mg; Se (Na₂SeO₃), 0.3 mg; I (KI), 0.4 mg.

**Table 1. Ingredients and nutrient composition of base diets (as-fed basis).**
Quantitative Real-Time PCR

The total RNA was extracted from the breast muscle using the RNAiso Plus reagent (TaKaRa, Dalian, China) as per the manufacturer’s instructions. The RNA concentration was quantified using a spectrophotometer (NanoDrop ONE, Thermo Fisher Scientific Inc., Madison, WI). About 1 μg RNA was reverse transcribed for cDNA using a PrimeScript RT reagent kit with gDNA eraser (TaKaRa) as per the manufacturer’s instructions. Real-time quantitative PCR was conducted using the primers listed in Table 2 that were designed with Primer Premier 5.0 Software (Premier Biosoft International, Palo Alto, CA). The housekeeping gene β-actin and GAPDH served as a control to normalize the mRNA expression level. Relative quantities of mRNA were calculated using the $2^{-\Delta\Delta C_T}$ method (Livak and Schmittgen 2001).

Statistical Analyses

Data were subjected to one-way ANOVA, linear, and quadratic using the JMP program version 13.0 (SAS, SAS Institute, Cary, NC), after testing the normality and homogeneity of variances with the Levene test. Significant differences between the groups were determined by the Tukey’s multiple range test after the ANOVA was significant. The level of significance was set at $P < 0.05$. All values were presented as mean ± SEM.

RESULTS

Growth Performance

As presented in Table 3, dietary Mn supplementation had no effect on the BW, ADFI, and ADG ($P > 0.05$). However, dietary Mn decreases the feed-to-gain ratio of day 1–14 ($P < 0.01$) and no influence on that of 35 d ($P > 0.05$). Interestingly, the BW and ADFI of day 1–35 were all fit to a quadratic curve.

Meat Quality

The meat quality of the Pekin ducks fed with diets containing different levels of Mn is summarized in Table 4. Dietary Mn significantly increased the $a^*$ (redness) value of the duck breast meat at 24 h ($P < 0.05$), and has the increased trend at 45 min ($P = 0.090$). However, Mn supplementation has no effect on the $b^*$ (yellowness) and $L^*$ (lightness) values ($P > 0.05$). Besides, as the Mn level increased, the drip loss and shear force of breast meat decreased ($P < 0.05$), and were all fit to linear and quadratic curves. However, IMF of the breast muscle increased with dietary Mn increase.

Table 2. Information of primers for RT-PCR.

| Gene name | Accession no | Primer sequences (5’ to 3’) | $T_m$ ($^\circ C$) | Product size (bp) |
|-----------|--------------|-----------------------------|------------------|------------------|
| MnSOD     | XM_027454289.1 | Forward: GCCTGCGAAAGGTGATGTTACA | 60 | 179 |
|           |              | Reverse: TTGCAAGGAAAGCAAGTTCACG | 60 | 154 |
| Trx2      | NM_001031410.1 | Forward: ATGATCACACAGACCTGCGAT | 60 | 154 |
|           |              | Reverse: GAGCAGGAAGGAAGGGTTACAA | 60 | 194 |
| Prdx3     | XM_027460711.1 | Forward: AGCAACAGATTACACGTCACCT | 60 | 194 |
|           |              | Reverse: TAGGGTACGTCGACCTGACAT | 60 | 194 |
| CAT       | XM_027458335.1 | Forward: GCCACATGGTGACTACCCTC | 60 | 117 |
|           |              | Reverse: TGGTTGAGTTTGGTGCCTTA | 60 | 117 |
| HO-1      | XM_005015345.4 | Forward: AAGGGCTGCTTTTCTCACC | 60 | 124 |
|           |              | Reverse: GCCCTCTCAAGAAGCTCCTT | 60 | 124 |
| ACC       | XM_027472149.1 | Forward: TAAGATGCACCTCTACCTGAGA | 60 | 189 |
|           |              | Reverse: TTAATGCGACCTCCACCTG | 60 | 189 |
| ME        | KF185113.1   | Forward: GCACCTCACCTCAACAGAGG | 60 | 228 |
|           |              | Reverse: CGAGAAGAAAGCTCACAAT | 60 | 228 |
| DGAT2     | XM_005024101.4 | Forward: ACAGTGAAAGAAGCTCAGTGA | 60 | 144 |
|           |              | Reverse: GCTGTATTTCCGTCTACGTGC | 60 | 144 |
| PPARγ     | XM_001310398.1 | Forward: GCCCAAGTTTGGATTCGGT | 60 | 201 |
|           |              | Reverse: AACACGTGGAGCACTCCTGG | 60 | 201 |
| FATP1     | XM_005018222.4 | Forward: TGGTGGGGCCAGATAATCAACA | 60 | 242 |
|           |              | Reverse: GTGAACCTACATCCTCTCCACT | 60 | 242 |
| LPL       | FJ185781.1   | Forward: AGTACTGCTGTGGCTTTTACAG | 60 | 191 |
|           |              | Reverse: AAGCATCAGACGACAGACTG | 60 | 191 |
| Cpt-1z    | XM_027457808.1 | Forward: ATCAACTGTTCCCTGCTGCT | 60 | 113 |
|           |              | Reverse: CAACAGTGGTCCTGCTGCT | 60 | 113 |
| PPARα     | NM_001310338.1 | Forward: CCCCCTTACCAGGCTTACAGT | 60 | 121 |
| β-actin   | NM_001310421.1 | Forward: CCTGGAGCTTTATCCCTCACA | 60 | 105 |
| GAPDH     | XM_027449739.1 | Forward: GGTGTGCTTCCCTGAGCTTTCA | 60 | 165 |
|           |              | Reverse: TCTGGTGGTCCATGTTGAC | 60 | 165 |

Abbreviations: ACC, acetyl-CoA carboxylase; CAT, catalase; Cpt1z, carnitine palmitoyl transferase 1z; DGAT2, diacylglycerol acyltransferase 2; FATP1, fatty acid transport protein 1; HO-1, heme oxygenase-1; LPL, lipoprotein lipase; ME, malic enzyme; MnSOD, manganese superoxide dismutase; PPARα, peroxisome proliferator–activated receptor alpha; PPARγ, peroxisome proliferator–activated receptor gamma; Prdx, peroxiredoxin; Trx2, thioredoxin 2.
Antioxidant Enzyme Activity Assays

The data of dietary Mn effect on the antioxidant enzyme activity of Pekin ducks are present in Table 5. Dietary Mn supplementation resulted in an increase in MnSOD and anti-superoxide anion activities but no significant difference. Moreover, dietary Mn supplementation significantly reduced the MDA content \( (P, 0.05) \), whereas at 240 mg in the Mn/kg group, there was an abnormal increase. There is no significant difference about anti-hydroxy radical and TAOC.

Antioxidant Enzyme–Related Gene Expression

We also measured the expression level of antioxidant genes, as shown in Figure 1. The expression of SOD was gradually upregulated as Mn supplementation increased \( (P, 0.05) \). Besides, the expression of thioredoxin 2 (Trx2) and peroxiredoxin 3 (Prdx3) also increased and resulted in a plateau with dietary Mn increase \( (P, 0.05) \). Intriguingly, the CAT expression increased by about 10-fold with dietary Mn increase.

Fatty Acid Profile of Breast Muscle

Fatty acid composition determined in the breast muscle is shown in Table 6. Dietary Mn increased \( (P, 0.05) \) the proportions of C20 family, such as C20:0, C20:1n9, C20:2, C20:3n3, and also fit to linear and quadratic curves. Besides, there is a tendency to increase C15:0 \( (P, 0.05) \), C17:0 \( (P, 0.07) \), and C18:1n9t \( (P, 0.05) \), whereas, increase in trend of C17:0 was fit to quadratic curve and C15:0 was fit to linear and quadratic curves. Dietary Mn supplementation did not influence the saturated fatty acid (SFA), monounsaturated fatty acid, and polyunsaturated fatty acid (PUFA) content \( (P > 0.05) \).

Tissues Manganese Concentration

The tibia, serum, heart, and breast muscle Mn contents in response to the experimental diets are shown in Figure 2. Ducks receiving higher level of Mn had greater tibia and breast muscle Mn level. On the other hand, serum Mn was not influenced by dietary Mn. At the same time, linear fitting analysis shows that the Mn
content of the tibia and breast muscle conforms to the linear curve and the Mn content of the heart conforms to the quadratic curve.

**Effects of Dietary Manganese Supplementation on Expression of Genes Involved in Synthesis, Absorption, and Oxidation in Breast Muscle**

The relative expression of genes involved in fatty acid de novo synthesis, transportation, and oxidation in the breast muscle can be seen in Figure 3. As shown, dietary Mn significantly increased (\(P < 0.05\)) the gene expressions in fatty acid synthesis and transportation or absorption, including acetyl-CoA carboxylase, malic enzyme, lipoprotein lipase, peroxisome proliferator-activated receptor gamma (PPAR\(\gamma\)), and decreased the expression of lipolysis gene such as carnitine palmitoyl transferase 1a (Cpt1a). At the same time, dietary Mn had no influence on expression of diacylglycerol acyltransferase 2, fatty acid transport protein 1 (FATP1), and peroxisome proliferator-activated receptor alpha (PPAR\(\alpha\)).

**DISCUSSION**

As the development of poultry feeding and breeding industry, the growth rate of poultry was faster than that 3 decades ago, but the requirement of trace mineral requirement is still not updated. The current NRC (1994)-recommended Mn level for optimal performance in ducks is 50 mg Mn/kg for 0–2 wk. It has been clearly established that dietary nutrients that include trace minerals of diets can affect their development and performance in the feeding period. Rats showed the impairment of reproduction and growth disorders (Orent and Mccollum, 1931) when feeding free Mn diets. Growth performance and incidence of leg abnormality were the earliest and most popular criteria for the evaluation of Mn requirement for broiler chicks. In this study, we

| Items          | 0       | 30      | 60      | 90      | 120     | 150     | 240     | SEM    | ANOVA Linear Quadratic |
|----------------|---------|---------|---------|---------|---------|---------|---------|--------|------------------------|
| MnSOD          | 190.71  | 204.96  | 204.01  | 213.14  | 230.04  | 218.52  | 208.60  | 28.282 | 0.980      | 0.588 | 0.615 |
| TAOC           | 0.62    | 0.68    | 0.78    | 0.73    | 0.64    | 0.53    | 0.59    | 0.163  | 0.952      | 0.539 | 0.782 |
| MDA            | 1.47a   | 0.93b   | 0.88c   | 0.74d   | 0.64b   | 0.66b   | 1.46abc | 0.214  | 0.047      | 0.097 | 0.002 |
| ASA            | 39.20   | 46.70   | 42.25   | 46.40   | 45.05   | 41.92   | 44.33   | 3.220  | 0.771      | 0.748 | 0.815 |
| AHR            | 35.71   | 35.14   | 35.01   | 31.43   | 37.98   | 32.36   | 33.50   | 2.750  | 0.778      | 0.571 | 0.835 |

Table 5. Effects of dietary gradient level manganese on antioxidant status of duck breast muscle.

Data are given as means and standard error of mean. Means within a row lacking a common superscript differ (\(P < 0.05\)).

Abbreviations: AHR, anti-hydroxy radical (U/mg protein) activity; ASA, anti-superoxide anion (U/mg protein) activity; MDA, malondialdehyde (nmol/mg protein); MnSOD, manganese superoxide dismutase (U/mg protein); TAOC, total antioxidant capacity (U/mg protein).

---

Figure 1. Effects of dietary manganese supplementation on the expression of antioxidant enzyme in the breast muscle of a duck. The mRNA expression of (A) manganese superoxide dismutase (MnSOD), (B) thioredoxin 2 (trx2), (C) peroxiredoxin (prdx), (D) heme oxygenase-1 (HO-1), (E) catalase (CAT) in the breast muscle were measured by RT-PCR. Date represent means with standard error of mean. Bars with different letters are statistically significant (\(P * 0.05\)) in different dietary manganese level.
did not find the leg abnormality caused by dietary Mn level. Birds could reach the maximum weight gain when fed casein-dextrose purified basal diet (containing 1.4 mg Mn/kg) supplemented with 14 mg Mn/kg (Southern and Baker, 1983; Li et al., 2011a; Brooks et al., 2012); however, there was no significant change in the growth performance as fed corn-soybean meal diet supplemented with inorganic or organic Mn (Stock and Latshaw, 1981). In other study of broilers, Mwangi et al. (2019) report that feeding marginally deficient Mn diets to broiler chicks after hatch does affect BW at 21 d old (Mwangi et al., 2019). Same as previous studies, the data presented herein demonstrated that dietary Mn supplementation has no influence on the growth performance; thus, we thought the abnormal decrease in the feed-to-gain ratio of day 1–14 at 150 mg in Mn/kg group may prove to be a special case.

The quantifiable properties of meat are indispensable for processors involved in the manufacture of value-added meat products, such as water-holding capacity (WHC), shear force, drip loss, cook loss, pH, shelf life, protein solubility, and fat-binding capacity (Mir et al., 2017). In this study, we measured the physical and chemical properties of the breast muscle, such as shear force, drip loss, color, pH, and IMF, and the results showed that dietary Mn significantly increased the a* value and IMF and decreased drip loss and shear force of the breast muscle. Although pH value has a great effect on meat quality that reflects the change of acidity in the process of muscle tissue fermentation and speed of glycogen fermentation after slaughtering, the stable pH value is conducive to the normal maturation of muscles (Watanabe et al., 1996). After slaughter, rapid pH fall in muscle results in myofibrillar protein denaturation in company with decreased protein solubility, which leads to poor WHC and deteriorated drip loss (Lesiów and Xiong, 2013). In this study, the decreased drip loss in the breast muscle may be attributed to a stable pH value (Witak, 2008). Color is the most important trait for the meat appearance (Joo et al., 2013), which is influenced by sex, genotype, and breed; besides, it also related to pH value (Fletcher, 1999). We found that Mn significantly increased the a* value, which is consistent with the action of zinc as our laboratory work (Wen et al., 2019).

Meat tenderness is a key factor determining consumer acceptability of cooked meat (Joo et al., 2013) and usually associated with the amount of IMF content and structure of a muscle fiber (Cai et al., 2018). Shear force is a reliable indicator inversely representing meat tenderness. As in the report of Chartrin et al. (2006), increasing IMF levels in the breast muscle increased tenderness with a correlation coefficient of 0.43. In our study, Mn significantly increased IMF and decreased shear force, which was similar to the studies performed by Yang et al. (2011) on broiler chickens.
As we know, the activities of antioxidant enzymes were significantly lower in the pale, soft, and exudative poultry meat (Carvalho et al., 2017). As reported by Jiang et al. (2007), the high WHC could alleviate lipid oxidation. In this study, the increase in MnSOD activity was not significant, as reported by Lu et al. (2007), that the

Figure 2. Effects of dietary manganese supplementation on manganese deposit in tissues of ducks. The manganese concentration of the tibia (A), breast muscle (B), serum (C), and heart (D) were measured by atomic absorbance spectrophotometry. Data represent means with standard error of mean. Bars with different letters are statistically significant ($P < 0.05$) in different dietary manganese level.

Figure 3. Effects of dietary manganese supplementation on expression of gene about fatty acid synthesis including (A) acetyl-CoA carboxylase (ACC), (B) malic enzyme (ME), (C) diacylglycerol acyltransferase 2 (DGAT2), and transportation or absorption gene, such as (D) lipoprotein lipase (LPL), (E) fatty acid transport protein 1 (FATP 1), and lipolysis gene including (F) peroxisome proliferator–activated receptor alpha (PPARα), (G) peroxisome proliferator–activated receptor gamma (PPARγ), (H) carnitine palmitoyl transferase 1α (Cpt1α). Data represent means with standard error of mean. Bars with different letters are statistically significant ($P < 0.05$) in different dietary manganese level.
Manganese is closely related to the level of reactive oxygen species (ROS) in the mitochondria (Zhang et al., 2020); we also confirmed in this study that Mn reduced lipid peroxidation induced by oxidative stress. Thus, we further measured the expression levels of antioxidant-related genes, including MnSOD, Trx 2, and Prdx 3, which expressed in mitochondria, and CAT synthesized from peroxisome. Thioredoxin 2 and Prdx 3 as a member of the thioredoxin system in mitochondria is involved in controlling the mitochondrial lipid peroxidation of cellular membrane. However, there is an increase in effect of MDA concentration with high dietary Mn in the longissimus thoracis of pig (Schwarz et al., 2017).

As shown in the results of fatty acid profile of the breast muscle, the SFA content in duck meat was higher than that in broilers, which is about 35% (Zhao et al., 2019). Compared with wild-living Mallard, Pekin duck breast muscle contained more SFA and monounsaturated fatty acids and less PUFA; age of birds and food resources may be the reason for the difference (Janiszewski et al., 2018). In addition, the saturation of fatty acids determines the degree of fat firmness, directly affecting the meat quality and nutritional value (Forte et al., 2018), and PUFA considered as the nutrition and healthy fatty acid. And surprisingly, our results have shown that Mn increased the proportions of C20 family ($P < 0.05$), including eicosanoic acid (C20:0), eicosenoic acid (C20:1n9), eicosadienoic acid (20:2), eicosatrienoic acid (C20:3n3), the metabolites of these fatty acid play an important role in physiological activities such as lipid metabolism (Endo et al., 2009), and the underlying mechanism need further study.

In previous studies, tissue mineral accumulation has been used to determine body mineral utilization, storage, and bioavailability; Mn concentrations in the liver, bone, and pancreas are frequently measured because they are by far the tissues richest in Mn (Luo et al., 1991). In this study, we confirmed that dietary Mn increased the Mn content of the tibia ash and breast muscle in linearly, which in accord with reports of Li et al. (2004) that also showed the bioavailability of different Mn resources. Our study has shown that the heart Mn conforms to the quadratic curve; this curve also was reported by Li et al. (2011a). However, the heart Mn displayed linear responses to dietary Mn when broiler was at 22–42 d of age (Lu et al., 2016). Besides, Mn source with different chelation strength could have an effect on the heart Mn concentrations, and the Mn with moderate chelation strength could be more effective than others (Li et al., 2011b). Those results suggested that the heart Mn concentration could be sensitive to dietary Mn.

As described by O’Hea and Leveille (1969), the 90% of fatty acids in body were synthesized by the liver, and about 80–85% of the fatty acids that accumulated in the adipose tissue are derived from plasma lipids (Hermier, 1997). Thus, we measure the change in mRNA expression of fatty acid de novo lipogenesis, transportation, and fatty acid oxidation in the breast muscle with dietary Mn supplementation, on account of our results that showed increased IMF with dietary Mn level. Acetyl-CoA carboxylase and malic enzyme played important roles in fatty acid de novo lipogenesis (Tian et al., 2019), those charge to biosynthesis of fatty acids by catalyst acetyl-CoA to malonyl-CoA and provides NADPH and oxaloacetic acid in the pathway of conversion of pyruvate to fatty acids, respectively. Therefore, our results proved that Mn improved the de novo lipogenesis in the breast muscle of ducks, combined with the slightly increased expression of diacylglycerol acyltransferase 2 ($P = 0.3289$). Lipoprotein lipase is a key enzyme of lipid metabolism and is synthesized mainly in adipose tissue, skeletal muscles, and mammary gland (Pascual et al., 2017), through catalyzes hydrolysis of TG and involved in the uptake of lipoprotein remnant. As report by Mario et al. (2004), FATP-1 levels in soleus were double those in gastrocnemius muscle, which has lower TG content than soleus. In this study, the expression of lipoprotein lipase consistent with dietary Mn level, companied with slightly increased expression of FATP1 ($P = 0.1198$), suggesting the fatty acid transportation was activated by Mn. Peroxisome proliferator–activated receptors and Cpt1α were associated with fatty acid oxidation in the mitochondria, Cpt1α catalyzes the long-chain fatty acids to acylcarnitine and across the mitochondrial membranes, and β-oxidation by mitochondria (Song et al., 2010). At the same time, PPARγ is essential for the deposition and formation of fat (Rosen et al., 1999). Our results demonstrate that Mn promotes fat deposition and inhibits fatty acid decomposition, which were evidenced by an increase in PPARγ expression and decrease in Cpt1α expression. Collectively, these findings indicate that dietary Mn is beneficial to the accumulation of fat via regulating mRNA expression of fatty acid de novo
lipogenesis, transportation, and fatty acid oxidation in the breast muscle.

Taken together, this study indicated that dietary Mn enhances the breast muscle quality through improving the ability of oxidative stability, the proportions of the C20 family, and accumulation of fat. Besides, the results also suggested that the heart Mn concentration could be sensitive to dietary Mn. The increased fatty acid de novo lipogenesis, transportation, and decreased fatty acid oxidation were involved in deposition of fat in the breast muscle caused by dietary Mn. This work provides important evidence concerning the effect of dietary Mn in meat duck breast muscle quality, which closely related to commercial value of duck.

ACKNOWLEDGMENTS

This work was supported by Sichuan Longda Animal Husbandry Science and Technology Co., Ltd. (No.009H2200).

Ting Yang and Gang Jia designed the experiment and completed this article. Xiaoyan Wang provided part service of fatty acid profile assay with gas chromatography and language edit service of this article. Min Wen did a favor with data statistics. The others author also helpful for this article.

DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

REFERENCES

Aguirre, J. D., and V. C. Culotta. 2012. Battles with iron: manganese in oxidative stress protection. J. Biol. Chem. 287:13541–13548.

Ahmed, S. T., M. M. Islam, A. B. Bostami, H. S. Mun, Y. J. Kim, and C. J. Yang. 2015. Meat composition, fatty acid profile and oxidative stability of meat from broilers supplemented with pomegranate (Punica granatum L.) by-products. Food Chem. 188:481–488.

Apple, J. K., W. J. Roberts, C. V. Maxwell, C. B. Boger, T. M. Fakler, and K. G. Friesen. 2004. Effect of supplemental manganese on mineral uptake by tissues and immune response in broiler chickens. Poult. Sci. 83:371–377.

Hermier, D. 1997. Lipoprotein metabolism and fattening in poultry. J. Nutr. 127:805S–808S.

Horning, K. J., S. W. Caito, K. G. Tipps, A. B. Bowman, and M. Aschner. 2015. Manganese is essential for Neuronal health. Ann. Rev. Nutr. 35:71–108.

Janiszewski, P., D. Murawska, V. Hanzal, M. Gesek, D. Michalik, and M. Zawacka. 2018. Carcass characteristics, meat quality, and fatty acid composition of wild-living mallards (Anas platyrhynchos L.). Poult. Sci. 97:709–715.

Jiang, Z., S. Jiang, C. Lin, P. Xi, D. Yu, and T. Wu. 2007. Effects of soybean isoflavone on growth performance, meat quality, and Antioxidation in male broilers. Poult. Sci. 86:1356–1362.

Joo, S. T., G. D. Kim, Y. H. Hwang, and Y. C. Ryu. 2013. Control of fresh meat quality through manipulation of muscle fiber characteristics. Meat Sci. 95:828–836.

Llesiów, T., and Y. L. Xiong. 2013. A simple, reliable and reproductive method to obtain experimental pale, soft and exudative (PSE) pork. Meat Sci. 93:489–494.

Li, C., L. Wang, J. Zhang, M. Huang, F. Wong, X. Liu, F. Liu, X. Cui, G. Yang, J. Chen, Y. Liu, J. Wang, S. Liao, M. Gao, X. Hu, X. Shu, Q. Wang, Z. Yin, Z. Tang, and M. Liu. 2014. CERKL interacts with mitochondrial TRX2 and protects retinal cells from oxidative stress-induced apoptosis. Biochim. Biophys. Acta. 1842:1121–1129.

Li, S., Y. Lin, L. Lu, L. Xi, Z. Wang, S. Hao, L. Zhang, K. Li, and X. Luo. 2011a. An estimation of the manganese requirement for broilers from 1 to 21 days of age. Biol. Trace Elem. Res. 143:939–948.

Li, C., L. Lu, S. Hao, Y. Wang, L. Zhang, S. Liu, B. Lin, K. Li, and X. Luo. 2011b. Dietary manganese modulates expression of the manganese-containing superoxide dismutase gene in chickens. J. Nutr. 141:189–194.

Li, S., X. Luo, B. Liu, T. D. Crenshaw, X. Kuang, G. Shao, and S. Y. Yu. 2004. Use of chemical characteristics to predict the relative bioavailability of supplemental organic manganese sources for broilers. J. Anim. Sci. 82:2352–2363.
