Effects of Manganese on Serum Lipid Metabolism, Antioxidant Indices, and Manganese Deposition in Organ Tissues of 1- to 4-Week-Old Wulong Geese (Postprint)

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Abstract

This study aimed to investigate the effects of different dietary manganese levels on serum lipid metabolism, antioxidant indices, and manganese deposition in organ tissues of 1- to 4-week-old Wulong geese, in order to determine the appropriate manganese supplementation level in goose diets. A total of 360 one-day-old Wulong geese with similar body weight were randomly allocated to six groups, with six replicates per group and ten geese per replicate (half male and half female). The control group (Group I) was fed a basal diet (manganese content of 20 mg/kg), while the experimental groups were fed experimental diets supplemented with 30 (Group II), 60 (Group III), 90 (Group IV), 120 (Group V), and 150 mg/kg manganese (Group VI) based on the basal diet. The experimental period lasted for four weeks. The results showed: 1) Serum triglyceride and total cholesterol contents in Groups IV, V, and VI were extremely significantly higher than those in Group I ($P<0.01$), and Group III was significantly higher than Group I ($P<0.05$). There was no significant difference in serum alkaline phosphatase activity among all groups ($P>0.05$). 2) Serum total antioxidant capacity and glutathione peroxidase activity in Groups IV, V, and VI were extremely significantly higher than those in Group I ($P<0.01$), and Group III was significantly higher than Group I ($P<0.05$); serum malondialdehyde content in Group V was extremely significantly lower than that in Groups I, II, and III ($P<0.01$), and Groups IV and VI were significantly lower than Groups I, II, and III ($P<0.05$). 3) Manganese deposition in serum, heart, liver, and pancreas in Groups IV, V, and VI was extremely significantly higher than that in Group I ($P<0.01$), and manganese deposition in serum, heart, and liver in Groups II and III was significantly higher than that in Group I ($P<0.05$). In conclusion, dietary manganese supplementation can increase serum triglyceride and total

cholesterol contents, enhance the antioxidant capacity of the body, and increase manganese deposition in organ tissues. It is recommended that the appropriate manganese supplementation level in goose diets be 90–120 mg/kg.

Full Text

Effects of Manganese on Serum Lipid Metabolism, Antioxidant Indices and Organ Tissue Manganese Deposition of Wulong Geese Aged from 1 to 4 Weeks

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Abstract

This experiment was conducted to investigate the effects of dietary manganese levels on serum lipid metabolism, antioxidant indices, and organ tissue manganese deposition in 1- to 4-week-old Wulong geese, and to determine the appropriate dietary manganese supplementation level. Three hundred sixty one-day-old Wulong geese with similar body weight were randomly allocated into six groups, each consisting of six replicates with ten geese per replicate (half male and half female). The control group (Group I) received a basal diet containing 20 mg/kg manganese, while the experimental groups received the basal diet supplemented with 30 (Group II), 60 (Group III), 90 (Group IV), 120 (Group V), and 150 mg/kg (Group VI) manganese, respectively. The experiment lasted for four weeks.

The results showed: (1) Serum triglyceride and total cholesterol contents in Groups IV, V, and VI were extremely significantly higher than those in Group I ($P<0.01$), while Group III was significantly higher than Group I ($P<0.05$). No significant differences were observed in serum alkaline phosphatase activity among all groups ($P>0.05$). (2) Serum total antioxidant capacity and glutathione peroxidase activity in Groups IV, V, and VI were extremely significantly higher than those in Group I ($P<0.01$), and Group III was significantly higher than Group I ($P<0.05$). Serum malondialdehyde content in Group V was extremely significantly lower than that in Groups I, II, and III ($P<0.01$), while Groups IV and VI were significantly lower than Groups I, II, and III ($P<0.05$). (3) Manganese deposition in serum, heart, liver, and pancreas of Groups IV, V, and VI was extremely significantly higher than that of Group I ($P<0.01$), and Groups II and III showed significantly higher manganese deposition in serum, heart, and liver compared to Group I ($P<0.05$). In conclusion, dietary manganese supplementation can increase serum triglyceride and total cholesterol

contents, enhance antioxidant capacity, and increase manganese deposition in organ tissues of Wulong geese. It is recommended that the suitable manganese supplementation level in goose diets is 90-120 mg/kg.

Keywords: manganese; Wulong geese; lipid metabolism; antioxidant capacity; manganese deposition

Introduction

Manganese (Mn) is widely distributed in nature, occurring in soil, minerals, plants, and deep-sea rock beds, and is an essential trace element for organisms. Its primary nutritional and physiological functions involve serving as an enzyme activator or component in carbohydrate, lipid, protein, and cholesterol metabolism. Manganese promotes growth and enhances immunity, and is indispensable for redox processes, tissue respiration, bone formation and growth, reproduction, embryonic development, blood formation, eggshell formation, and normal endocrine function in poultry. Thus, manganese plays an irreplaceable role in poultry nutrition, making research on manganese requirements crucial for the poultry industry.

Previous studies have demonstrated that manganese supplementation significantly increases cholesterol content in broiler chickens, indicating that manganese can elevate serum cholesterol levels. Research has also shown that manganese content varies across different tissues and organs, with the highest levels found in the liver, followed by the pancreas and heart, and the lowest in serum. Furthermore, different manganese levels affect antioxidant enzyme activity and lipid peroxide generation in immune organs. Among manganese sources, manganese sulfate has the highest bioavailability, followed by manganese oxide and manganese carbonate. Manganese deficiency in diets can cause skeletal deformities (perosis) in poultry. However, few studies have reported on manganese' s effects on antioxidant capacity. To date, research on manganese supplementation has focused primarily on chickens and ducks, with limited studies on geese. Therefore, this experiment used 1- to 4-week-old Wulong geese to investigate the effects of dietary manganese levels on serum lipid metabolism, antioxidant indices, and organ tissue manganese deposition, providing a foundation for determining appropriate manganese supplementation levels in goose diets.

Materials and Methods

Experimental Animals and Design Three hundred sixty healthy one-day-old Wulong geese with similar body weight were randomly allocated into six groups using a random numbering method. Each group contained six replicates with ten geese per replicate (half male and half female). The control group (Group I) received a basal diet containing 20 mg/kg manganese, while the experimental groups received the basal diet supplemented with 30 (Group II), 60

(Group III), 90 (Group IV), 120 (Group V), and 150 mg/kg (Group VI) manganese. The experimental period lasted four weeks. The geese were provided by the Laiyang Tiansen Huoyan Goose Breeding Center, a demonstration base of the National Waterfowl Industry Technology System. Manganese sulfate monohydrate ($MnSO_4 \cdot H_2O$, 33.2% manganese content) was purchased from Zhejiang Xinweipu Additive Co., Ltd.

Basal Diet The nutritional levels of the basal diet were formulated according to the NRC *Nutrient Requirements of Poultry* (1994 edition). The composition and nutrient levels of the basal diet are presented in Table 1 .

Table 1 Composition and nutrient levels of the basal diet (air-dry basis), %

Note: 1) The multivitamin and trace elements (without Mn) provided the following per kg of diet: nicotinic acid 65 mg, pantothenate 15 mg, folic acid 0.5 mg, VD_3 200 IU, VA 1,500 IU, VB_1 2.2 mg, VB_2 5.0 mg, VB_6 2 mg, VE 12.5 mg, VK_3 1.5 mg, biotin 0.2 mg, choline 1,000 mg, Fe 90 mg, Zn 85 mg, Cu 6 mg, I 0.42 mg, Se 0.3 mg, Co 2.5 mg. 2) Manganese was a measured value, while other nutrient levels were calculated values.

Management Before the experiment, the goose house was thoroughly disinfected. Throughout the experimental period, geese were raised indoors on deep litter in separate pens with free access to water and feed. Feed was provided in small amounts frequently, and daily feed intake was recorded. The growth status of the flocks was observed daily.

Sample Collection At the end of the fourth week, two geese were randomly selected from each replicate (72 geese total, half male and half female) after a 12-hour fast. Five milliliters of blood were collected from the wing vein and centrifuged at 3,000 r/min for 10 minutes to obtain serum. The heart, liver, and pancreas were collected after dissection, cleaned of attached tissues, rinsed three times with distilled water to remove contaminated blood, and stored at ultra-low temperature until analysis.

Laboratory Analyses **Serum lipid metabolism indices:** Serum triglyceride content was determined using a triglyceride (TG) assay kit; total cholesterol content was measured using a total cholesterol (CHOL) assay kit; and alkaline phosphatase (AKP) activity was determined using an alkaline phosphatase assay kit. All kits were purchased from Nanjing Jiancheng Bioengineering Institute.

Serum antioxidant indices: Serum total antioxidant capacity (T-AOC) was measured using a total antioxidant capacity assay kit; malondialdehyde (MDA) content was determined using an MDA assay kit; and glutathione peroxidase (GSH-Px) activity was measured using a glutathione peroxidase assay kit.

Manganese deposition in organ tissues: The heart, liver, and pancreas were dried in a 75°C oven to constant weight, then ground into fine powder using a mortar. Exactly 0.250 g of dried sample was placed in a Kjeldahl flask, digested with 10 mL nitric acid and 2.5 mL perchloric acid on a temperature-controlled electric furnace until no white smoke or reflux remained, then cooled. The digested sample was transferred without loss to a 25 mL volumetric flask and diluted to volume with distilled water. Manganese content was determined using atomic absorption spectrophotometry.

Statistical Analysis Data were analyzed using one-way ANOVA with LSD multiple comparisons in SPSS 17.0 software. Results are expressed as "mean ± standard deviation." Correlation analysis was performed using bivariate correlation analysis in SPSS 17.0. $P<0.05$ and $P<0.01$ were considered significant and extremely significant levels, respectively.

Results

Effects of Dietary Manganese Levels on Serum Lipid Metabolism Indices As shown in Table 2 , serum triglyceride and total cholesterol contents in Groups IV, V, and VI were extremely significantly higher than those in Group I ($P<0.01$), while Group III was significantly higher than Group I ($P<0.05$). No significant differences were observed between Groups I and II ($P>0.05$). Group V showed the highest serum triglyceride and total cholesterol contents. Serum alkaline phosphatase activity did not differ significantly among all groups ($P>0.05$).

Table 2 Effects of dietary manganese levels on serum lipid metabolism indices of Wulong geese

Note: In the same column, values with the same small letter or no letter superscripts indicate no significant difference ($P>0.05$), adjacent small letters indicate significant difference ($P<0.05$), and alternate small letters indicate extremely significant difference ($P<0.01$). The same applies to Table 3 and Table 4.

Effects of Dietary Manganese Levels on Serum Antioxidant Indices As shown in Table 3 , serum total antioxidant capacity and glutathione peroxidase activity initially increased and then decreased with increasing dietary manganese levels. Groups IV, V, and VI showed extremely significantly higher serum total antioxidant capacity and glutathione peroxidase activity than Group I ($P<0.01$), while Group III was significantly higher than Group I ($P<0.05$). Serum malondialdehyde content in Group V was extremely significantly lower than that in Groups I, II, and III ($P<0.01$), and Groups IV and VI were significantly lower than Groups I, II, and III ($P<0.05$). When dietary manganese supplementation reached 120 mg/kg, serum total antioxidant capacity and glutathione peroxidase activity peaked, while serum malondialdehyde content reached its lowest level.

These results indicate that the manganese nutritional requirement of geese has a certain threshold, as both excessive and deficient levels adversely affect the organism. Appropriate dietary manganese supplementation can enhance the antioxidant capacity of Wulong geese.

Table 3 Effects of dietary manganese levels on serum antioxidant indices of Wulong geese

Effects of Dietary Manganese Levels on Manganese Deposition in Serum and Organ Tissues As shown in Table 4 , manganese deposition in serum, heart, liver, and pancreas initially increased and then decreased with increasing dietary manganese levels. Groups IV, V, and VI showed extremely significantly higher manganese deposition in serum, heart, liver, and pancreas than Group I ($P<0.01$), while Groups II and III had significantly higher manganese deposition in serum, heart, and liver than Group I ($P<0.05$). Manganese deposition in serum and organ tissues of Wulong geese peaked when dietary manganese supplementation reached 120 mg/kg. The pancreas showed the highest manganese deposition, followed by the liver and heart, with the lowest levels in serum.

These results demonstrate that dietary manganese levels significantly affect manganese deposition in serum and tissues of Wulong geese.

Table 4 Effects of dietary manganese levels on manganese deposition in serum and organ tissue of Wulong geese

Correlation Analysis As shown in Table 5 , dietary manganese levels were extremely significantly positively correlated with serum triglyceride content, total cholesterol content, and alkaline phosphatase activity at 4 weeks of age ($P<0.01$).

Table 5 Correlation analysis of dietary manganese levels and serum lipid metabolism indices

*Note: ** indicates extremely significant correlation ($P<0.01$). The same applies below.*

As shown in Table 6 , dietary manganese levels were extremely significantly positively correlated with serum total antioxidant capacity and glutathione peroxidase activity ($P<0.01$), and extremely significantly negatively correlated with serum malondialdehyde content ($P<0.01$).

Table 6 Correlation analysis of dietary manganese levels and serum antioxidant indices

As shown in Table 7 , dietary manganese levels were extremely significantly positively correlated with manganese deposition in serum, heart, liver, and pancreas ($P<0.01$).

Table 7 Correlation analysis of dietary manganese levels and manganese deposition in serum and organ tissue

Discussion

Effects of Dietary Manganese Levels on Serum Lipid Metabolism Indices Triglycerides are related to animal growth, development, and immune function, and their content changes reflect lipid metabolism status. Previous research has shown that when manganese is deficient, the body automatically regulates lipoprotein decomposition to compensate for decreased blood cholesterol, and triglycerides from decomposed lipoproteins increase plasma triglyceride content. Other studies have demonstrated that dietary manganese supplementation has extremely significant effects on serum triglyceride content. Cholesterol is a component of cell membranes; some blood cholesterol is used for tissue cell structure, while another portion is converted to vitamin D_3, which promotes calcium absorption or steroid hormone metabolism. Clinical trials in humans have also proven that manganese can increase hepatic cholesterol synthesis. The present results show that serum triglyceride and cholesterol contents increased significantly with dietary manganese levels, reaching maximum values at 120 mg/kg manganese supplementation. Total serum cholesterol in all experimental groups was higher than in the control group, indicating that manganese deficiency reduces cholesterol synthesis. These findings are consistent with previous studies.

Effects of Dietary Manganese Levels on Serum Antioxidant Indices Poultry have vigorous metabolism and produce many free radicals, especially under intensive farming conditions where various environmental stressors can disrupt the balance of free radicals in the body and increase disease susceptibility. Therefore, compared with mammals, broiler ducks require a more powerful antioxidant system to eliminate excessive free radicals and maintain their stability and balance for health and normal growth. Manganese is closely related to poultry antioxidant function, and its supply is particularly important. Manganese is an active component of superoxide dismutase (SOD) and also affects the production of non-enzymatic antioxidant proteins in tissues; the manganese nutritional status of animals influences the antioxidant status of body tissues.

Total antioxidant capacity is a comprehensive indicator used to measure the functional status of the antioxidant system, reflecting the combined effects of various antioxidant enzymes in the body. The level of total antioxidant capacity directly indicates the response capacity of the enzymatic and non-enzymatic systems when facing external stimuli. When dietary manganese is deficient, the total antioxidant function of the organism decreases; appropriately increasing dietary manganese levels within a certain range can effectively improve total antioxidant capacity, though this capacity does not increase linearly with dietary manganese levels.

Malondialdehyde is a product of lipid peroxidation reactions in the body, pri-

marily produced by free radicals generated from enzymatic and non-enzymatic systems reacting with unsaturated fatty acids on cell membranes. It directly reflects the level of oxidative free radicals and the degree of cellular damage from oxidative attack. Glutathione peroxidase is an important peroxide-decomposing enzyme widely present in the organism and serves as one of the indicators of anti-peroxidation capacity.

Previous research has shown that the manganese nutritional status of animals affects the antioxidant status of body tissues. Other studies have reported that dietary supplementation with 60 mg/kg manganese can promote immune organ weight in broiler chickens. The present results demonstrate that dietary manganese supplementation at 60-120 mg/kg significantly increased serum total antioxidant capacity and glutathione peroxidase activity while significantly reducing malondialdehyde content, which aligns with previous findings.

Effects of Dietary Manganese Levels on Manganese Deposition in Organ Tissues Liver trace element content is an important indicator reflecting trace element absorption in the organism. The divalent metal transporter 1 (DMT1) is the intestinal absorption carrier for manganese, and its expression level is closely related to manganese source characteristics. Previous research suggests that myocardial manganese content can be used to evaluate changes in dietary manganese levels in broiler chickens. Studies have shown that pancreatic and myocardial manganese contents increase significantly with dietary manganese levels. Other research has demonstrated that manganese content in layer chicken tissues increases significantly with dietary manganese levels, and that dietary manganese levels significantly affect myocardial manganese content.

The absorption rate of dietary manganese in animals is very low. In poultry, manganese absorption occurs primarily in the duodenum; absorbed manganese enters the blood and is rapidly distributed to the liver, bone, and feathers, which become the main storage sites for manganese. Under normal conditions, manganese is excreted through bile but can also be excreted through the pancreas. Manganese distribution varies greatly among the same type of tissues in different locations due to differences in physiological function and metabolic level. Previous studies have shown that manganese deposition varies among organ tissues, with the highest content in the liver, followed by the pancreas and heart, and the lowest in serum.

The present results show that pancreatic manganese deposition increased with dietary manganese levels and was the highest among all tissues, indicating that pancreatic manganese deposition can serve as a sensitive indicator for evaluating manganese nutrition status. Different organ tissues showed varying manganese deposition levels, with the pancreas having the highest, followed by the liver and heart, and serum having the lowest, suggesting that trace element manganese distribution in animal tissues is related to tissue metabolic characteristics.

Conclusions

1. Appropriate dietary manganese supplementation levels can significantly increase serum triglyceride and total cholesterol contents.
2. Appropriate dietary manganese supplementation levels can significantly increase serum total antioxidant capacity and glutathione peroxidase activity.
3. Appropriate dietary manganese supplementation levels can significantly increase manganese deposition in serum, heart, liver, and pancreas.
4. The manganese nutritional requirement of geese has a certain threshold, as both excessive and deficient levels adversely affect the organism. It is recommended that the suitable manganese supplementation level in Wulong goose diets is 90-120 mg/kg.

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Note: Figure translations are in progress. See original paper for figures.