Synergistic Effects of Bacillus subtilis and Manganese on Serum Biochemical Indices, Antioxidant Capacity, and Tibial Development in 5-11 Week-Old Wulong Geese (Postprint)

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Abstract

This experiment aimed to investigate the synergistic effects of Bacillus subtilis and manganese on serum biochemical indices, antioxidant capacity, and tibia development in 5-11-week-old Wulong geese, and to explore the influence of dietary Bacillus subtilis supplementation on manganese requirement in meat geese. A total of 360 five-week-old Wulong geese were randomly allocated to 6 groups with 6 replicates per group and 10 geese per replicate (half male and half female). Group was fed a diet with 105 mg/kg manganese supplementation without Bacillus subtilis; Groups – were fed diets supplemented with 250 g/t Bacillus subtilis and manganese at levels of 0, 35, 70, 105, and 170 mg/kg, respectively. The experimental period lasted 7 weeks. The results showed: 1) In Groups – , serum alkaline phosphatase (AKP) activity and triglycerides (TG) and total cholesterol (CHOL) contents exhibited a trend of first increasing and then decreasing with increasing dietary manganese levels, with no significant differences among groups (P>0.05), and all were highest in Group . 2) In Groups – , serum total antioxidant capacity (T-AOC) and glutathione peroxidase (GSH-Px) activity showed a trend of first increasing and then decreasing with increasing dietary manganese levels; Group was significantly higher than Group (P<0.05) and extremely significantly higher than Group (P<0.01); serum malondialdehyde (MDA) content in Groups – showed a trend of first decreasing and then increasing with increasing dietary manganese levels, with Group being extremely significantly lower than Group (P<0.01). 3) Bone mineral density, bone strength, tibia weight, and tibia ash content in Groups – exhibited a trend of first increasing and then decreasing with increasing dietary manganese levels; bone mineral density, bone strength, tibia weight, and tibia calcium content in Group were significantly or extremely significantly higher than those in Group (P<0.05 or P<0.01); tibia manganese content in Groups , , , and was significantly or extremely significantly lower than that in Group (P<0.05 or P<0.01). In conclusion, dietary Bacillus subtilis supplementation can enhance the biological efficacy of manganese, further improve nutritional assimilation, strengthen antioxidant capacity, promote tibia development, and reduce tibia manganese content; under the condition of adding 250 g/t Bacillus subtilis to the diet of 5–11-week-old Wulong geese, the appropriate manganese supplementation level is recommended to be 70 mg/kg.

Full Text

Effects of Bacillus subtilis Cooperate with Manganese on Serum Biochemical Indices, Antioxidant Activity and Tibia Development of Wulong Geese Aged from 5 to 11 Weeks

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Abstract

This experiment was conducted to investigate the effects of Bacillus subtilis cooperate with manganese on serum biochemical indices, antioxidant activity, and tibia development of Wulong geese aged from 5 to 11 weeks, and to explore how dietary Bacillus subtilis supplementation affects manganese requirements in meat geese. A total of 360 five-week-old Wulong geese were randomly allocated into 6 groups with 6 replicates per group and 10 geese per replicate (half male and half female). Group I received a diet supplemented with 105 mg/kg manganese without Bacillus subtilis. Groups II–VI received diets supplemented with 250 g/t Bacillus subtilis and varying manganese levels of 0, 35, 70, 105, and 170 mg/kg, respectively. The experiment lasted for 7 weeks.

The results showed: (1) Serum alkaline phosphatase (AKP) activity and triglyceride (TG) and total cholesterol (CHOL) contents in groups II–VI exhibited a quadratic response to increasing dietary manganese levels, peaking in group IV, though differences among groups were not significant (P>0.05). (2) Serum total antioxidant capacity (T-AOC) and glutathione peroxidase (GSH-Px) activity in groups II–VI also showed a quadratic response, with group IV being significantly higher than group I (P<0.05) and extremely significantly higher than group II (P<0.01). Serum malondialdehyde (MDA) content displayed an inverse quadratic trend, with group IV being extremely significantly lower than group II (P<0.01). (3) Bone mineral density, bone strength, tibia weight, and tibia ash content in groups II–VI similarly showed quadratic responses, with group IV exhibiting significantly or extremely significantly higher bone mineral density, bone strength, tibia weight, and tibia calcium content compared to group I (P<0.05 or P<0.01). Tibia manganese content in groups II, III, IV, and V was significantly or extremely significantly lower than in group I (P<0.05 or P<0.01).

These findings indicate that dietary Bacillus subtilis supplementation can enhance manganese bioavailability, improve nutritional assimilation, strengthen antioxidant capacity, promote tibia development, and reduce tibia manganese content. Under the condition of 250 g/t Bacillus subtilis supplementation, the optimal dietary manganese level for 5-11 week-old Wulong geese is recommended to be 70 mg/kg.

Keywords: Bacillus subtilis; Wulong geese; manganese; serum biochemical indices; tibia development; antioxidant activity

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Manganese plays crucial nutritional and physiological roles as an enzyme activator or component in carbohydrate, lipid, protein, and cholesterol metabolism. It promotes growth and enhances immunity, and is indispensable for normal physiological functions in poultry including redox processes, tissue respiration, bone formation and growth, reproduction, embryonic development, blood formation, eggshell formation, and endocrine organ function [1]. Manganese deficiency readily leads to nutritional deficiency diseases. Poultry have higher manganese requirements than livestock. In poultry diets, corn contains minimal manganese, and poultry have relatively low intestinal absorption rates for manganese, making them more susceptible to manganese deficiency [2]. Research on manganese requirements is therefore significant for the poultry industry.

Previous studies have demonstrated that varying manganese levels affect antioxidant enzyme activity in immune organs and lipid peroxide generation [3, 4]. Luo et al. [5] found that dietary manganese supplementation significantly increased serum total cholesterol (CHOL) content in broiler chickens, indicating that manganese deficiency reduces cholesterol synthesis. Organic trace elements may have different post-absorption metabolism and higher bioavailability compared to their inorganic salts [6]. Ferket et al. [7] reported that dietary supplementation with manganese methionine hydroxy analogue chelate reduced tibial chondrodystrophy and lameness while increasing bone breaking strength in turkeys.

Microecological preparations have been extensively studied. Lactic acid bacteria produce antibacterial substances that inhibit intestinal putrefaction, improve intestinal environment, and consequently enhance health and longevity [8]. Yu et al. [9] demonstrated that appropriate Bacillus subtilis supplementation significantly increased thymus and bursa indices in broilers, exerting important effects on immune performance. Selvam et al. [10] found that surfactants secreted by Bacillus subtilis could inhibit phospholipase A2, a key enzyme in inflammatory responses, thereby improving colitis. As a microecological preparation, Bacillus subtilis can effectively improve intestinal flora composition and promote nutrient absorption. López et al. [11] noted that Bacillus strains possess strong metal adsorption capacity because metal ions can be immobilized through interaction with anions on bacterial cell surfaces. Jayaraman et al. [12] discovered that Bacillus subtilis could inhibit Clostridium perfringens-induced necrotic enteritis in broilers, improving intestinal health.

While numerous studies have examined the effects and mechanisms of manganese and Bacillus subtilis individually in poultry, research on how dietary Bacillus subtilis influences trace element absorption and its intervention mechanisms remains scarce. Studies on the synergistic effects of Bacillus subtilis and manganese on antioxidant capacity are particularly lacking, and no reports exist on their combined effects on goose tibia development. Therefore, this study utilized 5-11 week-old Wulong geese (Huoyan geese) to investigate the effects of dietary Bacillus subtilis and varying manganese levels on serum biochemical indices, antioxidant capacity, and tibia development, aiming to establish methods for minimizing dietary manganese supplementation and providing technical support for low-emission ecological nutrition.

1. Materials and Methods

1.1 Experimental Animals and Design A total of 360 healthy five-week-old Wulong geese with uniform body weight were randomly divided into 6 groups with 6 replicates per group and 10 geese per replicate (half male and half female). Group I received a basal diet supplemented with 105 mg/kg manganese [13] without Bacillus subtilis. Groups II–VI received basal diets supplemented with 250 g/t Bacillus subtilis and manganese levels of 0, 35, 70, 105, and 170 mg/kg, respectively. The experiment lasted for 7 weeks. Experimental geese were provided by the breeding base of the Institute of High Quality Waterfowl at Qingdao Agricultural University. Manganese sulfate monohydrate (98% purity) was purchased from Zhejiang Xinweipu Additive Co., Ltd.

1.2 Experimental Diets The basal diet was formulated according to NRC (1994) poultry nutrient requirements. The composition and nutrient levels of the basal diet are presented in Table 1 . Manganese content in the basal diet was measured as 20 mg/kg using plasma emission spectrometry.

1.3 Management Practices Before the experiment, the goose house was thoroughly disinfected. Geese were raised indoors on deep litter bedding with free access to water and feed throughout the trial period. Feed was provided in small amounts frequently, and the growth status of the flocks was monitored closely.

1.4 Sample Collection and Measurements 1.4.1 Serum Biochemical Indices

At the end of week 11, 2 geese were randomly selected from each replicate (72 geese total, half male and half female). Blood samples (5 mL) were collected from the wing vein and centrifuged at 3,000 r/min for 10 minutes to obtain serum. Serum alkaline phosphatase (AKP) activity was measured using an AKP assay kit, triglyceride (TG) content using a TG assay kit, and total cholesterol (CHOL) content using a CHOL assay kit. All kits were purchased from Nanjing Jiancheng Bioengineering Institute.

1.4.2 Antioxidant Capacity Determination

Using the serum samples from 1.4.1, total antioxidant capacity (T-AOC) was measured with a T-AOC assay kit, malondialdehyde (MDA) content with an MDA assay kit, and glutathione peroxidase (GSH-Px) activity with a GSH-Px assay kit. All kits were purchased from Nanjing Jiancheng Bioengineering Institute.

1.4.3 Tibia Development Measurements

At the end of week 11, 2 geese were randomly selected from each replicate (72 geese total, half male and half female) and slaughtered. Left and right tibias were separated. The right tibia was used to measure bone mineral density (BMD) using an Osteocore 3 digital 闪烁式锥形扫描骨密度仪 and bone strength using a WD-1 electronic universal testing machine. After drying at 105°C, tibia weight was recorded. Tibia ash, calcium, phosphorus, and manganese contents were determined according to GB/T 6438-92, potassium permanganate (KMnO4) redox titration method (GB 6436-86), molybdenum yellow colorimetry (GB 6437-86), and atomic absorption spectrometry, respectively.

1.5 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" . Orthogonal polynomial contrasts were used to analyze linear or quadratic responses of parameters to dietary manganese levels, and curve fitting was performed to determine the optimal dietary manganese level for 5-11 week-old geese. $P<0.05$ and $P<0.01$ were considered significant and extremely significant, respectively.

2. Results

2.1 Effects of Bacillus subtilis and Manganese on Serum Biochemical Indices As shown in Table 2 , serum AKP activity and TG and CHOL contents in groups II–VI exhibited a quadratic response to increasing dietary manganese levels, peaking in group IV, though differences among groups were not significant ($P>0.05$). These results suggest that dietary Bacillus subtilis supplementation can enhance manganese bioavailability and improve nutritional assimilation, thereby reducing dietary manganese requirements.

2.2 Effects of Bacillus subtilis and Manganese on Serum Antioxidant Capacity Table 3 shows that serum T-AOC and GSH-Px activity in groups II–VI displayed a quadratic response to dietary manganese levels, with group IV being significantly higher than group I ($P<0.05$) and extremely significantly higher than group II ($P<0.01$). Serum MDA content showed an inverse quadratic trend, with group IV being extremely significantly lower than group II ($P<0.01$). These findings indicate that at 250 g/t Bacillus subtilis supplementation, 70 mg/kg manganese can extremely significantly increase serum T-AOC and GSH-Px activity while extremely significantly decreasing MDA content. Dietary manganese significantly affects antioxidant capacity in geese, and Bacillus subtilis can further enhance manganese's antioxidant effects, reducing exogenous manganese requirements. However, quadratic curve fitting between antioxidant indices and dietary manganese levels was not significant ($P>0.05$).

2.3 Effects of Bacillus subtilis and Manganese on Tibia Development Table 4 demonstrates that bone mineral density, bone strength, tibia weight, and tibia ash content in groups II–VI showed quadratic responses to dietary manganese levels. Group IV exhibited significantly or extremely significantly higher bone mineral density, bone strength, tibia weight, and tibia calcium content compared to group I ($P<0.05$ or $P<0.01$). Tibia manganese content in groups II, III, IV, and V was significantly or extremely significantly lower than in group I ($P<0.05$ or $P<0.01$). These results indicate that at 250 g/t Bacillus subtilis, 70 mg/kg manganese promotes optimal tibia development while reducing tibia manganese content. However, quadratic curve fitting between tibia development indices and dietary manganese levels was not significant ($P>0.05$).

3. Discussion

3.1 Effects on Serum Biochemical Indices Triglycerides are associated with animal growth, development, and immune function, and their content reflects membrane lipid metabolism. Luo et al. [5] reported that to compensate for decreased blood cholesterol during manganese deficiency, the body automatically regulates lipoprotein decomposition, and TG from decomposed lipoproteins increases plasma TG content. Cholesterol is a cell membrane component; some blood cholesterol forms structural components in tissues, while another portion converts to vitamin D3, promoting calcium absorption or steroid hormone metabolism. Ding et al. [14] demonstrated that manganese promotes cholesterol synthesis from 14C-labeled acetate in cultured hepatocytes, as manganese serves as a cofactor for mevalonate kinase. Manganese deficiency inhibits mevalonate kinase activity, affecting two steps from acetate to mevalonate that require Mn^{2+}; pyrophosphate synthetase requires Mn^{2+}, and blocked pyrophosphate ester synthesis inhibits squalene production, thereby impeding cholesterol synthesis. Manganese deficiency obstructs cholesterol and its precursor synthesis, causing disorders in sex hormone synthesis. Curran and Azarnoff [15] showed that manganese stimulates cholesterol synthesis and increases cholesterol production in rat liver. Our results demonstrate that serum AKP activity and TG and CHOL contents increased then decreased with rising dietary manganese levels, reaching maximum values at 70 mg/kg manganese with 250 g/t Bacillus subtilis supplementation. This indicates that Bacillus subtilis can enhance manganese bioavailability and improve nutritional assimilation, thereby reducing dietary manganese requirements.

3.2 Effects on Antioxidant Capacity Manganese is a component of superoxide dismutase (SOD) and affects the production of non-enzymatic antioxidant proteins. Manganese nutritional status influences tissue antioxidant status [16]. Poultry have vigorous metabolism and produce numerous free radicals, especially under intensive farming conditions where various environmental stressors can disrupt the balance of free radicals, increasing disease susceptibility. Therefore, compared to mammals, meat ducks require a more robust antioxidant system to eliminate excessive free radicals and maintain stability and balance for health and normal growth. Manganese is particularly important for poultry antioxidant capacity [17].

Total antioxidant capacity (T-AOC) is a comprehensive indicator of antioxidant system function, reflecting the combined effects of various antioxidant enzymes and directly indicating the response capability of enzymatic and non-enzymatic systems to external stimuli [18]. Malondialdehyde (MDA) is a product of lipid peroxidation, generated when free radicals from enzymatic and non-enzymatic systems react with unsaturated fatty acids on cell membranes, directly reflecting free radical levels and the degree of cellular damage. Glutathione peroxidase (GSH-Px) is a crucial peroxide-decomposing enzyme widely present in the body and serves as an indicator of anti-peroxidation capacity [19]. Our results show that at 250 g/t Bacillus subtilis, 70 mg/kg manganese extremely significantly increased serum T-AOC and GSH-Px activity while extremely significantly decreasing MDA content, demonstrating advantages over group I (105 mg/kg manganese without Bacillus subtilis). These findings align with previous reports and indicate that Bacillus subtilis supplementation in 5–11 week-old goose diets can further enhance manganese' s antioxidant capacity, reducing exogenous manganese requirements.

3.3 Effects on Tibia Development Manganese is closely related to bone growth in livestock and poultry. Manganese deficiency in poultry diets causes disordered bone tissue formation and leg weakness. Tibia manganese content is the most sensitive indicator for evaluating manganese bioavailability. Ferket et al. [7] reported that manganese methionine hydroxy analogue chelate reduced tibial chondrodystrophy, lameness, and increased bone breaking strength in turkeys. Chen et al. [20] found that supplementing broiler diets based on soybean meal with 40, 70, 100, 200, and 2000 mg/kg manganese showed no significant differences in weight gain or feed conversion up to 200 mg/kg, but the incidence of slipped tendon disease decreased with increasing manganese levels. Reports indicate that broilers fed corn-soybean meal diets had 75.0% leg disease incidence at 3 weeks, which decreased to 27.2% with 25 mg/kg manganese supplementation and only 5.0% with 100 mg/kg manganese [21], confirming that corn and soybean meal contain far less manganese than required by broilers. Manganese deficiency affects cartilage matrix synthesis in bones, reducing hexosamine and hexuronic acid content, thereby impairing chondroitin sulfate synthesis and mucopolysaccharide formation, leading to skeletal lesions clinically manifested as joint swelling and tendon displacement. Our results demonstrate that at 250 g/t Bacillus subtilis, 70 mg/kg manganese promotes optimal tibia development while reducing tibia manganese content. The mechanism by which Bacillus subtilis reduces tibia manganese content requires further investigation.

Conclusions

1. Dietary Bacillus subtilis supplementation can enhance manganese bioavailability, improve nutritional assimilation, and reduce dietary manganese requirements.
2. Appropriate dietary manganese levels combined with Bacillus subtilis can further increase serum T-AOC and GSH-Px activity, decrease serum MDA content, and enhance antioxidant capacity.
3. Appropriate dietary manganese levels combined with Bacillus subtilis can promote tibia development and reduce tibia manganese content.
4. For 5–11 week-old Wulong geese, when dietary Bacillus subtilis is supplemented at 250 g/t, the optimal manganese level is recommended to be 70 mg/kg.

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