# Effects of Bacillus subtilis Supplementation in Diets with Different Manganese Levels on Growth Performance, Slaughter Performance, Antioxidant Capacity, and Serum Biochemical Indices of 12-16 Week-Old Wulong Geese (Postprint)

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**Date:** 2017-10-11T00:00:00+00:00

## Abstract

This experiment was conducted to investigate the effects of dietary Bacillus subtilis supplementation at different manganese levels on growth performance, slaughter performance, antioxidant capacity, and serum biochemical indices of 12- to 16-week-old Wulong geese, aiming to explore a method for formulating low-manganese diets. A total of 360 12-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 I was the adequate manganese supplementation group (manganese level 105 mg/kg, without Bacillus subtilis), while Groups II-VI were Bacillus subtilis supplementation groups (Bacillus subtilis level 250 g/t) with manganese supplementation levels of 0, 35, 70, 105, and 170 mg/kg, respectively. The experimental period lasted for 5 weeks. The results showed: 1) Body weight and average daily gain in Groups IV and V were significantly higher than those in Group I ($P<0.05$), and the feed conversion ratio in Group IV was significantly lower than that in Group I ($P<0.05$). 2) Dressing percentage, semi-eviscerated yield, eviscerated yield, and leg muscle percentage in Group IV were all significantly higher than those in Group I ($P<0.05$), while abdominal fat percentage in Groups IV, V, and VI was significantly lower than that in Group I ($P<0.05$). 3) Serum total antioxidant capacity in Group IV was significantly higher than that in Group I ($P<0.05$), serum malondialdehyde content in Group IV was significantly lower than that in Group I ($P<0.05$), and serum glutathione peroxidase activity in Groups IV and V was significantly higher than that in Group I ($P<0.05$). 4) There were no significant differences in serum alkaline phosphatase activity and triglyceride and total cholesterol contents among all groups ($P>0.05$). These results indicate that dietary Bacillus subtilis supplementation can significantly improve growth performance, slaughter performance, and antioxidant capacity, as well as significantly enhance manganese utilization, thereby reducing dietary manganese supplementation levels in 12- to 16-week-old Wulong geese. Under the condition of 250 g/t Bacillus subtilis supplementation in the diet, the appropriate manganese supplementation level is 70 mg/kg.

## Full Text

# Effects of Dietary *Bacillus subtilis* Along with Different Manganese Levels on Growth Performance, Slaughter Performance, Antioxidant Activity and Serum Biochemical Indices of Wulong Geese Aged from 12 to 16 Weeks

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### Abstract

This study investigated the effects of *Bacillus subtilis* along with different levels of manganese on growth performance, slaughter performance, antioxidant activity and serum biochemical indices of Wulong geese aged from 12 to 16 weeks, and aimed to find a method of producing diet with low manganese supplemental level. A total of 360 twelve-week-old Wulong geese were randomly divided into 6 groups with 6 replicates per group and 10 geese per replicate (half male and half female). Group served as the optimal manganese group (manganese supplementation level of 105 mg/kg, without *Bacillus subtilis*). Groups to were *Bacillus subtilis* supplementation groups with manganese supplementation levels of 0, 35, 70, 105, and 170 mg/kg, respectively, and all received 250 g/t *Bacillus subtilis*. The experiment lasted for 5 weeks. The results showed as follows: 1) the body weight and average daily gain in groups and were significantly higher than those in group ($P<0.05$), while the feed-to-gain ratio in group was significantly lower than that in group ($P<0.05$). 2) The dressed percentage, percentages of half-eviscerated yield, eviscerated yield and leg muscle in group were significantly higher than those in group ($P<0.05$), whereas the abdominal fat percentage in groups , and was significantly lower than that in group ($P<0.05$). 3) The serum total antioxidant capacity in group was significantly higher than that in group ($P<0.05$). The serum malondialdehyde content in group was significantly lower than that in group ($P<0.05$). The serum glutathione peroxidase activity in groups and was significantly higher than that in group ($P<0.05$). 4) There were no significant differences in alkaline phosphatase activity and the contents of triglyceride and total cholesterol in serum among all groups ($P>0.05$). In conclusion, adding *Bacillus subtilis* into the diet can promote growth performance, slaughter performance, and antioxidant activity of Wulong geese aged from 12 to 16 weeks, significantly increase the availability of manganese, and reduce the dietary supplemental level of manganese. The optimal supplemental level of manganese is 70 mg/kg under dietary supplementation with 250 g/t *Bacillus subtilis*.

**Key words:** *Bacillus subtilis*; manganese; geese; growth performance; antioxidant activity; serum biochemical indices

## Introduction

Manganese is widely distributed in nature, occurring in soil, minerals, plants, and other sources, and is an essential trace element for organisms. In livestock and poultry, manganese plays important roles as a key component of pyruvate carboxylase and arginine kinase, and as an activator of certain hydrolases, decarboxylases, transferases, and kinases. Manganese promotes growth and immunity, and is indispensable for various physiological processes including redox reactions, bone formation and growth, tissue respiration, reproduction, embryonic development, eggshell formation, blood formation, and normal endocrine function. Thus, manganese holds an irreplaceable position in poultry nutrition.

Kemmerer et al. [1] discovered that manganese is an essential trace element for normal growth and reproductive performance in rats and mice. Wilgus et al. [2] demonstrated that manganese can effectively prevent perosis, marking the first discovery of manganese' s important role in poultry. Furthermore, manganese deficiency readily leads to nutritional deficiency diseases such as impaired bone formation, bone shortening, and perosis. Compared with mammals, poultry have higher manganese requirements but lower intestinal absorption rates. Moreover, corn, a major component of poultry diets, contains very little manganese, making poultry more susceptible to manganese deficiency [3]. With the rapid development of animal production in China, the environmental pollution caused by livestock farming has become increasingly severe and has gradually attracted social attention. Heavy metal pollution from animal production is one of the most prominent issues in soil contamination. Heavy metals accumulate and transform in organisms after absorption, posing potential threats to human and animal health. Due to the cumulative, irreversible, and long-term consequences of heavy metal pollution, research in this field has been a hot topic in environmental and ecological sciences both domestically and internationally [4]. Manganese is one of the important heavy metal pollutants. Therefore, it is necessary to conduct more in-depth research on technologies to reduce dietary manganese supplementation levels.

Numerous studies have been conducted on microecological preparations both in China and abroad. Ouyang et al. [5] reported that *Lactobacillus* produces an antibacterial substance that can inhibit putrefactive products of intestinal flora, improve the intestinal environment, and thereby enhance health and prolong lifespan. Selvam et al. [6] found that surfactants secreted by *Bacillus subtilis* can inhibit phospholipase A2, a key enzyme in inflammatory responses, thereby ameliorating inflammatory responses in colitis. As a type of microecological preparation, *Bacillus subtilis* can effectively improve intestinal flora composition and promote nutrient absorption. López et al. [7] reported that *Bacillus* strains have strong metal adsorption capacity because metal ions can be immobilized through interaction with anions on the bacterial cell surface. Jayaraman et al. [8] found that *Bacillus subtilis* can inhibit necrotic enteritis caused by *Clostridium perfringens* in broiler chickens and improve intestinal health. To date, few studies have reported on the effects of dietary *Bacillus subtilis* supplementation on the digestion and absorption of trace elements in livestock and poultry. Research on the effect of *Bacillus subtilis* on manganese utilization efficiency remains blank, and its impact on goose slaughter performance has not been reported. Therefore, this experiment used 12- to 16-week-old Wulong geese (Huoyan geese) as experimental animals to investigate the effects of dietary *Bacillus subtilis* with different manganese levels on growth performance, slaughter performance, antioxidant activity, and serum biochemical indices, aiming to explore a method for minimizing manganese supplementation levels in poultry diets.

## 1. Materials and Methods

### 1.1 Experimental Animals and Design

A total of 360 healthy 12-week-old Wulong geese with similar body weight were selected and randomly divided into 6 groups with 6 replicates per group and 10 geese per replicate (half male and half female) using a random allocation numbering method. Group served as the optimal manganese group (manganese supplementation level of 105 mg/kg [12], without *Bacillus subtilis*). Groups to were *Bacillus subtilis* supplementation groups with manganese supplementation levels of 0, 35, 70, 105, and 170 mg/kg, respectively, and all received 250 g/t *Bacillus subtilis*. The experimental period lasted for 5 weeks. Experimental geese were provided by the breeding base of the Institute of High Quality Waterfowl at Qingdao Agricultural University. Manganese sulfate monohydrate used in the experiment was purchased from Zhejiang Xinweipu Additive Co., Ltd. (active ingredient content: 98%).

### 1.2 Experimental Diets

The nutrient levels of the basal diet were formulated according to the NRC (1994) nutrient requirements for poultry. The composition and nutrient levels of the basal diet are shown in Table 1 . The manganese content in the basal diet was measured to be 20 mg/kg using inductively coupled plasma optical emission spectrometry.

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

*Footnotes:*
1) The multivitamin and trace elements provided the following per kg of diet: VA 1,500 mg, VD3 200 IU, VE 12.5 mg, VK3 1.5 mg, VB1 2.2 mg, VB2 5.0 mg, nicotinic acid 65 mg, pantothenate 15 mg, VB6 2 mg, biotin 0.2 mg, folic acid 0.5 mg, choline 1,000 mg, Fe 85 mg, Zn 80 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.

### 1.3 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 but frequently, and the growth status of the geese was closely monitored.

### 1.4 Measurements and Methods

At the end of week 16, after an overnight fast, geese were weighed by replicate to calculate average daily gain (ADG) from 12 to 16 weeks of age. Daily feed consumption was recorded to determine average daily feed intake (ADFI). Mortality and culling were recorded daily, and the feed-to-gain ratio (F/G) and mortality rate were calculated accordingly [9].

At the end of week 16, after a 12-hour fast, 2 geese were randomly selected from each replicate (total of 72 geese, half male and half female) for slaughter. Dressed weight, half-eviscerated weight, eviscerated weight, abdominal fat weight, breast muscle weight, and leg muscle weight were measured according to the ‘Poultry Production Performance Terminology and Measurement Methods’ (NY/T 823-2004). Six slaughter performance indices were calculated: dressed percentage, half-eviscerated yield percentage, eviscerated yield percentage, abdominal fat percentage, leg muscle percentage, and breast muscle percentage.

At the end of week 16, blood samples (5 mL) were collected from the wing vein of 2 randomly selected geese per replicate (total of 72 geese, half male and half female) and centrifuged at 3,000 r/min for 10 min to obtain serum. Serum alkaline phosphatase (AKP) activity was measured using an AKP assay kit. Serum triglyceride (TG) content was measured using a TG assay kit. Serum total cholesterol (CHOL) content was measured using a CHOL assay kit. Serum total antioxidant capacity (T-AOC) was measured using a T-AOC assay kit. Serum malondialdehyde (MDA) content was measured using an MDA assay kit. Serum glutathione peroxidase (GSH-Px) activity was measured using a GSH-Px assay kit. All assay kits were purchased from Nanjing Jiancheng Bioengineering Institute.

### 1.5 Statistical Analysis

Data were analyzed using one-way ANOVA with LSD multiple comparison tests in SPSS 17.0 software. Results are expressed as ‘mean ± standard deviation’

. Orthogonal polynomial contrasts were used to analyze linear or curvilinear responses of various indices to dietary manganese supplementation levels, and curve fitting was employed to determine the optimal manganese supplementation level in *Bacillus subtilis*-supplemented diets for 12- to 16-week-old geese. P<0.05 and P<0.01 were considered statistically significant and highly significant, respectively.

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## 2. Results

### 2.1 Effects on Growth Performance

As shown in Table 2 , during the 12- to 16-week period, the body weight and average daily gain in groups and were significantly higher than those in group (P<0.05), while the feed-to-gain ratio in group was significantly lower than that in group (P<0.05). No significant differences were observed in average daily feed intake or mortality rate among groups (P>0.05).

Quadratic curve fitting and regression analysis revealed that the curve fitting between growth performance and dietary manganese supplementation levels was not significant (P>0.05).

Since no significant differences were found in average daily gain, feed-to-gain ratio, or average daily feed intake among groups , , and (P>0.05), and group achieved higher body weight and average daily gain along with a lower feed-to-gain ratio, the combination of 250 g/t *Bacillus subtilis* and 70 mg/kg manganese in the diet produced the best growth performance compared to the control group with 105 mg/kg manganese supplementation level.

**Table 2** Effects of dietary *Bacillus subtilis* along with different manganese levels on growth performance of Wulong geese

*Note:* In the same column, values with the same small or no letter superscripts mean no significant difference (P>0.05), while adjacent small letter superscripts mean significant difference (P<0.05), and alternate small letter superscripts mean highly significant difference (P<0.01). The same as below.

### 2.2 Effects on Slaughter Performance

As shown in Table 3 , during the 12- to 16-week period, the dressed percentage, percentages of half-eviscerated yield, eviscerated yield, and leg muscle in group were significantly higher than those in group (P<0.05), while the abdominal fat percentage in groups , and was significantly lower than that in group (P<0.05). The breast muscle percentage in group was higher than that in the control group, but the difference was not significant (P>0.05). No significant differences were observed among groups , , and in any slaughter performance indices except for dressed percentage (P>0.05).

Quadratic curve fitting and regression analysis revealed that the curve fitting between slaughter performance and dietary manganese supplementation levels was not significant (P>0.05).

These results indicate that for 12- to 16-week-old Wulong geese, dietary supplementation with 250 g/t *Bacillus subtilis* and 70 mg/kg manganese can achieve higher slaughter performance.

**Table 3** Effects of dietary *Bacillus subtilis* along with different manganese levels on slaughter performance of Wulong geese

### 2.3 Effects on Antioxidant Activity

As shown in Table 4 , during the 12- to 16-week period, serum T-AOC and GSH-Px activity in groups  to  showed a trend of increasing first and then decreasing, while serum MDA content showed a trend of decreasing first and then increasing. Serum T-AOC in group  was significantly higher than that in group  (P<0.05). Serum MDA content in group  was significantly lower than that in group  (P<0.05). Serum GSH-Px activity in groups  and  was significantly higher than that in group  (P<0.05).

Quadratic curve fitting and regression analysis revealed that the curve fitting between antioxidant capacity and dietary manganese supplementation levels was not significant (P>0.05).

These results indicate that for 12- to 16-week-old geese, dietary supplementation with 250 g/t *Bacillus subtilis* and 70 mg/kg manganese can significantly increase serum T-AOC, significantly decrease serum MDA content, and significantly increase serum GSH-Px activity.

**Table 4** Effects of dietary *Bacillus subtilis* along with different manganese levels on antioxidant activity of Wulong geese

### 2.4 Effects on Serum Biochemical Indices

As shown in Table 5 , during the 12- to 16-week period, no significant differences were observed in serum AKP activity or TG and CHOL contents among groups (P>0.05), although group  showed the highest values for serum AKP activity and TG and CHOL contents.

**Table 5** Effects of dietary *Bacillus subtilis* along with different manganese levels on serum biochemical indices of Wulong geese

## 3. Discussion

### 3.1 Effects on Growth Performance

Manganese primarily participates in the metabolism of various substances including fats, carbohydrates, and proteins in the body, and can promote animal growth and enhance reproductive performance. Zhu et al. [10] reported that the lowest feed-to-gain ratio was achieved when 120 mg/kg manganese was added to broiler diets. Zhang et al. [11] reported that dietary supplementation with 90-120 mg/kg manganese significantly increased average daily gain and decreased feed-to-gain ratio in 5- to 16-week-old Wulong geese. *Bacillus subtilis* is one of the 12 feed-grade microbial additives directly approved for animal feeding by the Chinese Ministry of Agriculture, and has demonstrated significant effects on improving animal growth performance and feed conversion efficiency [12-13]. Hooge et al. [13] confirmed that *Bacillus subtilis* preparations have growth-promoting effects in broilers and geese. Maneewan et al. [14] found that compared with the control group, *Bacillus subtilis* increased average daily gain in 1- to 28-day-old piglets, reduced the number of *E. coli* and *Salmonella* in the intestine, and decreased diarrhea rates. Molnár et al. [15] found that *Bacillus subtilis* increased average daily gain and improved feed conversion ratio in broiler chickens. The results of this study indicate that the *Bacillus subtilis* supplementation group achieved maximum average daily gain at a manganese supplementation level of 70 mg/kg, suggesting that dietary supplementation with 250 g/t *Bacillus subtilis* can reduce the manganese supplementation level in goose diets.

### 3.2 Effects on Slaughter Performance

Slaughter performance indices reflect differences in nutrient deposition among different tissues and within the same tissue, and many factors affect deposition levels. Zhang et al. [11] reported that dietary manganese supplementation tended to increase leg muscle percentage and decrease abdominal fat percentage in Wulong geese. Chen et al. [16] reported that supplementation with 100 mg/kg manganese tended to increase leg muscle percentage and decrease abdominal fat percentage in broilers. Lu et al. [17] reported that dietary supplementation with 100 mg/kg manganese decreased malic dehydrogenase (MDH) and lipoprotein lipase (LPL) activities while increasing hormone-sensitive lipase activity. Dietary manganese can affect abdominal fat deposition by regulating these enzyme activities to reduce fat synthesis and increase fat breakdown.

The results of this experiment indicate that when the manganese supplementation level was 70 mg/kg in the *Bacillus subtilis* groups, the dressed percentage, half-eviscerated yield percentage, eviscerated yield percentage, and leg muscle percentage of Wulong geese were significantly increased, suggesting that low-manganese diets supplemented with *Bacillus subtilis* can significantly improve goose slaughter performance. The mechanisms by which *Bacillus subtilis* affects manganese absorption and slaughter performance require further investigation.

### 3.3 Effects on Antioxidant Capacity

Poultry have vigorous metabolism and readily produce numerous free radicals, especially under intensive farming conditions where they are susceptible to various environmental stressors. This makes them more prone to disruption of the free radical balance and increased disease susceptibility. Therefore, compared with mammals, ducks require a more robust antioxidant system to eliminate excessive free radicals and maintain stability and balance, thereby preserving health and normal growth. Manganese has a close relationship with poultry antioxidant capacity, and manganese supplementation is of special importance [18]. Manganese is a component of superoxide dismutase (SOD) activity and also affects the production of non-enzymatic antioxidant proteins in tissues. Manganese nutritional status affects the antioxidant status of body tissues [19].

T-AOC 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. T-AOC levels directly indicate the response capacity of enzymatic and non-enzymatic systems when the body faces external stimuli [20]. MDA is a product of lipid peroxidation reactions in the body, primarily produced by the reaction of free radicals generated by enzymatic and non-enzymatic systems with unsaturated fatty acids on cell membranes. MDA directly reflects the level of oxidative free radicals and the degree of cellular damage [21]. GSH-Px is an important peroxide-decomposing enzyme widely present in the body and serves as an indicator of anti-peroxidation capacity [21]. The results of this experiment indicate that dietary supplementation with 250 g/t *Bacillus subtilis* and 70 mg/kg manganese significantly increased serum T-AOC, significantly decreased MDA content, and significantly increased GSH-Px activity in geese, which is consistent with the aforementioned research findings. This demonstrates that low-manganese diets supplemented with *Bacillus subtilis* are closely related to poultry antioxidant capacity.

### 3.4 Effects on Serum Biochemical Indices

TG is related to animal growth, development, and immune system function, and its content changes reflect membrane lipid metabolism. Luo et al. [22] reported that to compensate for decreased blood cholesterol content during manganese deficiency, the body automatically regulates lipoprotein breakdown, and TG derived from the breakdown of lipoproteins leads to increased serum TG content. Cholesterol is a component of cell membranes. Some blood cholesterol is transported to tissues to form structural components of cells, while another portion is converted into important sterol derivatives such as vitamin D3, which promotes calcium absorption, or participates in steroid hormone metabolism. Ding et al. [23] reported that manganese can promote the synthesis of cholesterol from 14C-labeled acetate in hepatocyte culture, as manganese is a cofactor for mevalonate kinase. Manganese deficiency inhibits mevalonate kinase activity. Two steps between acetate and mevalonate require manganese ions (Mn2+), and pyrophosphate synthetase requires Mn2+. Blockage of pyrophosphate ester synthesis inhibits squalene production and thus cholesterol synthesis. During manganese deficiency, cholesterol and its precursor synthesis are blocked, leading to obstacles in sex hormone synthesis. Curran et al. [24] reported that manganese can stimulate cholesterol synthesis and increase cholesterol production in rat liver. The results of this experiment indicate that serum AKP activity and TG and CHOL contents showed a trend of increasing first and then decreasing with increasing dietary manganese levels. Serum AKP activity and TG and CHOL contents were highest when dietary *Bacillus subtilis* supplementation was 250 g/t and manganese supplementation was 70 mg/kg.

## Conclusion

In conclusion, dietary supplementation with *Bacillus subtilis* can significantly improve growth performance, slaughter performance, and antioxidant capacity. Under the condition of dietary supplementation with 250 g/t *Bacillus subtilis*, the optimal manganese supplementation level is 70 mg/kg.

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