Combined Effects of Bacillus subtilis and Copper on Growth Performance, Carcass Characteristics, Nutrient Utilization, and Meat Quality in 5- to 16-Week-Old Wulong Geese (Postprint)

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Abstract

This experiment aimed to investigate the effects of synergistic action of Bacillus subtilis and copper on growth performance, slaughter performance, nutrient utilization, and meat quality of Wulong geese aged 5 to 16 weeks, and to explore a method for reducing dietary copper supplementation levels. A total of 360 five-week-old Wulong geese were selected and randomly divided into 6 groups, with 6 replicates per group and 10 geese per replicate (half male and half female). Group was the control group (copper supplementation level of 16 mg/kg, without Bacillus subtilis); Groups - were Bacillus subtilis supplementation groups (Bacillus subtilis supplementation level of 250 g/t), with copper supplementation levels of 0, 4, 8, 12, and 16 mg/kg, respectively. The experimental period lasted 12 weeks. The results showed that: 1) In the Bacillus subtilis supplementation groups, the average daily gain and feed-to-gain ratio were optimal when the copper supplementation level was 8 mg/kg. 2) The dressing percentage and semi-eviscerated yield of Groups , , and were significantly or extremely significantly higher than those of Group ($P<0.05$ or $P<0.01$). 3) The crude protein utilization rate of Group was significantly higher than that of Group ($P<0.05$), and the crude fiber utilization rate, acid detergent fiber utilization rate, and apparent digestibility of copper in Groups , , and were significantly or extremely significantly higher than those of Group ($P<0.05$ or $P<0.01$). 4) The nitrogen retention and nitrogen utilization rate of Group were significantly or extremely significantly higher than those of Group ($P<0.05$ or $P<0.01$), and fecal nitrogen was significantly lower than that of Group ($P<0.05$). In conclusion, dietary supplementation with Bacillus subtilis can improve copper utilization, thereby reducing dietary copper supplementation levels and improving growth performance, slaughter performance, and nutrient utilization; it is recommended that

under the condition of adding 250 g/t Bacillus subtilis to the diet of meat geese aged 5–16 weeks, the appropriate copper supplementation level is 8 mg/kg.

Full Text

Synergistic Effects of Bacillus subtilis and Copper on Growth Performance, Slaughter Performance, Nutrient Utilization and Meat Quality of Wulong Geese Aged 5 to 16 Weeks

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Abstract: This experiment was conducted to investigate the synergistic effects of *Bacillus subtilis* and copper on growth performance, slaughter performance, nutrient utilization, and meat quality of Wulong geese aged 5–16 weeks, and to explore a method for reducing dietary copper supplementation levels. A total of 360 five-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 I served as the control group (copper supplementation level of 16 mg/kg, without *Bacillus subtilis*); Groups II–VI were *Bacillus subtilis* supplementation groups (*Bacillus subtilis* at 250 g/t) with copper supplementation levels of 0, 4, 8, 12, and 16 mg/kg, respectively. The experiment lasted for 12 weeks. The results showed: 1) In the *Bacillus subtilis* supplementation groups, the best average daily gain and feed-to-gain ratio were achieved at a copper supplementation level of 8 mg/kg. 2) The dressed percentage and half-eviscerated yield percentage in Groups IV, V, and VI were significantly or extremely significantly higher than those in Group I ($P<0.05$ or $P<0.01$). 3) The crude protein utilization in Group IV was significantly higher than that in Group I ($P<0.05$), while the crude fiber utilization, acid detergent fiber utilization, and copper apparent digestibility in Groups IV, V, and VI were significantly or extremely significantly higher than those in Group I ($P<0.05$ or $P<0.01$). 4) The deposited nitrogen and nitrogen utilization in Group IV were significantly or extremely significantly higher than those in Group I ($P<0.05$ or $P<0.01$), and the nitrogen excretion was significantly lower than that in Group I ($P<0.05$). In conclusion, dietary supplementation with *Bacillus subtilis* can promote copper utilization, reduce dietary copper supplementation levels, and improve growth performance, slaughter performance, and nutrient utilization. The optimal supplementation levels are 250 g/t for *Bacillus subtilis* and 8 mg/kg for copper.

Key words: *Bacillus subtilis*; copper; geese; growth performance; slaughter performance; meat quality; nutrient; availability

Introduction

Copper serves as a catalyst in animal physiological metabolism and can promote animal growth by acting on enzymes related to nutrient and energy metabolism. It affects the synthesis of adrenal corticosteroids and catecholamines through insulin-like growth factor (IGF) [?]. In animal production, the growth-promoting effect of copper has received widespread attention; adding 250 mg/kg copper to piglet diets can improve growth rate and feed utilization efficiency [?]. However, while high-copper diets enhance livestock performance, they also lead to significantly increased copper content in excreta, causing environmental pollution [?]. Furthermore, high-dose copper supplementation may cause copper poisoning in livestock and increase copper deposition in animal products, directly threatening human health through the food chain [?]. Therefore, finding a method to reduce dietary copper supplementation levels is of significant importance for health and ecological nutrition.

Bacillus subtilis, as a type of microecological preparation, can effectively improve intestinal flora composition and promote nutrient absorption. Asunción et al. [?] reported that *Bacillus* strains have strong metal adsorption capacity because metal ions can be immobilized through interaction with anions on the bacterial cell surface. Zhao et al. [?] also demonstrated that *Bacillus subtilis* has a strong adsorption effect on copper ions. To date, few studies have reported on the effects of dietary *Bacillus subtilis* supplementation on trace element digestion and absorption in livestock and poultry, and research on the impact of *Bacillus subtilis* on copper utilization remains blank, with no reports on its effects on goose slaughter performance. Therefore, this experiment used 5–16-week-old Wulong geese as research subjects to investigate the synergistic effects of dietary *Bacillus subtilis* and copper supplementation on growth performance, slaughter performance, meat quality, and nutrient utilization, aiming to explore a method for reducing dietary copper supplementation levels.

1.1 Experimental Animals and Design

A total of 360 healthy five-week-old Wulong geese (Huoyan geese) with similar body weight were selected and randomly divided into 6 groups using a random allocation numbering method, with 6 replicates per group and 10 geese per replicate (half male and half female). Group I was the control group (based on the research results of Xu et al. [?], copper supplementation level was 16 mg/kg, without *Bacillus subtilis*); Groups II–VI were *Bacillus subtilis* supplementation groups with *Bacillus subtilis* added at 250 g/t, and copper supplementation levels of 0, 4, 8, 12, and 16 mg/kg, respectively. The experimental period lasted for 12 weeks. The experimental geese were provided by the breeding base of the Institute of High Quality Waterfowl at Qingdao Agricultural University. The *Bacillus subtilis* used in the experiment was in powder form with a viable count of 2$\times$10^{9}$ CFU/g, purchased from Shandong Sukahan Bioengineering Co., Ltd. The copper source used was copper sulfate pentahydrate, purchased from Zhejiang Xinweipu Additive Co., Ltd. (with an active ingredient content of 98.5%).

1.2 Experimental Diets

The basal diet was formulated according to the NRC (1994) poultry nutrient requirements. The composition and nutrient levels of the basal diet are shown in Table 1 . The copper content in the basal diet was measured to be 7.32 mg/kg using a plasma emission spectrometer.

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

Note: 1) The multivitamin and trace elements provided the following per kg of diet: VA 1,500 mg, VD_3 200 IU, VE 12.5 mg, VK_3 1.5 mg, VB_1 2.2 mg, VB_2 5.0 mg, nicotinic acid 65 mg, pantothenate 15 mg, VB_6 2 mg, biotin 0.2 mg, folic acid 0.5 mg, choline 1,000 mg, Fe 85 mg, Mn 80 mg, Zn 80 mg, I 0.42 mg, Se 0.3 mg, Co 2.5 mg. 2) Copper was a measured value, while the other nutrient levels were calculated values.

1.3 Management

Before the experiment, the goose house was thoroughly disinfected. Throughout the entire period, geese were raised indoors on thick litter in separate pens. Experimental geese had free access to water and feed, with frequent small feed additions. The growth status of the flocks was carefully monitored.

At the end of 16 weeks, the experimental geese were fasted and weighed by replicate to calculate the average daily gain (ADG) from 5 to 16 weeks. Daily feed consumption was recorded to calculate average daily feed intake (ADFI). Mortality and culling were recorded daily, and the feed-to-gain ratio (F/G) was calculated considering mortality and culling [**?**].

At the end of 16 weeks, after fasting and weighing, 2 geese were randomly selected from each replicate in each group (72 geese total from 6 experimental groups). Blood was collected from wing veins before slaughter. After 12 hours of pre-slaughter fasting, slaughter performance was measured according to *Poultry Production Performance Terminology and Measurement Statistics Methods* (NY/T 823—2004). Measurements included carcass weight, half-eviscerated weight, fully eviscerated weight, abdominal fat weight, breast muscle weight, and leg muscle weight, from which six slaughter performance indicators were calculated: dressed percentage, eviscerated yield percentage, half-eviscerated yield percentage, abdominal fat percentage, leg muscle percentage, and breast muscle percentage.

At the end of 16 weeks, meat quality was determined according to *Meat Products Testing Standards* (GB/T 9695.7—2008) after slaughter. The entire breast muscle was stripped from the sternum, and the anterior pectoralis major was taken as the meat sample. Meat color was measured using a Japanese automatic colorimeter, shear force was determined using a texture analyzer (TA-XT PLUS), pH was measured using a HANHA-HI9025 portable pH meter, and water loss rate was determined using a pressure meter.

At 14 weeks, 6 geese (3 male and 3 female) were randomly selected from each group and moved into metabolic cages (Patent No.: 200720177297) for feeding. The experimental phase consisted of a 4-day preliminary period, 1-day fasting period, and 3-day formal collection period, with free access to water and quantitative feeding. The total collection method was used to continuously collect excreta for 4 days. Fecal collection trays were placed under the metabolic cages and collected at fixed times daily. Nitrogen was fixed with hydrochloric acid, and samples were mixed and stored after feed was ground to 40 mm and dried at low temperature. Fecal samples were dried in an oven at 65–75 °C, rehydrated for 24 hours under natural conditions to prepare air-dried fecal samples, and then pulverized using a small universal grinder. Gross energy (GE) was determined using the bomb calorimetry method; crude protein (CP) content was detected using FOSS TECATOR QUALITY ASSURANCE equipment; crude fiber (CF), neutral detergent fiber (NDF), and acid detergent fiber (ADF) contents were determined using an ANKOM2000 Fiber Analyzer (NY14450) produced by ANKOM Company; crude ether extract (EE) content was determined using the ether extraction method; copper apparent digestibility was measured using a plasma emission spectrometer (ICP) produced by PE Company in the United States; nutrient utilization calculation formulas followed reference [**?**].

1.5 Statistical Analysis

Data were analyzed using one-way ANOVA with LSD method for multiple comparisons in SPSS 17.0 software. Experimental data were expressed as "mean ± standard deviation." $P<0.05$ and $P<0.01$ were considered as significant and extremely significant differences, respectively.

Results

2.1 Effects of Bacillus subtilis and Copper Synergy on Growth Performance of Geese

As shown in Table 2 , during weeks 5–16, the body weight (BW) of Groups IV and V was significantly or extremely significantly higher than that of Group I ($P<0.05$ or $P<0.01$). The average daily gain (ADG) of Groups IV and V was significantly or extremely significantly higher than that of Group I ($P<0.05$ or $P<0.01$). The feed-to-gain ratio (F/G) of Group IV was extremely significantly lower than that of Group II ($P<0.01$). No significant differences were observed in average daily feed intake (ADFI) or mortality rate among groups ($P>0.05$).

Quadratic curve fitting and regression equation analysis revealed that the curve fitting between growth performance and dietary copper supplementation level was not significant ($P>0.05$).

These results indicate that since there were no significant differences in body weight, feed-to-gain ratio, or average daily feed intake among Groups IV, V, and VI (P>0.05), and Group IV achieved higher body weight and average daily gain with a lower feed-to-gain ratio, the best growth performance was obtained with a dietary *Bacillus subtilis* supplementation level of 250 g/t and copper supplementation level of 8 mg/kg, which was superior to Group I with 16 mg/kg copper.

Table 2 Effects of *Bacillus subtilis* and copper synergy on growth performance of geese

Note: In the same column, values with the same small letter 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 extremely significant difference (P<0.01). The same as below.

2.2 Effects of Bacillus subtilis and Copper Synergy on Slaughter Performance of Geese

As shown in Table 3 , during weeks 5-16, the dressed percentage and half-eviscerated yield percentage of Groups IV, V, and VI were significantly or extremely significantly higher than those of Group I (P<0.05 or P<0.01), and showed an increasing trend with increasing dietary copper levels in the *Bacillus subtilis* supplementation groups. The eviscerated yield percentage, breast muscle percentage, and leg muscle percentage of all *Bacillus subtilis* supplementation groups were improved compared with Group I, showing an increasing trend with higher dietary copper levels, with Group VI being significantly higher than Group I (P<0.05). The abdominal fat percentage of Group V was significantly lower than that of Group I (P<0.05).

Comparison among Groups IV, V, and VI revealed no significant differences in any slaughter performance indicators (P>0.05). These results indicate that at a dietary *Bacillus subtilis* supplementation level of 250 g/t, a copper supplementation level of 8 mg/kg can achieve relatively high slaughter performance.

Table 3 Effects of *Bacillus subtilis* and copper synergy on slaughter performance of geese

2.3 Effects of Bacillus subtilis and Copper Synergy on Nutrient and Energy Utilization of Geese

As shown in Table 4 , during weeks 5-16, the CP utilization of Group IV was significantly higher than that of Group I (P<0.05), showing a 4.26% improvement. The CF utilization, ADF utilization, and copper apparent digestibility of Groups IV, V, and VI were significantly or extremely significantly higher than those of Group I (P<0.05 or P<0.01). No significant differences were observed in EE utilization between any *Bacillus subtilis* supplementation group and Group

I (P>0.05). The NDF utilization of Group IV was significantly higher than that of Group I (P<0.05).

As shown in Table 5 , during weeks 5-16, the deposited nitrogen and nitrogen utilization of Group IV were significantly or extremely significantly higher than those of Group I (P<0.05 or P<0.01), while nitrogen excretion was significantly lower than that in Group I (P<0.05). No significant differences were observed in nitrogen intake among groups (P>0.05).

As shown in Table 6 , during weeks 5-16, no significant differences were observed in GE intake, fecal GE, endogenous energy value, apparent metabolizable energy, true metabolizable energy, or GE utilization between any *Bacillus subtilis* supplementation group and Group I (P>0.05).

These results indicate that during weeks 5-16, at a dietary *Bacillus subtilis* supplementation level of 250 g/t, a copper supplementation level of 8 mg/kg can significantly improve CP utilization, CF utilization, copper apparent digestibility, and nitrogen utilization, significantly reduce nitrogen excretion, and extremely significantly improve ADF utilization.

Table 4 Effects of *Bacillus subtilis* and copper synergy on nutrient utilization of geese

Table 5 Effects of *Bacillus subtilis* and copper synergy on nitrogen utilization of geese

Table 6 Effects of *Bacillus subtilis* and copper synergy on energy utilization of geese

2.4 Effects of Bacillus subtilis and Copper Synergy on Meat Quality of Geese

As shown in Table 7 , during weeks 5-16, no significant differences were observed in meat quality between any *Bacillus subtilis* supplementation group and Group I (P>0.05). However, the b* value (yellowness), shear force, pH, and water loss rate of all *Bacillus subtilis* supplementation groups were lower than those of Group I, indicating that *Bacillus subtilis* supplementation had some influence on goose meat quality.

These results indicate that during weeks 5-16, the meat quality of geese fed diets supplemented with *Bacillus subtilis* was better than that of Group I fed the optimal copper supplementation level.

Table 7 Effects of *Bacillus subtilis* and copper synergy on meat quality of geese

Discussion

3.1 Effects of Bacillus subtilis and Copper Synergy on Growth Performance of Geese

Copper acts as a catalyst in animal physiological metabolism and can promote animal growth by interacting with enzymes related to nutrient and energy metabolism. Gan et al. [?] reported that adding 100–200 mg/kg copper to basal diets could significantly improve the daily gain of Angora rabbits. Xu et al. [?] reported that the optimal copper supplementation level for growth performance in 5–16-week-old Wulong geese was 16.25 mg/kg, and that copper promoted growth not by increasing feed intake but by improving feed utilization efficiency, which is consistent with the results of this experiment. *Bacillus subtilis*, as one of 12 feed-grade microbial additives directly approved for animal feeding by the Ministry of Agriculture, has obvious effects on improving animal growth performance and feed conversion efficiency [?, ?]. Hooge et al. [?] confirmed the growth-promoting effects of *Bacillus subtilis* preparations in broiler chickens and geese, while Lei et al. [?] demonstrated the effectiveness of *Bacillus subtilis* preparations in improving egg production rate and feed-to-egg ratio in laying hens and ducks.

This study shows that the best growth performance in *Bacillus subtilis* supplementation groups was achieved at a copper supplementation level of 8 mg/kg. Dietary *Bacillus subtilis* supplementation can improve copper utilization and reduce dietary copper supplementation levels. The synergistic growth-promoting effects of copper and *Bacillus subtilis* enabled geese to achieve higher growth performance. The mechanism of this synergistic effect warrants further investigation.

3.2 Effects of Bacillus subtilis and Copper Synergy on Slaughter Performance of Geese

Slaughter performance is a set of indicators that reflect differences in nutrient deposition among different tissues and within the same tissue, and many factors affect deposition quantity. Xu et al. [?] reported that adding 30 mg/kg copper to Wulong goose diets resulted in optimal slaughter performance at 16 weeks.

The results of this experiment indicate that all *Bacillus subtilis* supplementation groups showed better slaughter performance than Group I fed the optimal copper level reported by Xu et al. [?]. When copper supplementation exceeded 8 mg/kg, the dressed percentage and half-eviscerated yield percentage were significantly higher than those of Group I, demonstrating that *Bacillus subtilis* can significantly improve goose slaughter performance. Moreover, increasing copper supplementation levels within a certain range showed a trend toward improved slaughter performance. The mechanisms by which *Bacillus subtilis* affects copper absorption and slaughter performance require further research.

3.3 Effects of Bacillus subtilis and Copper Synergy on Nutrient Utilization and Metabolizable Energy of Geese

Animal nutrient utilization directly reflects metabolic rate and growth performance [?]. Kirchgessner et al. [?] demonstrated that appropriate copper ion concentrations can activate pepsin in vitro, increasing protein hydrolysis. Luo et al. [?] found that copper can significantly increase the activities of small intestinal lipase and phospholipase A in weaned piglets, enhancing essential fatty acid absorption and thus improving dietary EE digestibility.

Bacillus subtilis possesses strong protease, lipase, amylase, and other enzyme activities, can produce antibiotics and lactic acid to inhibit harmful bacterial proliferation, and has strong biological oxygen-deprivation capacity in the animal intestinal tract. These characteristics play important roles in promoting nutrient digestion and absorption, improving feed conversion efficiency, disease prevention, and growth promotion [?, ?, ?]. Research has reported that adding 0.10% *Bacillus subtilis* to piglet diets can significantly improve weight gain, feed conversion efficiency, and disease resistance in weaned piglets [?]. Additionally, almost all cellular processes require enzyme participation to improve efficiency. Similar to other non-biological catalysts, enzymes accelerate reaction rates by reducing the activation energy of chemical reactions, with most enzymes increasing reaction rates by millions of times. In fact, enzymes provide alternative pathways with lower activation energy requirements, enabling more reactant particles to possess kinetic energy equal to or greater than the activation energy, thereby accelerating reaction rates. *Bacillus subtilis* can synthesize enzymes such as α-amylase, protease, lipase, and cellulase, which function together with digestive enzymes in the animal digestive tract. It is precisely because of these mechanisms that appropriate dietary supplementation with *Bacillus subtilis* and copper can improve nutrient utilization and growth performance.

This experiment demonstrates that dietary supplementation with 250 g/t *Bacillus subtilis* and 8 mg/kg copper can significantly improve CP utilization, CF utilization, copper apparent digestibility, and nitrogen utilization, significantly reduce nitrogen excretion, and extremely significantly improve ADF utilization. These results are consistent with the aforementioned studies, indicating that the synergistic effect of *Bacillus subtilis* and copper can promote nutrient utilization and absorption, reduce copper emissions, and benefit ecological environmental protection. Zhou et al. [?] reported that copper is not absorbed by the body in ionic form but must first combine with some ligands to form absorbable chelates that are then absorbed by the small intestine into the body. Since *Bacillus subtilis* has adsorption effects on divalent copper ions, it is possible that bacterial proteins or amino acids secreted by *Bacillus subtilis* act as complexing agents that combine with copper ions, thereby promoting copper ion absorption. The complexation method and absorption mechanism of *Bacillus subtilis* and copper ions require further study.

3.4 Effects of Bacillus subtilis and Copper Synergy on Meat Quality of Geese

As a component of multiple enzymes, copper is a direct participant in body metabolism. For example, copper is a cofactor of tyrosinase; copper deficiency reduces tyrosinase activity and ATP production, causing skin and feather color fading [?, ?]. Proteins and enzymes related to copper antioxidation include ceruloplasmin and superoxide dismutase (SOD). Ceruloplasmin is the most abundant protein in plasma, with iron oxidase and antioxidant activities; SOD has strong antioxidant capacity, converting oxygen free radicals into hydrogen peroxide (H_2O_2) under its action, which is then degraded and cleared by catalase and glutathione peroxidase to complete the body' s antioxidant process [?]. Studies have shown that *Bacillus subtilis* has the ability to scavenge hydroxyl radicals, produce catalase, and resist lipid peroxidation [?, ?, ?]. Ren et al. [?] found that lower shear force indicates better meat quality.

This experiment shows that during weeks 5-16, when dietary *Bacillus subtilis* supplementation was 250 g/t, the meat quality of low-copper supplementation groups was better than that of Group I fed the optimal copper level. However, current reports on the effects of copper and *Bacillus subtilis* on goose meat quality are limited, allowing only a general range of meat color to be determined. Li et al. [?] measured Wulong goose muscle pH between 5.90-6.28, while Wang et al. [?] measured water loss rates of Wulong goose muscle between 27.84%-28.22%. This experiment showed pH values between 6.02-6.10 and water loss rates between 29.64%-31.95%, which are basically consistent with the aforementioned results.

Conclusions

1. The synergistic effect of *Bacillus subtilis* and copper had significant effects on average daily gain and feed-to-gain ratio of geese.
2. Dietary *Bacillus subtilis* supplementation significantly improved CP utilization, CF utilization, copper apparent digestibility, and nitrogen utilization; extremely significantly improved ADF utilization; and significantly reduced nitrogen excretion.
3. Dietary *Bacillus subtilis* supplementation can improve copper utilization, thereby reducing dietary copper supplementation levels and decreasing copper excretion.
4. It is recommended that for meat geese aged 5-16 weeks, when dietary *Bacillus subtilis* supplementation is 250 g/t, the appropriate copper supplementation level is 8 mg/kg.

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