Effects of Dietary Supplementation of Bacillus subtilis and Clostridium butyricum on Growth Performance, Slaughter Performance, Serum Biochemical Indices, and Antioxidant Capacity of Wulong Goslings (Postprint)

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Abstract

This experiment was conducted to investigate the effects of dietary supplementation of Bacillus subtilis and Clostridium butyricum on growth performance, slaughter performance, serum biochemical indices, and antioxidant capacity of Wulong goslings. A total of 200 healthy 1-day-old Wulong goslings with similar body weight were randomly divided into 4 groups, with 5 replicates per group and 10 goslings per replicate. Group (control group) was fed a basal diet, Group was fed the basal diet supplemented with 250 mg/kg Bacillus subtilis, Group was fed the basal diet supplemented with 250 mg/kg Clostridium butyricum, and Group was fed the basal diet supplemented with 250 mg/kg Bacillus subtilis + 250 mg/kg Clostridium butyricum. The experimental period lasted for 4 weeks. The results showed: 1) Compared with the control group, the final weight of goslings in Groups , , and increased by 7.78% ($P<0.05$), 3.12% ($P>0.05$), and 9.54% ($P<0.05$), respectively; the average daily gain increased by 7.89% ($P<0.05$), 3.39% ($P>0.05$), and 10.18% ($P<0.01$), respectively; and the feed conversion ratio decreased by 4.96% ($P<0.05$), 1.65% ($P>0.05$), and 6.20% ($P<0.05$), respectively. 2) Compared with the control group, the breast muscle percentage of goslings in Groups , , and increased by 2.10% ($P>0.05$), 2.80% ($P>0.05$), and 4.20% ($P<0.05$), respectively; the leg muscle percentage increased by 9.50% ($P<0.05$), 8.60% ($P<0.05$), and 10.86% ($P<0.05$), respectively; and the abdominal fat percentage decreased by 7.74% ($P<0.05$), 5.81% ($P>0.05$), and 10.97% ($P<0.05$), respectively. 3) Compared with the control group, the serum total antioxidant capacity of goslings in Groups , , and increased by 4.46% ($P>0.05$), 3.39% ($P>0.05$), and 12.33% ($P<0.05$), respectively; and the serum total superoxide dismutase activity increased by 4.94%

(P<0.05), 3.30% (P>0.05), and 7.69% (P<0.01), respectively. In conclusion, dietary supplementation of Bacillus subtilis and Clostridium butyricum can improve the growth performance, slaughter performance, and antioxidant capacity of Wulong goslings, and the combined use of the two is superior to single-strain supplementation.

Full Text

Effects of Dietary *Bacillus subtilis* and *Clostridium butyricum* on Growth Performance, Slaughter Performance, Serum Biochemical Indices, and Antioxidant Capacity of Wulong Goslings

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Abstract

This study investigated the effects of dietary *Bacillus subtilis* and *Clostridium butyricum* supplementation on growth performance, slaughter performance, serum biochemical indices, and antioxidant capacity of Wulong goslings. Two hundred healthy one-day-old Wulong goslings with similar body weight were randomly allocated into four groups with five replicates per group and ten goslings per replicate. Group I (control) received a basal diet, group II received the basal diet supplemented with 250 mg/kg *B. subtilis*, group III received the basal diet supplemented with 250 mg/kg *C. butyricum*, and group IV received the basal diet supplemented with 250 mg/kg *B. subtilis* plus 250 mg/kg *C. butyricum*. The experiment lasted for four weeks. The results showed: (1) Compared with the control group, the final body weight of goslings in groups II, III, and IV increased by 7.78% (P<0.05), 3.12% (P>0.05), and 9.54% (P<0.05), respectively; average daily gain increased by 7.89% (P<0.05), 3.39% (P>0.05), and 10.18% (P<0.01), respectively; and feed-to-gain ratio decreased by 4.96% (P<0.05), 1.65% (P>0.05), and 6.20% (P<0.05), respectively. (2) Compared with the control group, the percentage of breast muscle in groups II, III, and IV increased by 2.10% (P>0.05), 2.80% (P>0.05), and 4.20% (P<0.05), respectively; leg muscle percentage increased by 9.50% (P<0.05), 8.60% (P<0.05), and 10.86% (P<0.05), respectively; and abdominal fat percentage decreased by 7.74% (P<0.05), 5.81% (P>0.05), and 10.97% (P<0.05), respectively. (3) Compared with the control group, serum total antioxidant capacity in groups II, III, and IV increased by 4.46% (P>0.05), 3.39% (P>0.05), and 12.33% (P<0.05), respectively; serum total superoxide dismutase activity increased by 4.94% (P<0.05), 3.30% (P>0.05), and 7.69% (P<0.01), respectively. In conclusion, dietary supplementation with

B. subtilis and *C. butyricum* can improve growth performance, slaughter performance, and antioxidant capacity of Wulong goslings, with combined supplementation showing superior effects compared to single-strain supplementation.

Keywords: *Bacillus subtilis*; *Clostridium butyricum*; Wulong goslings; growth performance; slaughter performance; serum biochemical indices; antioxidant capacity

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Bacillus subtilis is a widely used microecological preparation, a spore-forming Gram-positive aerobic bacterium with strong survival capabilities in harsh environments. It produces various enzymes that facilitate animal digestion and absorption, and also generates numerous antibiotics that promote healthy animal growth. *Clostridium butyricum* is another beneficial spore-forming bacterium that thrives anaerobically and can colonize the digestive tract under oxygen-free conditions to exert beneficial effects. Its metabolic product, butyric acid, plays an important role in repairing intestinal inflammation in animals. Therefore, investigating the effects of *B. subtilis* and *C. butyricum* on animal health is of significant importance.

Pang et al. [1] found that adding *Clostridium butyricum* preparation to weaned piglet diets significantly reduced diarrhea rates. Shimbo et al. [2] demonstrated that *C. butyricum* effectively treats enteritis, diarrhea, and digestive disorders, and can also be used for malignant tumor prevention and treatment. Zheng et al. [3] showed that dietary *B. subtilis* supplementation significantly increased 42-day-old broiler body weight and improved feed utilization from 21 to 42 days of age. Liao [4] found that dietary *C. butyricum* supplementation significantly increased average daily gain in broilers during 1-21 days and 22-42 days, improved duodenal morphology at 21 and 42 days, and increased acetic acid, butyric acid, and total short-chain fatty acid (SCFA) content in cecal chyme at 21 days, while also increasing serum immunoglobulin M (IgM) content at 21 and 42 days.

Currently, numerous studies have reported on the effects of *B. subtilis* on animal growth, and research on *C. butyricum* for intestinal protection and treatment is also available. However, studies on the combined use of *B. subtilis* and *C. butyricum* regarding animal growth, development, and antioxidant capacity remain limited. Therefore, this experiment used Wulong geese as an animal model to investigate the effects of dietary *B. subtilis* and *C. butyricum* supplementation on growth performance, slaughter performance, serum biochemical indices, and antioxidant capacity of goslings, aiming to determine the efficacy of combined supplementation and provide technical support for developing novel microecological preparations.

1.1 Experimental Animals and Design

Two hundred healthy one-day-old Wulong goslings with similar body weight were randomly divided into four groups with five replicates per group and ten goslings per replicate. Group I (control) received a basal diet, group II received the basal diet supplemented with 250 mg/kg *B. subtilis*, group III received the basal diet supplemented with 250 mg/kg *C. butyricum*, and group IV received the basal diet supplemented with 250 mg/kg *B. subtilis* plus 250 mg/kg *C. butyricum*. The experimental period lasted four weeks. The experimental goslings were provided by the breeding base of the Institute of High Quality Waterfowl at Qingdao Agricultural University, while *B. subtilis* and *C. butyricum* were purchased from Shandong Sukahan Bio-Engineering Co., Ltd.

1.2 Basal Diet

The nutritional levels of the basal diet were formulated according to the NRC (1994) poultry nutrient requirements. The composition and nutrient levels of the basal diet are presented in Table 1 .

1.3 Feeding Management

Before the experiment, the goose house and equipment were thoroughly cleaned and disinfected, then fumigated for 24 hours and ventilated before starting the trial. Goslings were fed using a "small amount, frequent addition" approach with ad libitum access to feed and water. The brooding temperature was maintained at 28-30°C during the first week, 26-27°C during the second week, and 24-25°C during the third week, after which it was gradually reduced to ambient temperature. Lighting schedule was 23 hours during weeks 1-2, 18 hours during week 3, and natural lighting thereafter. The growth condition of goslings was closely monitored and recorded promptly.

1.4.1 Growth Performance Indices

Feed consumption was recorded by replicate with leftover feed recovered to calculate feed intake. At the end of the four-week experiment, goslings were weighed by replicate after 12 hours of feed withdrawal (with water available). Fasting body weight was recorded at 08:00 the following day. Average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G) were calculated for weeks 1-4.

1.4.2 Slaughter Performance Indices

At the end of the four-week feeding trial, ten goslings with body weight close to the group average were selected from each group (two per replicate, totaling 40 goslings), euthanized by jugular vein exsanguination, weighed after wet plucking and drying, and their slaughter performance was measured. Measurements included: carcass weight (weight after bleeding and feather removal), semi-eviscerated weight (carcass weight after removal of trachea, esophagus, crop, intestines, spleen, pancreas, gallbladder, reproductive organs, gizzard contents and cuticle), fully eviscerated weight (semi-eviscerated weight after removal of heart, liver, gizzard, abdominal fat, head, and feet), abdominal fat weight (abdominal plate fat and fat around the gizzard), breast muscle weight (breast muscle was separated at the anterior end by the biceps brachii muscle, starting from the shoulder joint and ending at the median sternal process; at both lateral ends of the sternum, the breast muscle was separated from the external abdominal muscle using the lateral sternal process edge as the boundary, and finally the entire breast muscle was stripped from the sternum), and leg muscle weight (leg muscle was first separated along the pubic bone edge, then bounded by the sartorius muscle at both ends and the semitendinosus muscle at the posterior end; along the edge of the coccygeal elevator muscle, the entire thigh and acetabulum were separated together with the muscle). Slaughter performance indices were calculated according to the following formulas [5]:

- Carcass percentage (%) = (carcass weight / pre-slaughter live weight) × 100
- Semi-eviscerated percentage (%) = (semi-eviscerated weight / pre-slaughter live weight) × 100
- Fully eviscerated percentage (%) = (fully eviscerated weight / pre-slaughter live weight) × 100
- Breast muscle percentage (%) = (weight of both breast muscles / fully eviscerated weight) × 100
- Leg muscle percentage (%) = (weight of both leg muscles / fully eviscerated weight) × 100
- Abdominal fat percentage (%) = [abdominal fat weight / (fully eviscerated weight + abdominal fat weight)] × 100

1.4.3 Serum Biochemical Index Determination

At the end of the four-week experiment, ten goslings with body weight close to the group average were selected from each group (two per replicate, totaling 40 goslings). Blood was collected from the wing vein, centrifuged at 3,000 r/min to obtain serum samples, which were aliquoted and stored at -40°C. Serum urea nitrogen (UN) content was determined using the urease method, alkaline phosphatase (AKP) activity by colorimetry, total protein (TP) content by the biuret method, and triglyceride (TG) content by the phosphoglycerol oxidase method. All kits were purchased from Nanjing Jiancheng Bioengineering Institute.

1.4.4 Serum Antioxidant Index Determination

At the end of the four-week experiment, ten goslings with body weight close to the group average were selected from each group (two per replicate, totaling 40 goslings). Blood was collected from the wing vein, centrifuged at 3,000 r/min to obtain serum samples, which were aliquoted and stored at -40°C. Serum total antioxidant capacity (T-AOC) was measured by colorimetry, total superoxide dismutase (T-SOD) activity by the hydroxylamine method, malondialdehyde (MDA) content by the thiobarbituric acid (TBA) method, and glutathione peroxidase (GSH-Px) activity by the dithiobisnitrobenzoic acid method. All kits were purchased from Nanjing Jiancheng Bioengineering Institute, and measurements were performed using a UV-1100 UV-Vis spectrophotometer at different wavelengths.

1.5 Statistical Analysis

Experimental data were preprocessed using Excel 2010 and analyzed using one-way ANOVA with LSD multiple comparison in SPSS 17.0 software for statistical and correlation analysis. $P>0.05$ was considered not significant, $P<0.05$ significant, and $P<0.01$ highly significant.

2.1 Effects of Dietary *Bacillus subtilis* and *Clostridium butyricum* on Growth Performance of Goslings

The effects of dietary *B. subtilis* and *C. butyricum* on growth performance are shown in Table 2 . The results demonstrated that dietary supplementation with *B. subtilis* and *C. butyricum* had highly significant effects on final body weight and average daily gain of goslings ($P<0.01$). Compared with the control group, the final body weight of goslings in groups II, III, and IV increased by 7.78% ($P<0.05$), 3.12% ($P>0.05$), and 9.54% ($P<0.05$), respectively; average daily gain increased by 7.89% ($P<0.05$), 3.39% ($P>0.05$), and 10.18% ($P<0.01$), respectively. No significant differences were observed in average daily feed intake among groups ($P>0.05$), though groups II, III, and IV showed increases of 2.35%, 1.44%, and 3.94%, respectively. Compared with the control group, feed-to-gain ratio decreased by 4.96% ($P<0.05$), 1.65% ($P>0.05$), and 6.20% ($P<0.05$) in groups II, III, and IV, respectively. Group IV exhibited the lowest mortality rate. These results indicate that dietary supplementation with *B. subtilis* and *C. butyricum* can improve body weight and average daily gain of goslings, with combined supplementation being superior to single-strain supplementation.

2.2 Effects of Dietary *Bacillus subtilis* and *Clostridium butyricum* on Slaughter Performance of Goslings

The effects of dietary *B. subtilis* and *C. butyricum* on slaughter performance are presented in Table 3 . The results showed that dietary supplementation had no significant effects on carcass percentage, semi-eviscerated percentage, or fully eviscerated percentage ($P>0.05$), but significantly affected breast muscle percentage, leg muscle percentage, and abdominal fat percentage ($P<0.05$). Compared with the control group, breast muscle percentage increased by 2.10% ($P>0.05$), 2.80% ($P>0.05$), and 4.20% ($P<0.05$) in groups II, III, and IV, respectively; leg muscle percentage increased by 9.50% ($P<0.05$), 8.60% ($P<0.05$), and 10.86% ($P<0.05$), respectively; and abdominal fat percentage decreased by 7.74% ($P<0.05$), 5.81% ($P>0.05$), and 10.97% ($P<0.05$), respectively. These findings demonstrate that dietary supplementation with *B. subtilis* and *C. butyricum* can increase breast and leg muscle percentages while decreasing abdominal fat percentage, with combined supplementation outperforming single-strain supplementation.

2.3 Effects of Dietary *Bacillus subtilis* and *Clostridium butyricum* on Serum Biochemical Indices of Goslings

The effects of dietary *B. subtilis* and *C. butyricum* on serum biochemical indices are shown in Table 4 . The results indicated that dietary supplementation had no significant effects on serum urea nitrogen, total protein, triglyceride content, or alkaline phosphatase activity in goslings (P>0.05).

2.4 Effects of Dietary *Bacillus subtilis* and *Clostridium butyricum* on Serum Antioxidant Indices of Goslings

The effects of dietary *B. subtilis* and *C. butyricum* on serum antioxidant indices are presented in Table 5 . The results revealed that dietary supplementation significantly affected serum total antioxidant capacity and superoxide dismutase activity (P<0.05). Compared with the control group, serum total antioxidant capacity in groups II, III, and IV increased by 4.46% (P>0.05), 3.39% (P>0.05), and 12.33% (P<0.05), respectively; serum total superoxide dismutase activity increased by 4.94% (P<0.05), 3.30% (P>0.05), and 7.69% (P<0.01), respectively. No significant differences were observed in serum malondialdehyde content or glutathione peroxidase activity among groups (P>0.05). These results suggest that dietary supplementation with *B. subtilis* and *C. butyricum* can enhance the antioxidant capacity of goslings, with combined supplementation being more effective than single-strain supplementation.

3.1 Effects of Dietary *Bacillus subtilis* and *Clostridium butyricum* on Growth Performance of Goslings

Probiotic preparations have been widely applied as effective functional feed additives in the poultry industry, with numerous studies demonstrating significant effects on growth performance. Cai et al. [6] reported that adding 60 mg/kg *Bacillus megaterium* to basal diets significantly increased gosling body weight and average daily feed intake compared to the control group. Zhang [7] found that 100 g/t *B. subtilis* supplementation yielded optimal broiler growth performance, increasing average daily feed intake by 7.29% and average daily gain by 6.42% compared to controls. Qi et al. [8] demonstrated that 500 mg/kg *B. subtilis* and 20 mg/kg colistin sulfate significantly increased 42-day-old broiler body weight and later-stage average daily gain while significantly reducing later-stage and overall feed-to-gain ratio without affecting other growth stages. Yin et al. [9] showed that 0.2% and 0.3% *B. subtilis* supplementation significantly increased broiler average body weight and average daily gain while reducing feed-to-gain ratio during early, late, and entire experimental periods. Sena et al. [10] found that Ross broiler growth performance increased linearly with increasing dietary *B. subtilis* levels (0, 0.15%, 0.30%, 0.40%). Pan et al. [11] reported that 0.1% *B. subtilis* preparation significantly increased broiler net weight gain and average daily gain, with improvements in average daily feed intake and feed conversion ratio. Maneewan et al. [12] found that *B. subtilis* increased average daily gain in 1-28 day-old piglets. Sun et al. [13] demonstrated that *Bacillus subtilis* natto increased average daily gain and feed conversion ratio in 7-day-old Holstein bull calves. Xiao et al. [14] showed that *C. butyricum* and *B. subtilis* significantly increased average daily gain and decreased feed-to-gain ratio at all stages in partridge shank broilers, with overall reductions of 6.49% and 6.00%, respectively. Chen et al. [15] reported that *B. subtilis* significantly improved Arbor Acres broiler growth performance and reduced feed-to-gain ratio. Jia [16] found that both antibiotic and *C. butyricum* groups significantly increased average daily gain in 1-21 day-old Cherry Valley ducks, with trends toward improved feed-to-gain ratio.

The current results align with these findings, showing that dietary *B. subtilis* and *C. butyricum* significantly affected gosling body weight and average daily gain. *C. butyricum* promotes goose growth and development primarily by producing B vitamins, vitamin K, amylase, protease, glucosidase, and cellulase, which stimulate the proliferation of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus* in the animal intestine, thereby improving nutrient utilization and accelerating growth. However, this study found no significant effect on average daily feed intake and only a non-significant trend toward improved feed-to-gain ratio, which may be attributed to differences in animal growth stages among studies.

3.2 Effects of Dietary *Bacillus subtilis* and *Clostridium butyricum* on Slaughter Performance of Goslings

Zhang [7] reported that 100 g/t *C. butyricum* supplementation yielded optimal broiler slaughter performance, increasing carcass percentage and fully eviscerated percentage by 0.42% and 3.97%, respectively, while decreasing abdominal fat percentage by 15.67%. Qi et al. [8] found no significant changes in broiler slaughter performance with 500 mg/kg *B. subtilis* or 20 mg/kg colistin sulfate supplementation. Cai et al. [6] showed that 60 mg/kg *B. megaterium* significantly increased fully eviscerated and semi-eviscerated percentages without affecting breast muscle, leg muscle, or abdominal fat percentages. Cui et al. [17] reported that *B. subtilis* supplementation significantly increased fully eviscerated and semi-eviscerated percentages by 3.38% and 1.83%, respectively. Deng et al. [18] found that *C. butyricum* significantly increased broiler breast muscle percentage and decreased abdominal fat percentage, with significant interactive effects when combined with xylo-oligosaccharides. Increased body weight and average daily gain are associated with feed intake and digestion; greater feed intake and better digestion lead to faster weight gain. Healthy, active goslings with increased exercise develop greater breast and leg muscle percentages while reducing fat deposition. The current findings are consistent with these results, showing that *B. subtilis* and *C. butyricum* supplementation increased body weight, average daily gain, breast muscle percentage, and leg muscle percentage while decreasing abdominal fat percentage. Moreover, combined supplementation was superior to single-strain supplementation, providing important insights for developing novel microecological preparations.

3.3 Effects of Dietary *Bacillus subtilis* and *Clostridium butyricum* on Serum Biochemical Indices of Goslings

Urea is the main end product of protein metabolism in animals, constituting the majority of blood non-protein nitrogen. Blood urea nitrogen originates from the liver and is excreted through the kidneys; elevated levels may indicate renal failure, nephritis, or urinary tract obstruction. Alkaline phosphatase, a zinc-containing glycoprotein, is commonly used to diagnose hepatobiliary and bone diseases. Serum total protein content primarily reflects liver synthetic function and protein loss due to kidney lesions, while serum triglyceride content indicates normal kidney function. Cao et al. [19] found that *C. butyricum* supplementation significantly reduced blood ammonia content in broilers. Jia et al. [20] reported that *C. butyricum* significantly increased serum total protein content in broilers, with 31.33% and 52.27% increases compared to the antibiotic group at 21 and 42 days, respectively, while significantly reducing blood ammonia content. Xin et al. [21] found that *Bacillus* preparation non-significantly reduced serum cholesterol content by 11.29% in laying hens. Fukushima et al. [22] reported that combined *B. subtilis* and *C. butyricum* supplementation reduced serum urea nitrogen and triglyceride content in goslings, consistent with the current results.

3.4 Effects of Dietary *Bacillus subtilis* and *Clostridium butyricum* on Serum Antioxidant Indices of Goslings

The body generates oxygen free radicals through enzymatic and non-enzymatic systems. Free radicals attack polyunsaturated fatty acids in biological membranes, initiating lipid peroxidation and forming lipid peroxides. Malondialdehyde content reflects the degree of lipid peroxidation and indirectly indicates cell damage. The strength of the antioxidant defense system is closely related to health status. Yu et al. [23] found that *B. subtilis* supplementation significantly or highly significantly increased serum and liver total antioxidant capacity and glutathione peroxidase activity while reducing serum and liver malondialdehyde and nitric oxide content in Ross 308 broilers. Rashid et al. [24] reported that *B. subtilis* increased serum glutathione peroxidase, peroxidase, glutathione reductase, and catalase activities while decreasing malondialdehyde content, thereby enhancing antioxidant capacity. Chen et al. [15] showed that *B. subtilis* increased serum glucose content and superoxide dismutase activity while reducing malondialdehyde content and alanine aminotransferase activity, without significantly affecting aspartate aminotransferase activity. Jia et al. [20] demonstrated that *C. butyricum* significantly enhanced serum glutathione peroxidase and total superoxide dismutase activities at 42 days, with glutathione peroxidase activity increasing by 60.00% compared to the antibiotic group, while the antibiotic group showed significantly increased serum total superoxide dismutase activity at 21 days compared to controls. The current findings are consistent with these studies, confirming that dietary *B. subtilis* and *C. butyricum* supplementation can enhance the antioxidant capacity of goslings.

4 Conclusion

Dietary supplementation with *Bacillus subtilis* and *Clostridium butyricum* can improve growth performance, slaughter performance, and antioxidant capacity of goslings, with combined supplementation demonstrating superior effects compared to single-strain supplementation.

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