Effects of Bacillus coagulans on Growth Performance, Immune Organ Indices, Serum Biochemical Parameters, and Intestinal Microbiota in Broiler Chickens (Postprint)

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Abstract

This experiment aimed to investigate the effects of Bacillus coagulans on growth performance, immune organ indices, serum biochemical parameters, and intestinal microflora in broiler chickens. A total of 10,000 healthy 1-day-old AV500 broiler chickens were selected and randomly divided into 2 groups with 5 replicates per group and 1,000 chickens per replicate. The control group was fed a basal diet, while the experimental group was fed the basal diet supplemented with 300 mg/kg Bacillus coagulans preparation. The experimental period lasted for 6 weeks. The results showed: 1) The mortality and culling rate of broiler chickens in the experimental group was significantly lower than that in the control group during 1-21 days and 22-42 days of age ($P<0.05$), decreasing by 61.7% and 52.42%, respectively; during 22-42 days of age, the average daily gain and average daily feed intake of broiler chickens in the experimental group were significantly higher than those in the control group ($P<0.05$). 2) At 21 days of age, the spleen index, thymus index, and bursa of Fabricius index of broiler chickens in the experimental group were significantly higher than those in the control group ($P<0.05$), while the intestinal index was significantly lower than that in the control group ($P<0.05$). At 42 days of age, the spleen index and thymus index of broiler chickens in the experimental group were significantly higher than those in the control group ($P<0.05$). 3) At both 21 and 42 days of age, the activities of alanine aminotransferase and alkaline phosphatase, as well as the contents of total protein and globulin in the serum of broiler chickens in the experimental group were significantly higher than those in the control group ($P<0.05$). 4) At both 21 and 42 days of age, the number of Bacillus coagulans in the cecal and ileal chyme of broiler chickens in the experimental group was significantly higher than that in the control group ($P<0.05$), while the number of

Escherichia coli was significantly lower than that in the control group (P<0.05). These results indicate that dietary supplementation with Bacillus coagulans can inhibit the growth of Escherichia coli in the intestine, improve immune organ indices, and reduce the mortality and culling rate of broiler chickens.

Full Text

Effects of Bacillus coagulans on Growth Performance, Immune Organ Indexes, Serum Biochemical Indexes and Intestinal Flora of Broilers

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Abstract

This experiment was conducted to investigate the effects of *Bacillus coagulans* on growth performance, immune organ indexes, serum biochemical indexes, and intestinal flora of broilers. A total of 10,000 healthy 1-day-old AV500 broilers were randomly divided into two groups with five replicates per group and 1,000 broilers per replicate. The control group was fed a basal diet, while the test group received the basal diet supplemented with 300 mg/kg *Bacillus coagulans* preparation. The experiment lasted for six weeks. The results showed: (1) The mortality rate of broilers in the test group was significantly lower than that in the control group at both 1–21 days and 22–42 days of age (P<0.05), decreasing by 61.7% and 52.42%, respectively; the average daily gain and average daily feed intake of broilers in the test group were significantly higher than those in the control group at 22–42 days of age (P<0.05). (2) At 21 days of age, the spleen index, thymus index, and bursa of Fabricius index of broilers in the test group were significantly higher than those in the control group (P<0.05), while the intestinal index was significantly lower (P<0.05). At 42 days of age, the spleen index and thymus index of broilers in the test group remained significantly higher than those in the control group (P<0.05). (3) The activities of alanine aminotransferase and alkaline phosphatase and the contents of total protein and globulin in serum of broilers in the test group were significantly higher than those in the control group at both 21 and 42 days of age (P<0.05). (4) The number of *Bacillus coagulans* in cecal and ileal digesta of broilers in the

test group was significantly higher than that in the control group at 21 and 42 days of age ($P<0.05$), while the *Escherichia coli* count was significantly lower ($P<0.05$). In conclusion, dietary supplementation with *Bacillus coagulans* can inhibit the growth of *E. coli* in the intestine, improve immune organ indexes, and reduce the mortality rate of broilers.

Keywords: *Bacillus coagulans*; broilers; immune organ indexes; serum biochemical indexes; intestinal flora

Introduction

Dietary supplementation with probiotic preparations can effectively regulate the microecological environment of the gastrointestinal tract, benefit microbial balance, and improve growth performance and disease prevention in livestock and poultry [1]. The advantages of probiotic preparations, such as lack of drug resistance and absence of residues, are unmatched by antibiotics. Excellent probiotic preparations must possess non-pathogenic and non-toxic strains, high stress resistance to gastric acid, bile salts, and high temperature/pressure, adhesion to intestinal epithelial cells, rapid growth and reproduction, ability to produce antibacterial substances, and good storage stability. *Bacillus coagulans* has gained increasing attention in animal feed applications for pigs and chickens in recent years due to these advantages [2–6, 7–10].

Bacillus coagulans is a lactic acid-producing spore-forming bacterium belonging to the Firmicutes phylum of intestinal lactic acid bacteria [11]. Its fermentation mode is homofermentative, and it possesses a broad antimicrobial spectrum while enhancing cellular and humoral immunity [12]. It can stimulate the development of immune organs and improve disease resistance in animals. The bacterium produces active substances such as proteases, lipases, amylases, and xylanases [13], promotes nutrient digestion and absorption, and generates vitamins, short-chain fatty acids, amino acids, organic acids, and growth-promoting factors that provide nutrition for animals [14–15]. It also promotes small intestinal peristalsis, improves intestinal digestive function, and creates favorable conditions for animal growth and development [16]. *Bacillus coagulans* remains stable in various feed forms, demonstrating high application value in the feed industry. Currently, research on the effects of *Bacillus coagulans* on intestinal microbiota in broilers is limited. Therefore, this study aimed to investigate the effects of dietary *Bacillus coagulans* supplementation on growth performance, immune organ indexes, serum biochemical indexes, and intestinal flora in broilers, providing a reference for its scientific application in broiler production.

Materials and Methods

1.1 Test Materials The *Bacillus coagulans* preparation contained viable bacteria at 1.0$×10^{9}$ CFU/g and was provided by Hubei Lutiandi Biotechnology Co., Ltd. The experimental animals consisted of 10,000 healthy 1-day-old AV500 broilers raised at the Xiangyang Shengfeng Agriculture and Animal Husbandry Co., Ltd. farm.

1.2 Experimental Design and Grouping The 10,000 healthy 1-day-old AV500 broilers were randomly divided into two groups with five replicates per group and 1,000 broilers per replicate. The control group received a basal diet, while the test group received the basal diet supplemented with 300 mg/kg *Bacillus coagulans* preparation. The basal diet was formulated according to NRC (1994) broiler feeding standards combined with actual production conditions. The composition and nutrient levels of the basal diet are shown in Table 1 . The experiment was conducted in two phases: 1–21 days and 22–42 days of age.

1.3 Management Practices The chicken house was heated with infrared lamps. Room temperature was maintained at 34°C on days 1–2, 32°C on days 3–7, 30°C on days 8–14, 27°C on days 15–20, and subsequently adjusted to 22°C. Relative humidity was maintained at 70% on days 1–7, 60% on days 8–20, and approximately 55% thereafter. Light intensity was 30 lx. Broilers were raised on netting with ad libitum access to water and feed. Manure was removed daily, and conventional disinfection and immunization procedures were followed.

1.4 Sample Collection and Measurements 1.4.1 Sample Collection

On days 21 and 42 of the experiment, after 12 h of fasting, 20 broilers were randomly selected from each replicate and weighed. They were then euthanized by jugular vein exsanguination and defeathered. The spleen, thymus, bursa of Fabricius, and intestine were dissected and weighed. Blood collected from the jugular vein was allowed to stand for 10 min before centrifugation at 3,000 r/min, and serum was stored at 4°C for biochemical analysis. Under aseptic conditions, cecal and ileal contents were quickly collected in sterile cryovials and stored in liquid nitrogen for microbial analysis.

1.4.2 Experimental Instruments

The instruments used included an AUY220 electronic analytical balance, a Shimadzu UV2550 UV-Vis spectrophotometer, a FOSS2300 automatic Kjeldahl nitrogen analyzer, a Roche MODULP800 automatic biochemical analyzer, an EDC-810 PCR amplifier, a JY300 horizontal electrophoresis apparatus, and a JY02S UV analyzer.

1.4.3 Growth Performance and Immune Organ Index Determination

Initial and final body weights were measured by replicate on the first and last days of the experiment (after 12 h fasting with free access to water). Daily

feed intake and culling/mortality were recorded to calculate average daily gain, average daily feed intake, and feed-to-gain ratio. Immune organ indexes were calculated as the ratio of organ weight to body weight, including spleen index, thymus index, bursa of Fabricius index, and intestinal index.

1.4.4 Serum Biochemical Index Determination

Serum biochemical indexes were measured using a Roche MODULP800 automatic biochemical analyzer.

1.4.5 Intestinal Microbial Count Determination

Real-time fluorescent quantitative PCR was used to detect *Bacillus coagulans* and *E. coli* numbers in intestinal contents. Primer sequences and parameters are shown in Table 2 , and primers were synthesized by Shanghai Sangon Biotech Co., Ltd. Intestinal content DNA was extracted according to the instructions of the Beijing Tiangen Genomic DNA Extraction Kit. Standard curves were constructed by recovering and purifying target DNA fragments from agarose gels to obtain *Enterococcus* and *E. coli* standards. Real-time fluorescent quantitative PCR was performed on bacterial standards, with the logarithm of different standard copy numbers as the x-axis and the initial cycle number (Ct) at which fluorescent signals appeared during PCR as the y-axis. Test samples were subjected to real-time fluorescent quantitative PCR to obtain Ct values, which were compared with the standard curve to determine bacterial numbers in each sample.

1.5 Statistical Analysis Data were processed using SPSS 16.0 software and analyzed by t-test. Results are expressed as "mean ± standard error."

Results

2.1 Effects of *Bacillus coagulans* on Broiler Growth Performance The effects of *Bacillus coagulans* on broiler growth performance are shown in Table 3 . The mortality rate of broilers in the test group was significantly lower than that in the control group at both 1–21 days and 22–42 days of age ($P<0.05$), decreasing by 61.7% and 52.42%, respectively. No significant differences were observed in average daily gain or average daily feed intake between the control and test groups at 1–21 days of age ($P>0.05$). However, at 22–42 days of age, both average daily gain and average daily feed intake in the test group were significantly higher than those in the control group ($P<0.05$). The feed-to-gain ratio did not differ significantly between groups throughout the experimental period ($P>0.05$).

2.2 Effects of *Bacillus coagulans* on Broiler Immune Organ Indexes The effects of *Bacillus coagulans* on broiler immune organ indexes are presented in Table 4 . At 21 days of age, the spleen index, thymus index, and bursa of

Fabricius index of broilers in the test group were significantly higher than those in the control group (P<0.05), while the intestinal index was significantly lower (P<0.05). At 42 days of age, the spleen index and thymus index of broilers in the test group remained significantly higher than those in the control group (P<0.05), but no significant differences were observed in the bursa of Fabricius index or intestinal index (P>0.05).

2.3 Effects of *Bacillus coagulans* on Broiler Serum Biochemical Indexes The effects of *Bacillus coagulans* on broiler serum biochemical indexes are shown in Table 5 . At both 21 and 42 days of age, the activities of alanine aminotransferase and alkaline phosphatase and the contents of total protein and globulin in serum of broilers in the test group were significantly higher than those in the control group (P<0.05). However, no significant differences were observed in aspartate aminotransferase activity or in the contents of albumin, triglycerides, high-density lipoprotein cholesterol, or low-density lipoprotein cholesterol between groups (P>0.05).

2.4 Effects of *Bacillus coagulans* on Broiler Intestinal Microflora Numbers The effects of *Bacillus coagulans* on broiler intestinal microflora numbers are presented in Table 6 . At both 21 and 42 days of age, the number of *Bacillus coagulans* in cecal and ileal digesta of broilers in the test group was significantly higher than that in the control group (P<0.05), while the *E. coli* count was significantly lower (P<0.05). As digesta moved along the intestinal tract, the *E. coli* count in the cecum at 42 days of age was markedly lower in both control and test groups.

Discussion

3.1 Effects of *Bacillus coagulans* on Broiler Growth Performance Numerous studies have demonstrated that dietary probiotic supplementation can improve animal growth performance and feed conversion efficiency [17–19]. Research has shown that adding *Bacillus coagulans* to broiler diets can improve feed conversion ratio [20–22]. The present study similarly found that during the 22–42 day period, broilers fed diets supplemented with *Bacillus coagulans* exhibited significantly higher average daily gain and average daily feed intake. The improved weight gain and feed conversion may be attributed to *Bacillus coagulans* directly producing nutrients such as vitamins, amino acids, and growth-promoting factors in the digestive tract, which increase small intestinal motility and improve digestive function, thereby promoting the digestion and absorption of various nutrients from the feed [14].

3.2 Effects of *Bacillus coagulans* on Broiler Immune Organ Indexes The thymus, bursa of Fabricius, and spleen are the most important immune organs in poultry, participating in both cellular and humoral immunity throughout

the body [23], and their weights can be used to evaluate the immune status of chicks [24]. This study found that at 21 days of age, the spleen index, thymus index, and bursa of Fabricius index of broilers in the test group were significantly higher than those in the control group, and at 42 days of age, the spleen index and thymus index remained significantly higher. Increased relative weights of the thymus, bursa of Fabricius, and spleen indicate enhanced immune function [25–26]. Reports have indicated that atrophy of the bursa of Fabricius prevents antibody production in chicks, while a well-developed bursa facilitates increased antibody levels [27]. Additionally, the significantly lower intestinal index in the test group at 21 days of age suggests that broilers allocated more energy to muscle tissue rather than intestinal growth, resulting in differences in intestinal index. The lack of significant differences in bursa of Fabricius index and intestinal index between groups at 42 days of age may be attributed to increased body weight and bursal atrophy during later growth stages, reducing the significance of organ index differences.

Differences in immune organ development may result from beneficial bacteria proliferating in the intestine and continuously synthesizing beneficial substances that act as antigens to stimulate and promote immune organ growth [28]. Zhao et al. [29] also suggested that *Bacillus coagulans* can function as an immune adjuvant at antigen recognition sites in the digestive tract, activating and modulating the immune system. Luo et al. [30] proposed that *Bacillus coagulans* can enhance the phagocytic and secretory functions of peritoneal mononuclear-macrophages and activate lymphocytes, strengthening humoral and cellular immunity and improving overall immune capacity. The stimulatory effect of microecological preparations on immune organs leads to differences in animal health status, which is directly reflected in mortality rates. In this study, dietary *Bacillus coagulans* supplementation significantly reduced broiler mortality during both 1–21 days and 22–42 days of age, demonstrating significant effects on growth characteristics and immune function.

3.3 Effects of *Bacillus coagulans* on Broiler Serum Biochemical Indexes The effects of *Bacillus coagulans* on serum biochemical indexes varied. Serum alanine aminotransferase activity within a certain range reflects the metabolic activity of hepatocytes. The significantly higher alanine aminotransferase activity in the test group indicates that *Bacillus coagulans* promotes liver physiological function. Serum alkaline phosphatase primarily originates from the liver and bones and can reflect hepatic and skeletal conditions [31]. Alkaline phosphatase is a marker enzyme for changes in small intestinal digestion and absorption function [32] and can defend against damage to intestinal mucosa by pathogenic lipopolysaccharides [33]. The significantly higher serum alkaline phosphatase activity in the test group is consistent with Lin et al. [34], who reported that *Bacillus coagulans* significantly increased serum alkaline phosphatase activity in yellow-feathered broilers at 28 and 70 days of age. The significantly higher total protein and globulin contents in the test group indicate good nutritional status, as serum total protein consists mainly of globulin

and albumin [35]. High-density and low-density lipoprotein cholesterol contents reflect cholesterol synthesis and dietary lipid absorption, while triglycerides are the primary form of energy storage and reflect lipid metabolism [36]. This study found no significant differences in serum triglycerides, high-density lipoprotein cholesterol, or low-density lipoprotein cholesterol contents between groups from 1–42 days of age, consistent with Zhang et al. [37] in gibel carp, suggesting that *Bacillus coagulans* supplementation did not significantly affect lipid metabolism.

3.4 Effects of *Bacillus coagulans* on Broiler Intestinal Microflora Numbers The effects of *Bacillus coagulans* on animal intestinal flora have received widespread attention. Dietary supplementation can reduce harmful bacteria and improve intestinal digestion and absorption by altering the microecological system [20,39]. This study used real-time fluorescent quantitative PCR to quantitatively analyze *Bacillus coagulans* and *E. coli* numbers in cecal and ileal digesta. Compared with the control group, the test group showed increased *Bacillus coagulans* numbers and decreased *E. coli* numbers in both cecal and ileal digesta, indicating that *Bacillus coagulans* colonized the broiler intestine and became the dominant flora through competitive exclusion, thereby reducing *E. coli* numbers and stably exerting probiotic effects.

Bacillus coagulans is a facultative anaerobe that enters the intestine as spores several hours after ingestion, then germinates and rapidly proliferates, consuming free oxygen [40]. Through competition for nutrients and binding sites on small intestinal mucosal epithelial cells, it reduces colonization by harmful pathogens. Additionally, *Bacillus coagulans* converts carbohydrates, particularly lactose, into lactic acid through fermentation, forming a biological barrier on the small intestinal epithelial cell surface that prevents pathogenic bacteria from approaching while lowering intestinal pH to inhibit growth of pathogens such as *E. coli*, *Salmonella*, and *Enterococcus* [3,41–44]. Furthermore, large amounts of antimicrobial substances such as coagulin and L-lactic acid secreted during its metabolic processes [45–47] also affect pathogenic bacterial proliferation. These combined actions regulate intestinal microflora balance and effectively exert probiotic effects, reducing disease occurrence.

In conclusion, dietary supplementation with *Bacillus coagulans* can inhibit intestinal *E. coli* growth, stimulate immune organ development, and reduce broiler mortality.

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