

Effects of Curcumin and *Bacillus licheniformis* on Growth Performance, Serum Antioxidant Function, Intestinal Microbial Count, and Immune Organ Index in Broiler Chickens: Postprint

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Abstract

This experiment aimed to investigate the effects of curcumin and *Bacillus licheniformis* on growth performance, serum antioxidant function, intestinal microbial populations, and immune organ indices in broiler chickens. A total of 450 one-day-old Arbor Acres (AA) broiler chickens were selected and randomly divided into 5 groups with 6 replicates per group and 15 chickens per replicate. The control group was fed a basal diet, while the experimental groups were fed the basal diet supplemented with 35 mg/kg antibiotics (D1 group), 200 mg/kg curcumin (D2 group), 100 mg/kg *Bacillus licheniformis* (D3 group), and 200 mg/kg curcumin + 100 mg/kg *Bacillus licheniformis* (D4 group), respectively. The experimental period lasted 42 days. The results showed: 1) Compared with the control group, the average daily gain of all experimental groups was significantly increased ($P < 0.05$); the average daily gain and final body weight of the D1 group were significantly higher than those of the D2 and D3 groups ($P < 0.05$), but showed no significant difference from the D4 group ($P > 0.05$); the feed conversion ratio of the D1 and D4 groups was significantly lower than that of the control group ($P < 0.05$). 2) Compared with the control and D1 groups, the D2, D3, and D4 groups all significantly increased the activities of superoxide dismutase (except for D3), glutathione peroxidase, and lysozyme in serum ($P < 0.05$); the serum malondialdehyde content in the D2, D3, and D4 groups was significantly lower than that in the control group ($P < 0.05$). 3) Compared with the control group, the numbers of *Lactobacillus* and *Bifidobacterium* in the intestine of the D4 group were significantly increased ($P < 0.05$), with no significant differences among the experimental groups ($P > 0.05$); the numbers of *Escherichia coli* and *Salmonella* in the intestine of the D1 and D4 groups were significantly decreased ($P < 0.05$). 4) Compared with the control group, the spleen and bursa

of Fabricius indices of the D4 group were significantly increased ($P < 0.05$). The results suggest that curcumin and *Bacillus licheniformis*, used alone or in combination, can improve the growth performance, immune function, and intestinal microbial environment of broiler chickens, with the combined use being superior to individual use, indicating a certain synergistic effect between the two.

Full Text

Effects of Curcumin and *Bacillus licheniformis* on Growth Performance, Serum Antioxidant Function, Intestinal Microbe Counts, and Immune Organ Indexes of Broilers

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Abstract

This experiment was conducted to investigate the effects of dietary supplementation with curcumin and *Bacillus licheniformis* on growth performance, serum antioxidant function, intestinal microbe counts, and immune organ indexes in broiler chickens. A total of 450 one-day-old Arbor Acres (AA) broilers were randomly allocated into five groups with six replicates per group and 15 birds per replicate. The control group received a basal diet, while the experimental groups received the basal diet supplemented with 35 mg/kg antibiotic (D1 group), 200 mg/kg curcumin (D2 group), 100 mg/kg *Bacillus licheniformis* (D3 group), or 100 mg/kg *Bacillus licheniformis* plus 200 mg/kg curcumin (D4 group). The trial lasted for 42 days. The results showed that: (1) Compared with the control group, all experimental groups exhibited significantly higher average daily gain (ADG) ($P < 0.05$). The ADG and final weight of the D1 group were significantly higher than those of the D2 and D3 groups ($P < 0.05$) but did not differ significantly from the D4 group ($P > 0.05$). The feed-to-gain ratio of the D1 and D4 groups was significantly lower than that of the control group ($P < 0.05$). (2) Compared with the control and D1 groups, the D2, D3, and D4 groups showed significantly increased serum activities of lysozyme (LZM), superoxide dismutase (SOD) (except for D3), and glutathione peroxidase (GSH-Px) ($P < 0.05$). Serum malondialdehyde (MDA) content in the D2, D3, and D4 groups was significantly lower than in the control group ($P < 0.05$). (3) Compared with the control group, the D4 group showed significantly higher counts of *Lactobacillus* and *Bifidobacterium* in the intestine ($P < 0.05$), with no significant differences among the experimental groups ($P > 0.05$). The D1 and D4 groups exhibited significantly reduced intestinal counts of *Escherichia coli* and *Salmonella* ($P < 0.05$). (4) Compared with the control group, the D4 group demonstrated significantly elevated spleen and bursa of Fabricius indexes ($P < 0.05$). These findings indicate that dietary supplementation with curcumin and *Bacillus licheniformis*,

either individually or in combination, can improve growth performance, immune function, and intestinal microbial environment in broilers, with the combined treatment showing superior effects to individual supplementation, suggesting a synergistic interaction between the two additives.

Keywords: broiler; curcumin; *Bacillus licheniformis*; immune organ; intestinal microbe

Introduction

With rapid socioeconomic development, the living standards of Chinese residents have progressively improved, leading to increased per capita consumption of meat and eggs. Broiler production constitutes a significant component of China's animal agriculture, with annual production reaching approximately 7 billion birds. To enhance production efficiency and maintain animal health, sub-therapeutic doses of antibiotics have traditionally been used as antibiotic growth promoters (AGPs) in feed [?]. However, growing research on the effects of antibiotics has revealed notable drawbacks: while inhibiting pathogenic bacteria, antibiotics also suppress beneficial gut microbiota, causing microbial imbalance; prolonged and extensive use promotes antibiotic resistance; and antibiotic residues in animal waste and animal products can accumulate in humans through the food chain, posing direct health risks. Consequently, identifying substances that can enhance animals' natural defense mechanisms represents an effective strategy to reduce antibiotic dependence [?].

Curcumin serves as an important food additive in the food industry, functioning as an antioxidant to prevent spoilage [?]. Research has demonstrated that curcumin possesses extensive pharmacological properties. Sa et al. [?] reported that curcumin exhibits preventive and therapeutic effects against various diseases. Priyadarsini et al. [?] and Miriyala et al. [?] found that curcumin can interrupt free radical reactions and scavenge excess free radicals in vivo. Hatcher et al. [?] also noted that curcumin acts as both a free radical scavenger and hydrogen donor, displaying both pro-oxidant and antioxidant activities. These unique physiological functions position curcumin as a novel functional feed additive. *Bacillus licheniformis*, a probiotic microorganism, plays roles in regulating animal microecological balance, reducing intestinal diseases, enhancing immune function, promoting beneficial gut bacteria growth, and improving animal product quality. Zhang et al. [?] reported that *B. licheniformis* produces various antimicrobial substances during its metabolic processes, exerting strong inhibitory effects on common pathogenic bacteria. Benyacoub et al. [?] demonstrated that bacilli can stimulate the immune system and enhance immune function, making them effective immunomodulators.

Therefore, beyond establishing optimal rearing environments and implementing proper sanitation and biosecurity measures, feeding nutritionally efficient diets with appropriate functional additives is crucial. This study investigated

the effects of dietary supplementation with curcumin and *B. licheniformis*, individually and in combination, on broiler growth performance, immune organ indexes, serum antioxidant function, and intestinal microbe counts, aiming to provide a theoretical basis for their further application and promotion.

1.1 Experimental Design

A total of 450 one-day-old Arbor Acres (AA) broilers (43.15 ± 0.17 g) were obtained from Kaifeng Hefeng Animal Husbandry Co., Ltd. and randomly divided into five groups with six replicates per group and 15 birds per replicate, with no significant differences in initial body weight among groups ($P > 0.05$). A basal diet was formulated as a powdered complete feed according to the *Feeding Standard of Broilers* (NY/T 33-2004), with composition and nutrient levels shown in Table 1. The control group received the basal diet, while experimental groups received the basal diet supplemented with 35 mg/kg antibiotic (D1 group), 200 mg/kg curcumin (D2 group), 100 mg/kg *B. licheniformis* (D3 group), or 100 mg/kg *B. licheniformis* plus 200 mg/kg curcumin (D4 group). The feeding trial was conducted at the Weishi Experimental Base of Henan Academy of Agricultural Sciences and lasted 42 days. During the trial, broilers had ad libitum access to feed and water, and were vaccinated according to standard procedures.

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

1.2 Materials and Sources

The curcumin used in this experiment was Baiweisu (Guangzhou Kehu Biological Technology Research Center) containing 10% curcumin. *Bacillus licheniformis* was obtained from Alpharma Inc. (total viable count of 2×10^{10} CFU/g). The antibiotic was Su Da Fei (Phibro Animal Health Corporation) containing 50% virginiamycin.

1.3 Measurements

1.3.1 Growth Performance Determination Initial body weight was recorded when birds were housed. At 42 days of age, final body weight and feed intake were measured after a 12-hour fasting period (feed was withdrawn at 20:00 on the day before weighing, and birds were weighed at 08:00 the following day). Feed provided and remaining feed were recorded for each replicate to calculate average daily feed intake (ADFI), average daily gain (ADG), and feed-to-gain ratio (F/G).

1.3.2 Serum Antioxidant Indices Determination On day 42, one broiler with body weight closest to the replicate mean was selected from each replicate for blood collection from the wing vein. Blood samples were centrifuged at 3,000 rpm for 15 minutes, and serum was aliquoted and stored at -80°C . Serum

malondialdehyde (MDA) content and activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and lysozyme (LZM) were measured using assay kits from Nanjing Jiancheng Bioengineering Institute according to the manufacturer's instructions on a microplate reader (BioTek, USA).

1.3.3 Intestinal Microbe Counts Determination After blood collection, broilers were euthanized by anesthesia with sodium pentobarbital solution (30 mg/mL). The abdominal cavity was immediately opened, and the intestinal tract was removed and placed on ice. The digestive tract was ligated with sterile cotton thread at various segments to separate the duodenum, jejunum, ileum, and cecum. Contents from the middle sections of each segment were collected on ice under sterile conditions and mixed uniformly. Under sterile conditions, 0.5 g of the mixed content was transferred to a flask containing 4.5 mL sterile physiological saline and glass beads (10^{-1} dilution). After thorough mixing, 0.5 mL of this dilution was transferred to a tube containing 4.5 mL sterile physiological saline, and serial dilutions from 10^{-2} to 10^{-7} were prepared for microbial enumeration. *Lactobacillus*, *Bifidobacterium*, *Escherichia coli*, and *Salmonella* counts were determined using the plate count method.

1.3.4 Immune Organ Index Determination The spleen, thymus, and bursa of Fabricius were dissected from the euthanized broilers, with adherent tissues removed and blood blotted with filter paper before weighing fresh weight. Immune organ index was calculated using the following formula:

Immune organ index (%) = (Fresh weight of immune organ / Pre-slaughter fasting live weight) \times 100

1.4 Data Processing

All experimental data were analyzed using SPSS 20.0 software via one-way ANOVA. When significant differences were detected among groups, Tukey's HSD test was used for multiple comparisons. Significance was declared at $P < 0.05$, and data are presented as "mean \pm standard error."

2.1 Effects of Curcumin and *Bacillus licheniformis* on Growth Performance of Broilers

As shown in Table 2, for ADG, all experimental groups were significantly higher than the control group ($P < 0.05$). The D1 group exhibited significantly higher ADG than the D2 and D3 groups ($P < 0.05$) but showed no significant difference from the D4 group ($P > 0.05$). The D2 and D4 groups had significantly higher ADFI than the control, D1, and D3 groups ($P < 0.05$). For F/G, the D1 and D4 groups were significantly lower than the control group ($P < 0.05$), with the D1 group showing the lowest value but not differing significantly from the D4 group ($P > 0.05$). Regarding final weight, all four experimental groups were significantly

higher than the control group ($P < 0.05$), with the D1 and D4 groups increasing by 11.59% and 10.97%, respectively, and both being significantly higher than the D2 and D3 groups ($P < 0.05$).

Table 2 Effects of curcumin and *Bacillus licheniformis* on growth performance of broilers

2.2 Effects of Curcumin and *Bacillus licheniformis* on Serum Antioxidant Function of Broilers

Table 3 shows that serum MDA content was highest in the control group, being significantly higher than in the D2, D3, and D4 groups ($P < 0.05$). Serum LZM activity in the control and D1 groups was significantly lower than in the other groups ($P < 0.05$). The D1 group showed the lowest SOD activity, which did not differ significantly from the control group ($P > 0.05$), while the D2 group exhibited the highest SOD activity, significantly exceeding the control and D1 groups ($P < 0.05$). Compared with the control group, all experimental groups showed elevated serum GSH-Px activity, with the D2, D3, and D4 groups being significantly higher ($P < 0.05$).

Table 3 Effects of curcumin and *Bacillus licheniformis* on serum antioxidant indexes of broilers

2.3 Effects of Curcumin and *Bacillus licheniformis* on Intestinal Microbe Counts of Broilers

Table 4 demonstrates that all experimental groups had significantly higher intestinal *Lactobacillus* counts than the control group ($P < 0.05$), while the D2, D3, and D4 groups showed significantly higher *Bifidobacterium* counts ($P < 0.05$). The D1 group had the lowest *E. coli* and *Salmonella* counts, with no significant differences among the D2, D3, and D4 groups ($P > 0.05$).

Table 4 Effects of curcumin and *Bacillus licheniformis* on intestinal microbe counts of broilers

2.4 Effects of Curcumin and *Bacillus licheniformis* on Immune Organ Indexes of Broilers

Table 5 reveals that compared with the control group, all experimental groups showed varying degrees of improvement in immune organ indexes. However, only the D4 group exhibited significantly increased spleen and bursa of Fabricius indexes ($P < 0.05$, increasing by 27.27% and 57.14%, respectively), while other groups showed no significant changes ($P > 0.05$).

Table 5 Effects of curcumin and *Bacillus licheniformis* on immune organ indexes of broilers

Discussion

3.1 Effects of Curcumin and *Bacillus licheniformis* on Growth Performance of Broilers

The application of curcumin as a plant extract additive in animal feed has become increasingly common. In this study, the D4 group showed significantly improved ADFI and ADG compared with other groups, indicating that dietary supplementation with curcumin alone or in combination with *B. licheniformis* can enhance broiler growth performance and feed utilization efficiency. Zhu et al. [?] reported that curcumin reduced fat content in chicken thigh meat, increased overall weight gain, and decreased F/G. Hu et al. [?] found that dietary curcumin supplementation benefited feed intake, growth, meat quality, and egg production rate in chickens. Peng [?] and Yang et al. [?] suggested that curcumin may improve feed digestibility and intake by stimulating the production or enhancing the activity of gastrointestinal digestive enzymes, thereby promoting growth performance. Previous studies have indicated that stress conditions inhibit broiler development and reduce growth performance, while dietary curcumin can alleviate these negative effects. Zhang et al. [?] found that curcumin supplementation helped mitigate heat stress impacts on broilers, and Gowda et al. [?] demonstrated that different levels of curcuminoids significantly alleviated aflatoxin-induced reductions in broiler performance. Therefore, stress mitigation may represent an important mechanism through which curcumin improves broiler growth performance.

The results also showed that the D3 group had significantly higher ADG than the control group, indicating that *B. licheniformis* supplementation significantly improved growth performance. Manickam et al. [?] reported that dietary supplementation with bacilli increased 6-week body weight by 9.54% and reduced F/G by 7.45% in broilers under six weeks of age. This effect may be related to altered nitrogen metabolism, as Yang et al. [?] demonstrated that bacilli can regulate nitrogen metabolism, and Manabe et al. [?] reported that *B. licheniformis* can modulate the secretion of key rate-limiting enzymes in nitrogen metabolism, such as xanthine oxidase and glutamine synthetase. Dietary bacilli supplementation has also been shown to improve apparent protein and energy metabolism and enhance breast muscle water-holding capacity and tenderness [?]. In this study, the D2 and D3 groups had significantly lower ADG than the D1 group, while the D1 and D4 groups showed no significant difference, suggesting that the combined supplementation of curcumin and *B. licheniformis* was more effective than individual supplementation, possibly due to synergistic interactions, though the underlying mechanisms require further investigation.

3.2 Effects of Curcumin and *Bacillus licheniformis* on Serum Antioxidant Function of Broilers

Superoxide dismutase (SOD) plays a crucial role in maintaining the oxidant-antioxidant balance by scavenging superoxide anion radicals and protecting cells

from damage [?], while MDA content reflects the degree of lipid peroxidation and indirectly indicates cellular damage. This study demonstrated that dietary supplementation with curcumin and *B. licheniformis* significantly affected serum MDA content and activities of catalase (CAT), SOD, and GSH-Px. Hu et al. [?] reported that human consumption of curcumin significantly increased serum SOD and GSH-Px activities while reducing MDA content by 1.07%, confirming its antioxidant effects. Li et al. [?] found that dietary turmeric powder significantly increased serum SOD, CAT, and GSH-Px activities while reducing MDA content in fast-growing Lingnan yellow chickens, indicating improved antioxidant function. This may occur because curcumin can partially or completely reverse oxidative stress-induced depletion of intracellular glutathione (GSH), thereby enhancing antioxidant capacity [?].

Previous studies have shown that surface antigens or metabolites of bacilli can stimulate phagocytic activity of granulocytes, serving as immune enhancers that improve disease resistance. Benyacoub et al. [?] reported that bacilli can stimulate the immune system and enhance immune function. Duc et al. [?] suggested that bacilli act on lymphoid tissues of mesenteric lymph nodes after entering the intestine, increasing levels of immunoglobulin G (IgG) and secretory immunoglobulin A (sIgA).

3.3 Effects of Curcumin and *Bacillus licheniformis* on Intestinal Microbe Counts of Broilers

The intestinal microbiota constitutes a vital component of the avian digestive system, and its composition directly influences broiler growth performance and immune function. In this study, the D4 group showed no significant difference in harmful bacterial counts compared with the D1 group, while beneficial bacterial counts were slightly elevated, indicating that dietary supplementation with curcumin and *B. licheniformis* exerted probiotic effects and inhibited harmful microbial communities. Research has demonstrated that plant extracts possess strong antimicrobial properties. Wang [?] found that oregano essential oil exhibited potent antibacterial activity, preventing or limiting the growth of pathogenic microorganisms in the animal digestive system. Zheng et al. [?], Sun et al. [?], and Zhong et al. [?] reported that curcumin has broad-spectrum antibacterial effects, strongly inhibiting *Staphylococcus aureus*, *E. coli*, and *Salmonella*, thereby improving intestinal health.

Zhao et al. [?] reported that dietary supplementation with *Bacillus coagulans* inhibited *E. coli* growth and reduced mortality in broilers. Qi et al. [?] found that dietary *Bacillus subtilis* increased *Lactobacillus* counts in the cecum and maintained microbial balance. Teo and Tan [?] reported that dietary *B. subtilis* supplementation in corn-soybean meal diets reduced ileal *E. coli* counts. This effect may be attributed to the fact that pathogenic bacteria such as *E. coli* and *S. aureus* are primarily aerobic, while bacilli rapidly proliferate in the intestine in spore or vegetative form, consuming local oxygen and creating an anaerobic environment that prevents survival of harmful aerobic bacteria, thereby consol-

idating the dominance of beneficial microbes [?].

3.4 Effects of Curcumin and *Bacillus licheniformis* on Immune Organ Indexes of Broilers

Normal organ development and morphological integrity reflect an animal's health status. Fluharty et al. [?] found that dietary nutrient levels can influence relative organ weights. Zhang [?] reported that 50 and 100 mg/kg curcumin significantly increased bursa weight and bursa index in 21-day-old broilers, while 200 mg/kg curcumin significantly increased spleen weight and spleen index in 42-day-old broilers. Hu et al. [?] found that 250 mg/kg curcumin significantly increased thymus index but did not affect bursa or spleen indexes. This may be because high-density rearing conditions increase stress and accumulate oxygen free radicals, which damage biological membranes and impair nerves, tissues, and organs, while curcumin can scavenge free radicals and improve antioxidant function [?], thereby influencing immune organ development.

The current results showed that the D4 group had significantly higher spleen and bursa indexes than other groups, while thymus index did not increase significantly. Shi [?] and Sun et al. [?] reported that dietary *B. licheniformis* supplementation significantly increased thymus and bursa indexes in broilers, which differs slightly from our findings. This discrepancy may be because bacilli promote immune organ development and increase T and B lymphocyte populations in the spleen, stimulating humoral immunity through IgA, IgG, and IgM, thereby reaching adequate immune levels and preventing further increases in thymus index [?].

Conclusion

Dietary supplementation with curcumin and *Bacillus licheniformis*, either individually or in combination, can improve growth performance and immune function in broilers. The combined supplementation demonstrates superior effects compared with individual supplementation, indicating a synergistic interaction between the two additives.

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