Effects of Clostridium butyricum on Growth Performance, Antioxidant Capacity, Immune Function, and Serum Biochemical Indices in Broiler Chickens: Postprint

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Abstract

This experiment aimed to investigate the effects of Clostridium butyricum on growth performance, antioxidant capacity, immune function, and serum biochemical indices in Ross 308 broiler chickens. A total of 540 healthy 1-day-old Ross 308 broiler chickens were randomly divided into 3 groups with 6 replicates per group and 30 birds per replicate. The experimental groups were: control group, fed a basal diet without antibiotics; antibiotic group, fed the basal diet supplemented with 10 mg/kg colistin sulfate and 50 mg/kg zinc bacitracin; and Clostridium butyricum group, fed the basal diet supplemented with 3×108 CFU/kg Clostridium butyricum. The experimental period lasted 42 days. The results showed: 1) Compared with the control group, the body weight, average daily gain (ADG), and average daily feed intake (ADFI) of broiler chickens in both the Clostridium butyricum group and antibiotic group were significantly increased ($P<0.05$). 2) Compared with the control group, the serum glutathione peroxidase (GSH-Px) and total superoxide dismutase (T-SOD) activities in 42-day-old broiler chickens in the Clostridium butyricum group were significantly enhanced ($P<0.05$), with GSH-Px activity being 60.00% higher than that in the antibiotic group ($P<0.05$); the serum T-SOD activity in broiler chickens in the antibiotic group at 7 and 21 days of age was significantly higher than that in the control group ($P<0.05$). 3) At 21 days of age, the serum immunoglobulin A (IgA) content in broiler chickens in the Clostridium butyricum group and antibiotic group was increased by 28.70% ($P<0.05$) and 26.46% ($P<0.05$), respectively, compared with the control group. At 7, 21, and 42 days of age, the serum immunoglobulin G (IgG) content in broiler chickens in the Clostridium butyricum group was increased by 36.60% ($P<0.05$), 37.77% ($P<0.05$), and 27.03% ($P<0.05$), respectively, compared with the control group. At 7 and 42

days of age, the serum IgG content in broiler chickens in the antibiotic group was significantly increased compared with the control group (P<0.05). At 21 and 42 days of age, the serum immunoglobulin M (IgM) content in broiler chickens in the Clostridium butyricum group was significantly increased compared with the control group (P<0.05), and was 7.92% (P>0.05) and 47.62% (P<0.05) higher than that in the antibiotic group, respectively. 4) Compared with the control group, the serum total protein (TP) content in broiler chickens in the Clostridium butyricum group was significantly increased (P<0.05), and at 21 and 42 days of age, the TP content was 31.33% (P<0.05) and 52.27% (P<0.05) higher than that in the antibiotic group, respectively. Compared with the control group, the blood ammonia content in broiler chickens in the Clostridium butyricum group at 21 and 42 days of age was significantly decreased (P<0.05). In conclusion, dietary supplementation with Clostridium butyricum can enhance serum antioxidant capacity and strengthen immune function, promote protein metabolism, thereby improving growth performance in broiler chickens, and reduce ammonia emission from broiler chickens.

Full Text

Effects of Clostridium butyricum on Growth Performance, Antioxidant Capacity, Immune Function and Serum Biochemical Parameters of Broilers

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Abstract

This experiment was conducted to investigate the effects of *Clostridium butyricum* on growth performance, antioxidant capacity, immune function, and serum biochemical parameters of broilers. A total of 540 one-day-old healthy Ross 308 broilers were randomly assigned to 3 groups with 6 replicates per group and 30 birds per replicate. The dietary treatments consisted of: a control group fed a basal diet without antibiotics; an antibiotic group fed the basal diet supplemented with 10 mg/kg colistin sulfate and 50 mg/kg bacitracin zinc; and a *Clostridium butyricum* group fed the basal diet supplemented with 3×10 CFU/kg *Clostridium butyricum*. The trial lasted 42 days. The results showed: 1) Compared with the control group, body weight, average daily gain, and average daily feed intake were significantly increased in both the *Clostridium butyricum* and antibiotic groups (P<0.05). 2) The *Clostridium butyricum* group exhibited significantly enhanced serum glutathione peroxidase (GSH-Px) and total superoxide dismutase (T-SOD) activities at 42 days of age (P<0.05), with GSH-Px

activity 60.00% higher than that of the antibiotic group (P<0.05). The antibiotic group showed significantly increased serum T-SOD activity at 7 and 21 days of age compared with the control group (P<0.05). 3) At 21 days of age, serum immunoglobulin A (IgA) content in the *Clostridium butyricum* and antibiotic groups increased by 28.70% (P<0.05) and 26.46% (P<0.05), respectively, compared with the control group. Serum immunoglobulin G (IgG) content in the *Clostridium butyricum* group increased by 36.60% (P<0.05), 37.77% (P<0.05), and 27.03% (P<0.05) at 7, 21, and 42 days of age, respectively, compared with the control group. The antibiotic group also showed significantly increased serum IgG content at 7 and 42 days of age (P<0.05). Serum immunoglobulin M (IgM) content in the *Clostridium butyricum* group was significantly higher than the control group at 21 and 42 days of age (P<0.05), and 7.92% (P>0.05) and 47.62% (P<0.05) higher than the antibiotic group, respectively. 4) Serum total protein (TP) content in the *Clostridium butyricum* group was significantly elevated compared with the control group (P<0.05), with increases of 31.33% (P<0.05) and 52.27% (P<0.05) compared with the antibiotic group at 21 and 42 days of age, respectively. Serum ammonia content in the *Clostridium butyricum* group was significantly reduced at 21 and 42 days of age (P<0.05). These results indicate that dietary supplementation with *Clostridium butyricum* can enhance serum antioxidant capacity and immune function, promote protein metabolism, improve growth performance, and reduce ammonia emissions in broilers.

Keywords: broilers; *Clostridium butyricum*; growth performance; antioxidant capacity; immune function; serum biochemical parameters

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Introduction

With the increasing intensification of broiler production, the impact of stress on animal performance has become more pronounced. Oxidative stress, in particular, often leads to increased metabolic consumption in poultry, resulting in impaired growth and development, reduced meat quality, and in severe cases, various diseases that cause significant economic losses to the poultry industry. Consequently, investigating nutritional strategies to improve broiler growth and mitigate the negative effects of oxidative stress has become an important research priority.

Clostridium butyricum, also known as butyric acid bacteria, was approved by the Chinese Ministry of Agriculture in July 2009 as a new generation of microbial feed additive. Research has demonstrated that *Clostridium butyricum* provides

nutritional benefits, promotes metabolism, and maintains intestinal health in humans and animals, showing remarkable efficacy in treating inflammation, acute and chronic diarrhea, and irritable bowel syndrome caused by intestinal flora imbalance. However, few studies have reported on the effects of *Clostridium butyricum* on antioxidant capacity and immune function in broilers. Therefore, this experiment was designed to investigate the effects of dietary *Clostridium butyricum* supplementation on growth performance, antioxidant capacity, immune function, and serum biochemical parameters in broilers, providing a scientific basis for its application in animal production.

Materials and Methods

1.1 Experimental Material

The *Clostridium butyricum* preparation was provided by Zhejiang Huijia Biological Technology Co., Ltd., with a viable count of 10 CFU/kg.

1.2 Experimental Design

A total of 540 one-day-old healthy Ross 308 broilers with an average body weight of (42.59±0.13) g were randomly allocated to 3 groups with 6 replicates per group and 30 birds per replicate (half male and half female). The dietary treatments were: a control group fed a basal diet without antibiotics, an antibiotic group fed the basal diet supplemented with 10 mg/kg colistin sulfate and 50 mg/kg bacitracin zinc, and a *Clostridium butyricum* group fed the basal diet supplemented with 3×10 CFU/kg *Clostridium butyricum*. The basal diet was a powdered compound feed formulated according to the NRC (1994) nutrient requirements for broilers, with composition and nutrient levels shown in Table 1 . The trial lasted 42 days. Birds were raised on net floors with ad libitum access to feed and water, and managed according to conventional farm procedures and vaccination programs.

Table 1 Composition and nutrient levels of basal diets (air-dry basis), %

Items	1 to 21 days of age	22 to 42 days of age
Ingredients		
Corn	56.70	62.50
Soybean meal	30.00	25.00
Extruded soybean	5.00	5.00
Import fish meal	3.00	2.50
CaHPO	1.80	1.60
Limestone	1.20	1.20
NaCl	0.30	0.30
DL-Met	0.20	0.15
L-Lys	0.10	0.15

Items	1 to 21 days of age	22 to 42 days of age
Premix[1])	1.70	1.60
Total	**100.00**	**100.00**
Nutrient levels[2])		
ME/(MJ/kg)	12.50	12.80
CP	21.50	19.50
Lys	1.20	1.05
Met	0.50	0.40
Ca	1.00	0.90
AP	0.45	0.40

[1]) The premix provided the following per kg of diet: VA 1,500 IU, VD 200 IU, VE 10 IU, VK 0.5 mg, VB 0.01 mg, VB 3.0 mg, VB 1.5 mg, D-pantothenic acid 10 mg, folic acid 0.5 mg, nicotinic acid 30 mg, biotin 0.15 mg, Cu 8 mg, Fe 80 mg, Zn 40 mg, Mn 60 mg, Se 0.15 mg, I 0.18 mg.
[2]) ME was a calculated value, while the others were measured values.

1.3 Detection Indicators

At 1, 21, and 42 days of age, birds were weighed by replicate after fasting. Daily feed supply and residual feed were recorded throughout the experiment. At 7, 21, and 42 days of age, 6 birds with similar body weight were selected from each group, and blood samples were collected via jugular vein puncture. Serum was separated by centrifugation at 3,000 r/min for 15 min at 4 °C, and the supernatant was aliquoted into 1.5 mL Eppendorf tubes and stored at -20 °C. Serum total protein (TP), albumin (ALB), ammonia content, total antioxidant capacity (T-AOC), malondialdehyde (MDA) content, and activities of glutathione peroxidase (GSH-Px) and total superoxide dismutase (T-SOD) were measured. Serum immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM) contents were determined by enzyme-linked immunosorbent assay (ELISA). All assay kits were purchased from Nanjing Jiancheng Bioengineering Institute, and measurements were performed according to the manufacturer' s instructions.

1.4 Statistical Analysis

Data were analyzed using one-way ANOVA with SPSS 16.0 statistical software. Results are expressed as "mean ± standard error." Differences were considered significant at $P<0.05$.

Results

2.1 Growth Performance

The effects of *Clostridium butyricum* on broiler growth performance are presented in Table 2 . Compared with the control group, dietary supplementation with either antibiotics or *Clostridium butyricum* significantly increased body weight, average daily gain, and average daily feed intake at all growth stages ($P<0.05$), with no significant differences between the two treatment groups ($P>0.05$). The antibiotic group showed a significantly lower feed-to-gain ratio during 22-42 days of age compared with the control group ($P<0.05$). These results demonstrate that dietary *Clostridium butyricum* supplementation significantly improved broiler growth performance.

Table 2 Effects of *Clostridium butyricum* on growth performance of broilers

Items	Groups	Days of age	Control	Antibiotic	*Clostridium butyricum*
Body weight (BW), g		1	42.58±0.64	42.76±0.75	42.43±0.57
		21	733.83±10.74	778.18±26.62	764.13±20.61
		42	2,118.21±52.23	2,235.40±22.42	2,276.65±44.78
Average daily gain (ADG), g		1-21	32.92±0.20	35.02±0.50	34.37±0.39
		22-42	65.92±0.97	69.39±0.86	72.03±0.61
		1-42	49.42±0.48	52.21±0.53	53.20±0.43
Average daily feed intake (ADFI), g		1-21	50.53±0.42	52.92±0.54	52.17±0.51
		22-42	136.90±0.71	144.03±2.08	150.37±1.42
		1-42	93.55±0.30	97.64±1.37	100.44±0.97
Feed-to-gain ratio		1-21	1.54±0.01	1.51±0.01	1.53±0.01
		22-42	2.10±0.02	2.04±0.01	2.07±0.03
		1-42	1.91±0.03	1.87±0.01	1.89±0.02

In the same row, values with different small letter superscripts indicate significant difference (P<0.05), while values with the same or no letter superscripts indicate no significant difference (P>0.05). The same applies below.

2.2 Antioxidant Capacity

The effects of *Clostridium butyricum* on serum antioxidant capacity in broilers are shown in Table 3 . Compared with the control group, dietary *Clostridium butyricum* supplementation significantly increased serum GSH-Px and T-SOD activities at 42 days of age (P<0.05), with GSH-Px activity 60.00% higher than that of the antibiotic group (P<0.05). The antibiotic group showed significantly increased serum T-SOD activity at 7 and 21 days of age compared with the control group (P<0.05). No significant differences were observed in serum T-AOC and MDA content between the *Clostridium butyricum* group and the control group (P>0.05), although T-AOC showed an increasing trend. These findings indicate that dietary *Clostridium butyricum* supplementation can enhance serum antioxidant capacity in broilers.

Table 3 Effects of *Clostridium butyricum* on serum antioxidant capacity of broilers

Items	Groups	Days of age	Control	Antibiotic	*Clostridium butyricum*
GSH-Px (mol/L)		7	512.07±59.27	279.31±15.80	294.82±20.90
		21	752.06±20.16	463.79±66.41	806.21±50.51
		42	775.86±31.58	389.68±24.84	943.45±13.53
T-AOC (U/mL)		7	16.07±0.94	24.01±2.35	19.12±0.91
		21	14.68±1.11	18.96±0.95	19.82±2.79
		42	18.71±1.17	19.07±2.91	20.74±0.49
T-SOD (U/mL)		7	113.38±2.37	128.61±3.15	116.57±4.53
		21	174.38±5.86	198.46±4.19	183.08±8.70
		42	215.35±6.92	235.57±5.93	263.14±13.01
MDA (nmol/mL)		7	2.31±0.07	2.74±0.12	2.74±0.30
		21	2.56±0.07	2.55±0.12	2.53±0.09
		42	2.37±0.12	2.67±0.01	2.28±0.05

2.3 Immune Function

The effects of *Clostridium butyricum* on serum immune parameters in broilers are presented in Table 4 . At 21 days of age, serum IgA content in the *Clostridium butyricum* and antibiotic groups increased by 28.70% (P<0.05) and 26.46% (P<0.05), respectively, compared with the control group. Serum IgG content in the *Clostridium butyricum* group increased by 36.60% (P<0.05), 37.77% (P<0.05), and 27.03% (P<0.05) at 7, 21, and 42 days of age, respectively, compared with the control group. The antibiotic group also showed significantly increased serum IgG content at 7 and 42 days of age (P<0.05). Serum IgM content in the *Clostridium butyricum* group was significantly higher than the control group at 21 and 42 days of age (P<0.05), and 7.92% (P>0.05) and 47.62% (P<0.05) higher than the antibiotic group, respectively. These results demonstrate that *Clostridium butyricum* can increase serum immunoglobulin content in broilers.

Table 4 Effects of *Clostridium butyricum* on serum immune parameters of broilers (mg/mL)

Items	Groups	Days of age	Control	Antibiotic	*Clostridium butyricum*
IgA		7	3.84±0.01	3.12±0.07	3.60±0.07
		21	2.23±0.08	2.82±0.14	2.87±0.03
		42	1.98±0.18	1.75±0.07	2.23±0.17
IgG		7	2.35±0.11	2.96±0.09	3.21±0.10
		21	1.88±0.09	1.94±0.02	2.59±0.08
		42	1.48±0.06	1.92±0.11	1.88±0.06
IgM		7	1.77±0.05	1.69±0.08	1.56±0.09
		21	1.45±0.02	1.59±0.11	1.70±0.01
		42	1.28±0.18	1.26±0.05	1.86±0.05

2.4 Serum Biochemical Parameters

The effects of *Clostridium butyricum* on serum biochemical parameters in broilers are shown in Table 5 . Serum total protein (TP) content in the *Clostridium butyricum* group was significantly higher than the control group (P<0.05), with increases of 31.33% (P<0.05) and 52.27% (P<0.05) compared with the antibiotic group at 21 and 42 days of age, respectively. In contrast, serum TP content in the antibiotic group showed a decreasing trend with age, being 21.09% lower than the control group at 42 days of age (P<0.05). Serum ammonia content in the *Clostridium butyricum* group was significantly reduced at 21 and 42 days of age compared with the control group (P<0.05). No significant effect of *Clostridium butyricum* on serum albumin (ALB) content was observed (P>0.05), whereas the antibiotic group significantly decreased serum ALB content at 42 days of age (P<0.05). These results indicate that dietary *Clostridium butyricum*

supplementation can significantly increase serum TP content and reduce serum ammonia content in broilers.

Table 5 Effects of *Clostridium butyricum* on serum biochemical parameters of broilers

Items	Groups	Days of age	Control	Antibiotic	*Clostridium butyricum*
ALB (g/L)		7	15.30±0.59	15.73±0.49	16.15±0.16
		21	18.77±0.28	19.98±0.74	21.26±1.22
		42	18.76±0.48	14.21±0.42	19.86±1.49
TP (g/L)		7	38.89±0.94	48.56±0.94	50.57±0.75
		21	42.91±1.14	43.44±0.52	57.05±2.37
		42	52.68±1.14	41.57±0.25	63.30±1.47
Serum ammonia (mol/L)		7	465.38±18.83	437.20±21.28	450.55±11.82
		21	376.06±4.34	450.64±7.60	339.04±11.83
		42	289.41±15.24	349.63±12.75	216.11±16.75

Discussion

3.1 Effects of *Clostridium butyricum* on Broiler Growth Performance

Previous studies have investigated the effects of *Clostridium butyricum* on animal growth performance. Zhang et al. found that 1% *Clostridium butyricum* significantly increased average daily gain in weaned piglets, and when the dosage was increased to 3%, the feed-to-gain ratio decreased significantly. Meng et al. reported that simultaneous supplementation of *Clostridium butyricum* and *Bacillus subtilis* in growing-finishing pig diets significantly improved apparent digestibility of gross energy and nitrogen, resulting in significantly improved growth performance. Hua et al. also observed that various combinations of *Clostridium butyricum* with *Bacillus coagulans* and *Bacillus megaterium* significantly enhanced piglets' digestion and absorption of dietary protein and fiber, with the greatest improvement in apparent crude protein digestibility achieved when all three strains were added together. Liao et al. reported that different dosages of *Clostridium butyricum* significantly increased average daily gain in 1-21 day-old broilers, with no significant differences compared with the antibiotic group, and no significant differences in average daily feed intake or feed-to-gain

ratio among all treatment groups. Zhao et al. found that dietary *Clostridium butyricum* supplementation significantly increased average daily gain and average daily feed intake in broilers, significantly enhanced intramuscular fat content at 42 days of age, increased liver fatty acid synthase and breast muscle lipoprotein lipase activities, and accelerated fat deposition. However, Zhang et al. and Amerah et al. reported that dietary *Clostridium butyricum* supplementation had no significant effect on animal growth performance. These discrepancies suggest that the efficacy of probiotic preparations is influenced by factors such as strain type and fermentation level, management practices, animal health status, and supplementation dosage. The results of this study are consistent with those of Zhao et al., demonstrating that *Clostridium butyricum* can significantly increase average daily gain and average daily feed intake at all growth stages in broilers, with effects comparable to antibiotics.

The growth-promoting effects of *Clostridium butyricum* are closely related to its biological characteristics. As an anaerobic spore-forming bacterium, it can exist in spore form under adverse environmental conditions, exhibiting strong acid and bile salt tolerance that enables effective colonization in the intestine. Its primary metabolite, butyric acid, is the main energy source for colonic epithelial cells and plays a crucial role in maintaining intestinal morphological integrity and promoting intestinal cell proliferation and maturation. Butyric acid also stimulates pancreatic secretion, enhances jejunal brush border enzyme activity, promotes glucagon-like peptide-2 (GLP-2) production, and expands intestinal absorption area, thereby improving nutrient digestion and absorption capacity. In addition to butyric acid, *Clostridium butyricum* produces various enzymes such as amylase, glucosidase, and cellulase, as well as vitamins B and K during metabolism, all of which contribute to improved growth performance in livestock and poultry.

3.2 Effects of *Clostridium butyricum* on Serum Antioxidant Indices in Broilers

Under normal conditions, a dynamic balance exists between free radical generation and the antioxidant defense system in animals. However, when animals are diseased or subjected to stress, excessive free radical production causes oxidative damage. T-SOD and GSH-Px are important antioxidant enzymes that play crucial roles in scavenging superoxide radicals and peroxides and preventing or reducing hydroxyl radical formation. T-AOC is a comprehensive indicator of antioxidant function, while MDA content reflects the degree of lipid peroxidation mediated by oxygen free radicals. Yu et al. reported that *Bacillus subtilis* effectively protected broilers from oxidative damage by enhancing serum and liver T-SOD and GSH-Px activities, significantly increasing T-AOC and decreasing MDA content. Li et al. found that supplementation with 0.5% *Bacillus* in grass carp diets for 4 weeks significantly enhanced T-AOC and GSH-Px activity in liver and serum. The present study found that both antibiotics and *Clostridium butyricum* significantly enhanced serum GSH-Px and T-SOD activities in

broilers, with the promoting effect of *Clostridium butyricum* becoming more pronounced and stable than antibiotics with increasing age. These results indicate that *Clostridium butyricum*, like other probiotics, can improve antioxidant capacity in broilers, with effects superior to antibiotics and more beneficial for animal health.

Both *Clostridium butyricum* and its metabolites butyric acid and hydrogen possess antioxidant properties. *Clostridium butyricum* can produce T-SOD and NADH/NADPH peroxidase, directly scavenging reactive oxygen species. Zhang et al. demonstrated that sodium butyrate significantly enhanced catalase (CAT) activity in broiler breast muscle and significantly reduced MDA content, thereby alleviating oxidative stress induced by corticosterone. Ohsawa et al. first reported that hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals.

3.3 Effects of *Clostridium butyricum* on Broiler Immune Function

Immunoglobulins are important anti-infective substances in the body, primarily present in serum, containing specific and non-specific antibodies with various functions including antibacterial, antiviral, and antitoxin activities. Poultry contain three important immunoglobulins (IgA, IgG, and IgM). Yang et al. reported that dietary *Clostridium butyricum* supplementation (2×10 and 3×10 CFU/kg) significantly increased serum IgA and IgG contents in Lingnan yellow-feathered broilers during 14-42 days of age, and serum IgM content also increased significantly during 21-42 days of age. Zhang et al. demonstrated in a challenge study with *Escherichia coli* K88 that *Clostridium butyricum* significantly increased serum IgA and yolk immunoglobulin (IgY) contents on day 14 post-challenge, with IgM content reaching significant levels on day 21. The present study yielded similar results, showing that *Clostridium butyricum* had a much greater effect on serum immunoglobulin content than antibiotics, significantly altering serum IgA, IgG, and IgM contents, with particularly pronounced effects on IgG.

Furthermore, *Clostridium butyricum* modulates immune responses through other mechanisms, such as repairing damaged mucosa and controlling inflammatory bowel disease. Hayashi et al. found that *Clostridium butyricum* directly stimulated macrophage accumulation in inflamed intestinal sites and induced macrophage secretion of large amounts of the anti-inflammatory cytokine interleukin-10 (IL-10) through the Toll-like receptor 2/myeloid differentiation factor 88 (TLR2/MyD88) signaling pathway. Gao' s in vitro study also indicated that IL-10 is crucial for the immunomodulatory effects induced by *Clostridium butyricum*, which strengthens intestinal mucosal immune tolerance by significantly increasing IL-10 and heat shock protein 70 (HSP70) expression. However, unlike Hayashi et al., Gao found that *Clostridium butyricum* had no significant effect on MyD88 mRNA levels, suggesting that it activates the MyD88-independent signaling pathway mediated by TLR2.

3.4 Effects of *Clostridium butyricum* on Broiler Serum Biochemical Parameters

Elevated serum TP and ALB contents reflect vigorous protein metabolism, indicating improved absorption and utilization of amino acids and proteins, enhanced hepatic protein synthesis, increased tissue protein deposition, and improved animal performance. Ammonia in animals primarily originates from amino acid deamination and can also be produced by urease from intestinal bacteria. Low levels of blood ammonia are beneficial as it can participate in metabolism through the reverse reaction of deamination, whereas high ammonia levels are considered toxic both within the body and in the environment. Zhang et al. reported that dietary supplementation with *Bacillus subtilis* UBT-MO2 alone reduced ammonia emissions by 26.5% and hydrogen sulfide (H S) emissions by 7.9% in broilers; combinations of *Bacillus subtilis* with *Clostridium butyricum* and *Lactobacillus acidophilus* were also effective in reducing ammonia loss from excreta. Cao et al. found that *Clostridium butyricum* supplementation alone significantly reduced serum ammonia content in broilers. The present study yielded similar results, showing that compared with the control and antibiotic groups, *Clostridium butyricum* supplementation significantly increased serum TP content and significantly decreased serum ammonia content, with a trend toward increased ALB content. This may be because *Clostridium butyricum* entering the intestine ferments carbohydrates in the colon, producing large amounts of short-chain fatty acids that increase intestinal acidity and create a chemical barrier, inhibiting fermentation gas production by putrefactive bacteria and reducing harmful amine and ammonia production. Meanwhile, the low-acid environment favors the growth and proliferation of beneficial bacteria such as bifidobacteria, enhances digestive enzyme activity, and greatly improves nutrient absorption and utilization, particularly protein.

In conclusion, the results of this study demonstrate that dietary *Clostridium butyricum* supplementation can significantly improve broiler growth performance, enhance serum antioxidant capacity and immune function, promote protein metabolism, and help reduce ammonia emissions.

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