Effects of Composite Probiotic Preparation on Growth Performance, Slaughter Performance, and Immune Indices in Broiler Chickens: Postprint

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Abstract

Compound probiotic preparation; Genetically engineered bacteria; Growth performance; Slaughter performance; Immune indicators; Broiler chickens

Full Text

Effects of Compound Probiotics on Growth Performance, Slaughter Performance and Immune Indices of Broilers

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Abstract

This experiment was conducted to investigate the effects of different proportions of compound probiotics on growth performance, slaughter performance, and immune indices of Arbor Acres (AA) broilers. A total of 320 one-day-old healthy male AA broiler chicks were randomly allocated into 4 groups with 4 replicates per group and 20 broilers per replicate. Broilers in the control group (Group I) were fed a basal diet, while those in Groups II, III, and IV were fed the basal diet supplemented with 1,000 mg/kg of compound probiotics at different ratios. The experiment lasted for 42 days. The results showed: 1) During days 1–42, Group III exhibited the highest average daily feed intake and average daily gain, along with the lowest feed-to-gain ratio, which were significantly different from Group I ($P<0.05$). 2) The breast muscle percentage in Groups III and IV was significantly higher than that in Group I ($P<0.05$). 3) At both 21 and 42 days of age, serum immunoglobulin A content in Group III was significantly higher than in

Group I (P<0.05). These findings indicate that dietary supplementation with compound probiotics can improve growth performance, slaughter performance, and serum immunoglobulin content in broilers, with the most effective formulation being 1,000 mg/kg at a ratio of 1:2:1:1 (*Bacillus subtilis*:*Saccharomyces cerevisiae*:*Lactobacillus acidophilus*:*Bifidobacterium lactis*).

Keywords: compound probiotics; gene-engineering bacteria; growth performance; slaughter performance; immune indices; broilers

Introduction

With continuous improvements in living standards, animal husbandry has developed rapidly, and the safety of livestock products has received widespread attention. However, the extensive and irrational use of antibiotics, chemical drugs, and hormones in livestock production in China has seriously compromised the safety of animal products. Probiotic preparations are novel biological active agents composed of live microorganisms, mainly including lactic acid bacteria, bacilli, yeasts, and enterococci. *Bacillus subtilis* can secrete various peptides and peptide-derived antimicrobial substances. The *Bacillus subtilis* antimicrobial peptide used in this study is a novel antimicrobial peptide expressed in a genetically engineered *Bacillus subtilis* strain developed by fusing maggot antimicrobial peptide gene fragments using the splicing overlap extension PCR (SOEing PCR) method. Previous research has demonstrated that supplementing broiler diets with compound probiotics composed of *Bacillus pumilus*, *Lactobacillus plantarum*, and yeast can significantly reduce feed-to-gain ratio, mortality, and culling rates, though slaughter performance remains unchanged. Other studies have shown that feeding probiotics to chicks increases the number of immunoglobulin G (IgG), immunoglobulin M (IgM), and immunoglobulin A (IgA) producing cells in the bursa of Fabricius and spleen, indicating that probiotics can enhance humoral immune function in immune organs. Additionally, dietary supplementation with probiotic and antimicrobial peptide complexes has been found to significantly improve growth performance and immune function while reducing ammonia and hydrogen sulfide concentrations in poultry houses and decreasing mortality rates. While numerous studies have investigated probiotics or antimicrobial peptides individually, no reports have examined the combined use of probiotics and antimicrobial peptide-containing genetically engineered bacteria in broiler production. Therefore, this experiment utilized probiotics and antimicrobial peptide-containing genetically engineered bacteria as test materials, adding three different proportions of compound probiotics (determined through preliminary artificial gastrointestinal fluid tolerance tests) to AA broiler diets to evaluate their effects on growth performance, slaughter performance, and immune indices, thereby identifying the optimal formulation to provide data supporting practical application in the broiler industry.

Materials and Methods

1.1 Experimental Design and Materials

This experiment employed a single-factor completely randomized design. A total of 320 one-day-old healthy male AA broiler chicks (purchased from a hatchery in Dehui City, Jilin Province) were randomly divided into 4 groups with 4 replicates per group and 20 broilers per replicate. Initial body weight showed no significant differences among groups ($P>0.05$). The control group (Group I) received a basal diet, while Groups II, III, and IV received the basal diet supplemented with 1,000 mg/kg of compound probiotics at ratios of 2:1:1:1, 1:2:1:1, and 1:1:1.5:1.5 (*Bacillus subtilis*:*Saccharomyces cerevisiae*:*Lactobacillus acidophilus*:*Bifidobacterium lactis*), respectively. The experimental period lasted 42 days.

The compound probiotics used in this study were solid powders primarily composed of *Bacillus subtilis*, *Saccharomyces cerevisiae*, *Lactobacillus acidophilus*, and *Bifidobacterium lactis*, with total viable counts of 9.00×10 CFU/g for *B. subtilis*, 4.00×10 CFU/g for *S. cerevisiae*, 1.00×10^{1} CFU/g for *L. acidophilus*, and 1.00×10^{1} CFU/g for *B. lactis*. The *B. subtilis*, *S. cerevisiae*, and *L. acidophilus* strains were prepared in our laboratory, with *B. subtilis* being a genetically engineered strain expressing antimicrobial peptide CC34. *Bifidobacterium lactis* was provided by Shaanxi Sciphar Biotechnology Co., Ltd.

1.2 Experimental Diets

The basal diet was formulated according to the NRC (1994) *Nutrient Requirements of Poultry* and China' s *Feeding Standard of Chicken* (NY/T 33—2004). Feed ingredients were purchased from Daqing Hefeng Bayi Agricultural University Animal Technology Co., Ltd. The diet was in powder form, pulverized and mixed in our laboratory. The composition and nutrient levels of the basal diet are presented in Table 1 .

1.3 Management Practices

Before chick arrival, the poultry house was thoroughly cleaned and disinfected. Since the experiment was conducted in spring, preheating began two days before chick placement. Within two hours of arrival, chicks were provided with a low-concentration glucose solution (5%) at moderate temperature. Broilers were raised in cages at a stocking density of 20 birds/m^2 during days 1–21 and 10 birds/m^2 during days 22–42, with free access to water and feed. House temperature, humidity, and lighting duration were strictly controlled according to conventional management requirements, with adequate ventilation ensured. Water troughs were cleaned and disinfected daily before water replacement, and cooled boiled water was provided throughout the trial. Chicks were vaccinated against Newcastle disease and infectious bursal disease according to routine immunization procedures.

1.4 Measurements

1.4.1 Growth Performance Feed consumption was recorded weekly by replicate. Body weight was measured at 1, 7, 14, 21, 28, 35, and 42 days of age at 08:00 after 12 hours of fasting (with free access to water). Weekly body weight and feed intake per replicate were recorded to calculate average daily feed intake (ADFI, g), average daily gain (ADG, g), and feed-to-gain ratio (F/G) for each growth stage.

1.4.2 Slaughter Performance At 42 days of age, three healthy broilers with similar body weight were randomly selected from each replicate (12 birds per group), fasted for 12 hours (with free access to water), weighed, and slaughtered using conventional methods. Slaughter performance indices were calculated using the following formulas:

- Dressing percentage (%) = (carcass weight/live weight) × 100
- Semi-eviscerated percentage (%) = (semi-eviscerated weight/live weight) × 100
- Eviscerated percentage (%) = (eviscerated weight/live weight) × 100
- Breast muscle percentage (%) = (weight of both breast muscles/eviscerated weight) × 100
- Leg muscle percentage (%) = (weight of both leg muscles/eviscerated weight) × 100

1.4.3 Immune Indices At 21 and 42 days of age, three broilers were randomly selected from each replicate, and 5 mL of blood was collected from the heart. Serum was separated by centrifugation at 3,000 rpm for 10 minutes, transferred to 1.5 mL centrifuge tubes, and stored at -20°C for subsequent analysis. After blood collection, broilers were slaughtered and the spleen, thymus, and bursa of Fabricius were excised and weighed. Immune organ index was expressed as the relative weight of immune organs to body weight (g/kg BW). Serum IgG and IgA contents were determined by enzyme-linked immunosorbent assay (ELISA) using kits purchased from Suzhou Calvin Biotechnology Co., Ltd.

1.5 Statistical Analysis

Growth performance data were analyzed using replicate means (4 values per group), while slaughter performance and immune indices were analyzed using 12 samples per group. Data were initially processed using Excel 2007 and subjected to one-way ANOVA using SAS 8.0 software. Significant differences were further analyzed using Duncan' s multiple comparison test. Results were expressed as "mean ± standard deviation," with $P<0.05$ considered statistically significant.

Results

2.1 Effects of Compound Probiotics on Growth Performance

The effects of compound probiotics on ADFI are presented in Table 2 . No significant differences were observed among groups during days 1-14 and 36-42 (P>0.05). During days 15-21, ADFI in Group III was significantly higher than in other groups (P<0.05). During days 22-28, Groups III and IV showed significantly higher ADFI than other groups (P<0.05). During days 29-35, Groups II, III, and IV all exhibited significantly higher ADFI than Group I (P<0.05). Overall, during days 1-42, Group III had the highest ADFI, which was not significantly different from Group IV (P>0.05) but significantly higher than Group I (P<0.05).

The effects on ADG are also shown in Table 2. No significant differences were found among groups during days 1-7 (P>0.05). During days 8-42, ADG in Group III was significantly higher than in Groups I and IV (P<0.05), though during days 15-21, no significant difference was observed between Groups II and III (P>0.05). Overall, Group III achieved the highest ADG during days 1-42, significantly exceeding other groups (P<0.05) by 21.46%, 7.98%, and 9.97%, respectively.

The effects on feed-to-gain ratio are presented in Table 2. No significant differences were detected among groups during days 1-7 and 29-35 (P>0.05). During days 8-14 and 36-42, Group III showed significantly lower feed-to-gain ratio than other groups (P<0.05). During days 15-21, Groups III and IV had significantly higher feed-to-gain ratio than other groups (P<0.05). During days 22-28, Groups II and III exhibited significantly lower feed-to-gain ratio than Groups I and IV (P<0.05). Overall, no significant difference was observed between Groups II and III during days 1-42 (P>0.05), with Group III showing the lowest feed-to-gain ratio, significantly lower than Groups I and IV (P<0.05) by 9.25% and 7.10%, respectively.

Table 2 Effects of compound probiotics on growth performance of broilers

Items	Days of age	Group I	Group II	Group III	Group IV	P-value
ADFI (g)	1-14	22.86±0.48	23.77±0.86	23.08±0.66	23.30±0.48	-
	15-21	73.54±2.62	73.55±0.38	82.70±1.54	77.47±0.14	<0.0001
	22-28	116.96±9.29	114.32±0.52	129.33±0.36	128.06±0.26	-
	29-35	130.73±11.14	147.78±7.29	154.14±10.98	148.53±10.66	-
	36-42	156.88±4.19	165.29±7.47	166.63±5.78	165.97±1.30	-
	1-42	93.97±2.22	98.13±1.86	103.24±2.09	101.15±1.70	<0.0001
ADG (g)	1-7	12.21±0.52	12.45±0.60	12.86±0.63	12.85±0.73	-
	8-14	33.36±0.46	34.64±0.26	36.68±0.37	34.68±0.83	<0.0001

Items	Days of age	Group I	Group II	Group III	Group IV	P-value
	15–21	54.70±2.51	55.63±0.52	57.95±1.56	54.25±0.42	-
	22–28	68.68±2.52	76.73±5.10	88.63±6.98	76.98±0.40	<0.0001
	29–35	78.24±2.60	91.97±0.22	98.46±1.78	91.88±3.30	<0.0001
	36–42	78.16±2.08	94.57±5.67	100.63±1.17	88.77±3.44	<0.0001
	1–42	54.23±0.61	61.00±1.41	65.87±0.61	59.90±1.25	<0.0001
F/G	1–7	1.87±0.08	1.91±0.11	1.80±0.10	1.82±0.07	-
	8–14	1.88±0.08	1.85±0.01	1.73±0.02	1.83±0.04	-
	15–21	1.35±0.04	1.32±0.02	1.43±0.05	1.43±0.01	-
	22–28	1.71±0.16	1.49±0.09	1.47±0.11	1.66±0.01	-
	29–35	1.67±0.10	1.61±0.08	1.56±0.09	1.62±0.13	-
	36–42	2.01±0.03	1.75±0.03	1.66±0.05	1.87±0.06	<0.0001
	1–42	1.73±0.03	1.61±0.04	1.57±0.05	1.69±0.05	-

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

2.2 Effects of Compound Probiotics on Slaughter Performance

The effects of compound probiotics on slaughter performance are presented in Table 3 . Groups II, III, and IV showed varying degrees of improvement in dressing percentage, semi-eviscerated percentage, and eviscerated percentage compared with Group I, though differences were not significant (P>0.05). Breast muscle percentage in Groups III and IV was significantly higher than in Group I (P<0.05), increasing by 4.11% and 4.60%, respectively. No significant differences were observed in leg muscle percentage among groups (P>0.05), though Group IV achieved the highest value, showing improvements of 4.25%, 4.52%, and 4.30% compared with other groups.

Table 3 Effects of compound probiotics on slaughter performance of broilers (%)

Items	Group I	Group II	Group III	Group IV	P-value
Dressing percentage	92.27±0.68	93.08±1.07	93.28±1.16	92.91±0.68	-
Semi-eviscerated percentage	85.20±1.31	85.97±1.14	86.07±1.42	85.32±1.12	-

Items	Group I	Group II	Group III	Group IV	P-value
Eviscerated percentage	78.67±1.40	79.23±1.14	79.04±1.51	78.73±1.48	-
Breast muscle percentage	24.55±0.82	24.77±0.94	25.56±1.47	25.68±1.26	-
Leg muscle percentage	19.06±1.24	19.01±1.11	19.05±0.79	19.87±0.50	-

2.3 Effects of Compound Probiotics on Immune Indices

The effects of compound probiotics on immune organ indices are shown in Table 4 . At both 21 and 42 days of age, no significant differences were observed in spleen index, thymus index, or bursa of Fabricius index among groups ($P>0.05$), though Groups II, III, and IV showed higher values than Group I.

The effects on serum IgG and IgA contents are also presented in Table 4. No significant differences were found in serum IgG content among groups at either 21 or 42 days of age ($P>0.05$). However, Group III achieved the highest IgG content, showing increases of 48.54%, 21.24%, and 6.05% at 21 days, and 50.64%, 25.46%, and 22.54% at 42 days compared with other groups. At 21 days, no significant differences in serum IgA content were observed among Groups II, III, and IV ($P>0.05$), though Group III showed the highest value, significantly exceeding Group I ($P<0.05$) by 93.88%. At 42 days, Group III exhibited significantly higher IgA content than all other groups ($P<0.05$), with increases of 63.83%, 42.59%, and 30.51%, respectively.

Table 4 Effects of compound probiotics on immune indices of broilers

Items	Days of age	Group I	Group II	Group III	Group IV	P-value
Spleen index (g/kg)	21	0.96±0.26	1.05±0.26	1.04±0.030	0.97±0.42	-
	42	1.35±0.39	1.43±0.33	1.62±0.39	1.41±0.49	-
Thymus index (g/kg)	21	3.82±0.42	4.05±0.51	4.21±0.60	4.18±0.43	-
	42	3.32±1.13	3.45±0.66	3.73±0.74	3.84±0.77	-

Items	Days of age	Group I	Group II	Group III	Group IV	P-value
Bursa index (g/kg)	21	1.78±0.78	1.80±0.39	1.84±0.34	1.85±0.80	-
	42	0.99±0.49	0.99±0.32	1.02±0.45	0.99±0.57	-
IgG (g/L)	21	3.42±0.85	4.19±1.75	5.08±1.71	4.79±2.19	-
	42	3.14±0.89	3.77±1.27	4.73±2.20	3.86±2.15	-
IgA (g/L)	21	0.49±0.14	0.66±0.28	0.95±0.52	0.81±0.44	-
	42	0.47±0.12	0.54±0.12	0.77±0.26	0.59±0.24	-

Discussion

3.1 Effects of Compound Probiotics on Growth Performance

Both compound probiotics and antimicrobial peptides can improve animal performance, promote growth and development, and enhance feed conversion efficiency. The compound probiotics used in this study consisted of *Bacillus subtilis* (a genetically engineered strain containing antimicrobial peptide CC34), *Saccharomyces cerevisiae*, *Lactobacillus acidophilus*, and *Bifidobacterium lactis*. *Bacillus subtilis* is an aerobic bacterium, mostly beneficial, that primarily inhibits harmful aerobic bacteria by reducing free oxygen in the intestine, thereby creating an anaerobic environment favorable for obligate anaerobes such as lactobacilli and bifidobacteria. With a single-layer cell membrane, *B. subtilis* facilitates easy recovery and purification of secreted antimicrobial peptides. It also produces various digestive enzymes including lipases, proteases, and amylases, as well as non-starch polysaccharide enzymes such as cellulase and glucanase, which decompose multiple nutrients in the diet to provide abundant substrates for beneficial bacteria. Yeasts possess fermentative properties that promote digestion and accelerate beneficial bacterial growth. Yeast cells are rich in protein, fat, sugar, and B vitamins, which can be digested and absorbed by enzymes produced by bacilli to provide substantial nutrients. Additionally, yeasts can supplement selenium and enhance immunity. Lactic acid bacteria and bifidobacteria can synthesize vitamins required by animals and convert carbohydrates into organic acids such as lactic acid, acetic acid, propionic acid, and butyric acid, which reduce pH in the diet and animal intestine, activate digestive enzymes including trypsin, amylase, lipase, and total protease, promote excretion, and inhibit intestinal putrefaction.

Previous research has shown that dietary supplementation with 0.1% and 0.2% compound probiotics containing *Lactobacillus plantarum*, *L. acidophilus*, *Bacillus licheniformis*, *B. subtilis*, *Bacillus cereus*, and *S. cerevisiae* significantly increased average daily gain and survival rate while substantially decreasing feed-to-gain ratio, with the 0.1% level showing the best overall results. In the present study, during days 1-7, no significant differences were observed in ADFI, ADG, or feed-to-gain ratio among groups, likely due to stress from long-distance transportation, immature digestive systems in early growth stages, and the time required for adaptation to the environment and dietary changes, as well as for exogenous probiotics to colonize the intestine. The genetically engineered strain containing antimicrobial peptide CC34 used in this study was in powder form, and as an extracellular secretion type, required time to revive in the intestine before secreting antimicrobial peptides to promote broiler growth. Typically, feed-to-gain ratio is lowest in the first week and increases with age. The relatively high feed-to-gain ratio during the first two weeks in this experiment may be attributed to small chick size and the use of feed buckets placed inside cages, which caused significant feed wastage due to the birds' scratching behavior. From week 3 onward, hanging feeders were used, reducing feed wastage and lowering the feed-to-gain ratio. Throughout the entire 1–42 day experimental period, Groups II, III, and IV showed significantly increased ADFI and ADG and reduced feed-to-gain ratio compared with the control group. This may be because, after adaptation to the environment and diet, the compound probiotics, antimicrobial peptide-containing genetically engineered bacteria, and their various metabolites and antimicrobial peptides facilitated nutrient digestion and utilization, improved the intestinal microecological environment, and promoted healthy growth, thereby enhancing growth performance. Group III showed the best results, possibly because the double proportion of *S. cerevisiae* imparted a special aromatic flavor that stimulated the birds' olfactory senses, increasing appetite and ADFI. Additionally, yeast can enhance intestinal mucosal barrier function, reduce mucosal permeability, and improve intestinal mucosal nutrition, promoting nutrient digestion and utilization and thereby improving growth performance.

3.2 Effects of Compound Probiotics on Slaughter Performance

Dressing percentage, breast muscle percentage, and leg muscle percentage are important indicators for evaluating broiler meat production performance and key metrics for assessing slaughter performance. Previous studies have demonstrated that compound probiotics can increase dressing percentage, leg muscle percentage, and breast muscle percentage in 21–35-day-old broilers in a dose-dependent manner. In this experiment, dietary supplementation with compound probiotics improved slaughter performance by increasing semi-eviscerated percentage, eviscerated percentage, dressing percentage, breast muscle percentage, and leg muscle percentage to varying degrees. This may be because antimicrobial peptides not only promote growth but also regulate nutrient deposition in muscles. Additionally, all four probiotic strains can enhance protein synthesis in broilers, and fermentation of dietary components and bacterial metabolism in the gastrointestinal tract produce various short-chain fatty acids, thereby improving slaughter performance. Group IV, with

1.5 times the proportion of lactic acid bacteria, showed increased breast and leg muscle percentages compared with the control group, though the specific mechanism requires further investigation, consistent with findings reported by Guo Yuansheng.

3.3 Effects of Compound Probiotics on Immune Indices

Immune organs are sites for immune cell development, proliferation, and differentiation, and their development and functional strength play a decisive role in avian immune competence. Therefore, immune organ index is a reliable indicator of avian immune status. Avian immune organs primarily include the spleen, thymus, and bursa of Fabricius. Previous research has shown that supplementing broiler diets with lactic acid bacteria, butyric acid bacteria, and compound probiotics produces increasingly significant effects with continuous application and increasing bird age. In this study, dietary supplementation with different proportions of compound probiotics did not significantly affect immune organ indices but showed a trend toward improvement, consistent with Lin Qian et al.' s report that probiotics had no significant effect on immune organ indices in yellow-feathered broilers.

Serum immunoglobulin content is an important marker reflecting changes in animal immune function. IgG primarily mediates humoral immunity, and avian serum IgG possesses multiple activities against bacteria, viruses, and exotoxins, playing a crucial role in humoral immunity. IgA primarily participates in mucosal local immunity by binding to pathogenic microorganisms and preventing their adhesion to cell surfaces, thereby functioning in local anti-infection processes. Previous studies have indicated that appropriate supplementation with compound microecological preparations can increase serum IgG content, elevate secretory IgA (sIgA) content in cecal contents, enhance Newcastle disease hemagglutination inhibition antibody titers, and ultimately strengthen immunity. Other research has reported that compound probiotics can significantly increase thymus and bursa indices at 21 days and thymus index at 42 days, significantly elevate serum IgG content at 21 days and IgA content at 42 days, and significantly enhance peripheral blood T lymphocyte proliferation at 21 days. In this study, compound probiotics increased serum immunoglobulin content at both 21 and 42 days, with Group III showing the best results. This may be because yeast is rich in protein, B vitamins, and amino acids, which can enhance disease resistance in livestock and poultry. However, the double proportion of *S. cerevisiae* was only one contributing factor. Although probiotics exhibit good immunomodulatory effects, not all beneficial flora promotes animal immune defense systems. The compound probiotics used in this experiment were mixtures of probiotics and antimicrobial peptide-containing genetically engineered bacteria at different ratios, and synergistic interactions may exist among the strains. Given the limited research in this area, the specific mechanisms require further verification.

Conclusion

Dietary supplementation with different proportions of compound probiotics can improve growth performance, slaughter performance, and serum immunoglobulin content in broilers to varying degrees. The most effective formulation was 1,000 mg/kg at a ratio of 1:2:1:1 (*Bacillus subtilis*:*Saccharomyces cerevisiae*:*Lactobacillus acidophilus*:*Bifidobacterium lactis*).

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