Effects of Lactic Acid Bacteria and Yeast Composite Preparation on Growth Performance, Slaughter Performance, and Intestinal Health of Broiler Chickens (Postprint)

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Date: 2017-10-23T00:00:00+00:00

Abstract

This study aimed to investigate the effects of a lactic acid bacteria and yeast compound preparation (LS) and its combination with virginiamycin (VM) on growth performance, slaughter performance, and intestinal health of Arbor Acres (AA) broiler chickens. A total of 400 healthy 1-day-old AA male chicks were allocated into 4 groups according to the principle of similar body weight, with 5 replicates per group and 20 birds per replicate. The control group was fed a basal diet, while the experimental groups were fed experimental diets supplemented with 30 mg/kg VM (VM group), 15 mg/kg VM + 1,000 mg/kg LS (VM+LS group), and 1,000 mg/kg LS (LS group), respectively. The experimental period was 42 days. The results showed that: 1) The body weight of 42-day-old broilers in the VM, VM+LS, and LS groups was significantly higher than that in the control group ($P<0.05$); the average daily gain and feed conversion ratio of broilers aged 22-42 and 1-42 days in the VM+LS and LS groups were significantly better than those in the control group ($P<0.05$). 2) The breast muscle percentage and eviscerated carcass percentage of 42-day-old broilers in the VM+LS group were significantly higher than those in the control group ($P<0.05$). 3) Compared with the control group, the jejunal mucosal morphology of 21-day-old broilers in the LS group was significantly improved ($P<0.05$), and the duodenal villus height and villus height to crypt depth ratio were significantly increased ($P<0.05$); the duodenal and jejunal villus height and villus height to crypt depth ratio of 42-day-old broilers in the VM+LS and LS groups were significantly increased ($P<0.05$). 4) The cecal Escherichia coli count and Escherichia coli/Lactobacillus ratio of 42-day-old broilers in the VM+LS and LS groups were significantly lower than those in the control group ($P<0.05$). In conclusion, LS and its combined use with VM can both improve intestinal health and promote growth in broiler

chickens.

Full Text

Effects of Lactobacillus and Saccharomycetes Compound Preparation on Growth Performance, Slaughter Performance, and Intestinal Health of Broilers

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Abstract

This study investigated the effects of a Lactobacillus and Saccharomycetes compound preparation (LS) and its combination with virginiamycin (VM) on growth performance, slaughter performance, and intestinal health of Arbor Acres (AA) broilers. Four hundred one-day-old healthy AA male chicks were randomly allocated into four groups with five replicates each (20 birds per replicate). The control group received a basal diet, while experimental groups received basal diets supplemented with 30 mg/kg VM (VM group), 15 mg/kg VM + 1,000 mg/kg LS (VM+LS group), or 1,000 mg/kg LS (LS group). The 42-day trial yielded several key findings. First, body weight at 42 days of age was significantly higher in VM, VM+LS, and LS groups compared to the control ($P<0.05$), with VM+LS and LS groups showing superior average daily gain (ADG) and feed-to-gain ratio (F/G) during both 22–42 days and 1–42 days ($P<0.05$). Second, the VM+LS group exhibited significantly higher breast muscle yield and eviscerated yield at 42 days compared to the control ($P<0.05$). Third, LS supplementation significantly improved jejunal mucosal morphology at 21 days ($P<0.05$), increasing duodenal villus height and villus height-to-crypt depth ratio (V/C) while VM+LS and LS groups showed significantly enhanced duodenal and jejunal villus height and V/C values at 42 days ($P<0.05$). Fourth, VM+LS and LS groups had significantly lower cecal *Escherichia coli* counts and *E. coli*/Lactobacillus ratios at 42 days ($P<0.05$). These results demonstrate that LS alone or combined with VM improves intestinal health and promotes growth performance in broilers.

Keywords: probiotics; virginiamycin; broilers; growth performance; intestinal health

Introduction

In modern livestock production, antibiotics are widely used as growth promoters. However, due to concerns about antimicrobial resistance and drug residues, restricting or banning feed antibiotics has become an inevitable trend, making research on antibiotic alternatives a hot topic in animal nutrition with important implications for maintaining livestock health and ecological balance. The ability of Lactobacillus to adhere to intestinal mucosal surfaces is a prerequisite for antagonizing pathogenic microbial colonization and growth, and plays an important role in immune regulation and promoting repair of damaged mucosa. Lactobacillus and Saccharomycetes mutually promote growth through complementary metabolites and quorum sensing, enabling their compound preparation to grow better in intestinal environments than either organism alone and exert more complete probiotic effects. Dietary or water supplementation with Lactobacillus or Saccharomycetes significantly increases broiler body weight, reduces F/G, increases villus height and V/C values, enhances beneficial bacteria, and reduces harmful bacteria, with effects superior to antibiotic treatments. Nevertheless, research on Lactobacillus-Saccharomycetes compound preparations as antibiotic substitutes or in combination with antibiotics remains insufficient. This study therefore aimed to investigate the effects of such a compound preparation alone and combined with virginiamycin on growth performance, slaughter performance, and intestinal health of AA broilers, providing a theoretical basis for rational application of this probiotic preparation in broiler production.

Materials and Methods

1.1 Experimental Materials

The Lactobacillus and Saccharomycetes compound preparation contained *Lactobacillus* (BCRC 16092) at 2.5×10 CFU/g (actual measured 2.3×10 CFU/g) and *Saccharomycetes* (BCRC 20262) at 1.3×10 CFU/g (actual measured 1.3×10 CFU/g), provided by Xiamen Honghegu Biotechnology Co., Ltd. Virginiamycin (50% active ingredient) was purchased from Phibro Animal Health Corporation.

1.2 Experimental Design and Management

Four hundred healthy one-day-old AA male broilers with similar initial body weight [(40.3±5.0) g] were randomly divided into four groups with five replicates each (20 birds per replicate). The control group received a basal diet, while experimental groups received basal diets supplemented with 30 mg/kg virginiamycin (VM group), 15 mg/kg virginiamycin + 1,000 mg/kg LS (VM+LS group), or 1,000 mg/kg LS (LS group). Birds were raised in four-tier cage systems for 42 days, divided into starter (1–21 days) and finisher (22–42 days) phases. The basal diet composition and nutrient levels are shown in Table 1 , formulated according to NRC (1994), Chinese Chicken Feeding Standards

(NY/T 33‒2004), and the AA Broiler Management Guide. Diets were prepared as mash, then cold-pelleted (maximum temperature ~65°C) and rapidly cooled to minimize heat damage to the probiotic preparation before feeding as pellets.

Throughout the trial, birds had free access to feed and water. Lighting consisted of natural light plus artificial supplementation: 24 h daily for days 1-7, then 23 h from day 8 onward. Room temperature was maintained at 33°C for the first 3 days, then reduced by 2°C weekly until reaching 24°C, which was maintained thereafter. Management followed the AA Broiler Management Guide with routine vaccination and disinfection. Temperature and humidity were recorded continuously, and mortality was documented daily.

1.3 Measurement Indices and Methods

1.3.1 Growth Performance Daily observations recorded growth, morbidity, and mortality by replicate, including feed consumption. Birds were weighed by replicate after overnight fasting at 1, 21, and 42 days of age to calculate average body weight, average daily feed intake (ADFI), average daily gain (ADG), F/G, and mortality for periods 1-21, 22–42, and 1–42 days.

1.3.2 Slaughter Performance At 21 and 42 days, two broilers per replicate with body weight close to the replicate average were selected, slaughtered by jugular venous exsanguination, and dissected to separate breast muscle, leg muscle, and abdominal fat for weighing. Eviscerated yield, breast muscle yield, leg muscle yield, and abdominal fat percentage were calculated according to the National Poultry Breeding Commission methods:

- Eviscerated yield (%) = 100 × eviscerated weight / pre-slaughter weight
- Breast muscle yield (%) = 100 × breast muscle weight / eviscerated weight
- Leg muscle yield (%) = 100 × leg muscle weight / eviscerated weight
- Abdominal fat percentage (%) = 100 × abdominal fat weight / (eviscerated weight + abdominal fat weight)

Pre-slaughter weight was measured after 12-hour feed withdrawal.

1.3.3 Intestinal Mucosal Morphology At 21 and 42 days, one broiler per replicate with body weight close to the average was slaughtered to isolate duodenum, jejunum, and ileum. Middle segments (~1 cm) were gently rinsed with cold saline and fixed in pH 7.4 formalin at 4°C. Fixed tissues were processed for paraffin sections: dehydration → transparency → paraffin infiltration → embedding → discontinuous sectioning (6–8 μm thickness) → hematoxylin-eosin (HE) staining → mounting. From each intestinal segment, one slide was selected from every 20 sections (five slides per segment) and observed under light microscopy. Five non-consecutive fields per slide (with intact, straight villi) were randomly selected, with three measurements per field for villus height and crypt depth to calculate V/C values. Nine values were used for each metric. Slide preparation and measurements were performed by Beijing Jialanhai Biotechnology Co., Ltd.

1.3.4 Cecal Microbial Populations At 21 and 42 days, one broiler per replicate was fasted (with water) for 12 hours, weighed, euthanized by cardiac air injection, and cecal segments were collected and stored in liquid nitrogen. Plate colony counting detected cecal microbiota. Samples (1 g) were aseptically mixed with 9 mL sterile saline, serially diluted to 10 -10 , and 0.1 mL aliquots were spread on plates in triplicate. *E. coli* were cultured on EMB agar at 37°C for 24 h aerobically; Lactobacilli were cultured on MRS agar at 37°C for 48 h in CO . Plates with 30-300 colonies were counted, with results expressed as log CFU/g cecal content.

1.4 Statistical Analysis

Data are expressed as "mean ± standard deviation." SPSS 16.0 was used for analysis. Growth performance data were analyzed using the Repeated Measures procedure in General Linear Model. If sphericity test yielded $P>0.05$, one-way ANOVA was performed; if $P<0.05$, Multivariate analysis was used. Mortality was analyzed by chi-square test. Other indices were analyzed by one-way ANOVA among four treatments, with Duncan' s multiple comparison applied when significant differences were detected ($P<0.05$).

Results

2.1 Effects on Growth Performance

As shown in Table 2 , during days 1-21, ADG, ADFI, F/G, mortality, and 21-day body weight did not differ significantly among groups ($P>0.05$). During days 22-42, ADG was significantly higher and F/G significantly lower in VM+LS and LS groups compared to the control ($P<0.05$), with no significant difference between these two groups ($P>0.05$). Notably, LS group had significantly lower F/G than the VM group ($P<0.05$), while VM group did not differ from control or VM+LS groups ($P>0.05$). At 42 days, body weight was significantly higher in VM, VM+LS, and LS groups than the control ($P<0.05$), with VM+LS group significantly exceeding LS and VM groups ($P<0.05$). Over the entire 1-42 day period, ADG was significantly higher in all treatment groups versus control ($P<0.05$), with VM+LS group superior to VM and LS groups ($P<0.05$), while VM+LS and LS groups showed significantly lower F/G than control ($P<0.05$). These results indicate that 1,000 mg/kg LS can replace or partially replace antibiotics, with the combination of 15 mg/kg VM and LS showing superior growth-promoting effects compared to either treatment alone.

2.2 Effects on Slaughter Performance

Table 3 shows that dietary LS and its combination with VM did not significantly affect slaughter performance at 21 days ($P>0.05$). However, at 42 days, VM+LS group exhibited significantly higher eviscerated yield than the control ($P<0.05$), while LS group' s eviscerated yield did not differ significantly from control or VM+LS groups (P>0.05). Breast muscle yield was significantly higher in VM, VM+LS, and LS groups compared to control (P<0.05), with no significant differences among these three groups (P>0.05). These findings demonstrate that 1,000 mg/kg LS alone or combined with 15 mg/kg VM significantly improves slaughter performance in 42-day-old broilers.

2.3 Effects on Intestinal Mucosal Morphology

Table 4 reveals that at 21 days, LS supplementation significantly increased duodenal villus height and V/C value (P<0.05), significantly elevated jejunal villus height and V/C value while reducing crypt depth (P<0.05), and significantly improved ileal V/C value in VM+LS and LS groups (P<0.05). At 42 days, VM+LS and LS groups showed significantly higher duodenal and jejunal villus height and V/C values compared to control (P<0.05). The VM group did not differ significantly from control in intestinal morphology at either age (P>0.05). These results indicate that dietary supplementation with 15 mg/kg VM + 1,000 mg/kg LS or 1,000 mg/kg LS alone improves small intestinal mucosal morphology in 42-day-old broilers, with LS also showing beneficial effects at 21 days.

2.4 Effects on Cecal Microbial Populations

As presented in Table 5 , at 21 days, cecal *E. coli* and Lactobacilli counts and their ratio did not differ significantly among groups (P>0.05). At 42 days, however, VM+LS and LS groups exhibited significantly lower *E. coli* counts and *E. coli*/Lactobacillus ratios compared to control (P<0.05), though Lactobacilli counts did not differ significantly (P>0.05). The VM group showed no significant changes in cecal microbiota at either age (P>0.05). These findings demonstrate that 15 mg/kg VM + 1,000 mg/kg LS or 1,000 mg/kg LS alone improves intestinal microecology and promotes intestinal health.

Discussion

3.1 Effects on Growth Performance

Average daily gain and feed efficiency are crucial indicators of probiotic efficacy and economic viability. Previous research demonstrated that dietary Lactobacillus at 10 CFU/kg significantly increased 42-day body weight and reduced F/G during days 1-42, while Lactobacillus in drinking water significantly improved ADG and reduced F/G. Similarly, dietary live yeast at 1×10 CFU/g significantly increased broiler body weight, confirming that both organisms improve growth performance. Our results align with these findings, showing that 1,000 mg/kg LS significantly enhanced growth performance, with LS group achieving higher ADG and lower F/G during days 22–42 compared to the VM group.

Virginiamycin irreversibly inhibits bacterial protein synthesis, leading to bacterial death, and is typically used to prevent and treat Gram-positive infections. Limited research exists on combined probiotic-antibiotic use. Our study partially replaced VM with LS, finding that 15 mg/kg VM + 1,000 mg/kg LS produced significantly higher ADG during days 1–42 and greater 42-day body weight than 30 mg/kg VM alone, suggesting synergistic effects that warrant further investigation.

3.2 Effects on Slaughter Performance

Lactobacillus has been shown to significantly increase eviscerated yield and breast muscle yield in broilers. One study reported that 5,000 mg/kg Lactobacillus increased breast muscle yield by 7.15% and 4.38% compared to control and 100 mg/kg chlortetracycline groups, respectively. Research on combined probiotic-antibiotic effects on slaughter performance is scarce. Our results show that LS alone and LS+VM increased breast muscle yield by 4.33% and 4.57% compared to control, significantly improving slaughter performance at 42 days. While 5–20 mg/kg VM reportedly does not affect slaughter performance, our 30 mg/kg VM significantly increased breast muscle yield. Reducing VM to 15 mg/kg while adding 1,000 mg/kg LS significantly improved both eviscerated yield and breast muscle yield, suggesting potential synergistic effects requiring further study.

3.3 Effects on Intestinal Health

The intestine is the primary site for digestion and absorption in broilers. Increased villus height enhances digestive-absorptive function, while shallower crypt depth indicates more mature intestinal development. Elevated V/C values reflect improved intestinal function and mucosal structure. Numerous studies demonstrate that dietary Lactobacillus significantly increases villus height and V/C values while reducing crypt depth. Our findings are consistent, showing that 1,000 mg/kg LS significantly increased jejunal villus height and V/C values at both 21 and 42 days. Compared to 30 mg/kg VM, LS significantly improved intestinal morphology, indicating superior maintenance of intestinal structural health.

Virginiamycin kills bacteria by inhibiting ribosomal synthesis, while Lactobacillus protects intestinal mucosa by stimulating mucin-2 expression. Their combined action may synergistically improve intestinal health. Previous research showed that Lactobacillus significantly increased villus height and reduced crypt depth, while antibiotics did not significantly affect duodenal morphology. Similarly, our VM group showed no significant changes in intestinal morphology, whereas the VM+LS group exhibited significantly increased duodenal and jejunal villus height and V/C values at 42 days, demonstrating that combined treatment improves mucosal morphology and enhances nutrient absorption capacity, thereby improving growth performance.

Intestinal microbiota balance depends primarily on beneficial bacteria (Lactobacillus, Bifidobacterium) that produce bacteriocins and metabolites while competing for space and nutrients, preventing pathogen overgrowth and improving performance. Studies confirm that dietary Lactobacillus significantly increases cecal Lactobacillus counts. Our results show that LS significantly reduced cecal *E. coli* counts and *E. coli*/Lactobacillus ratios, consistent with previous research using 2,000 mg/kg plant-derived Lactobacillus. The VM group showed no significant changes in cecal *E. coli*, possibly because VM primarily stabilizes the anterior intestinal microbiota.

Virginiamycin kills some Gram-positive bacteria by inhibiting ribosomal synthesis, while Lactobacillus and Saccharomycetes reduce pathogenic bacteria through antagonism, interference, and barrier effects. Their combined action increases beneficial bacteria, reduces harmful bacteria, and improves intestinal health. Previous research demonstrated that 20 mg/kg VM significantly increased microbiota stability in the anterior small intestine. Our study shows that VM+LS combination significantly reduced cecal *E. coli* counts and *E. coli*/Lactobacillus ratios, indicating that 1,000 mg/kg LS + 15 mg/kg VM synergistically improves intestinal microbiota stability and absorption capacity, thereby enhancing broiler growth performance.

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