Effects of Lactobacillus acidophilus-Fermented Cottonseed Meal on Growth Performance, Slaughter Performance, and Serum Biochemical Indices of Yellow-Feathered Broilers (Postprint)

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Abstract

This experiment aimed to investigate the effects of Lactobacillus acidophilus fermented cottonseed meal on the growth performance, slaughter performance, and serum biochemical indices of yellow-feathered broilers. The experiment selected 180 healthy 21-day-old yellow-feathered broiler roosters, which were randomly divided into 3 groups: Group (control group), Group , and Group , with 6 replicates per group and 10 birds per replicate. The control group was fed a basal diet containing 6% cottonseed meal, Group was fed an experimental diet containing 6% Lactobacillus acidophilus fermented cottonseed meal, and Group was fed an experimental diet with 7$×10^{4}$ CFU/g of Lactobacillus acidophilus added to the basal diet containing 6% cottonseed meal. The experimental period was divided into two phases: 21–42 days of age and 43–64 days of age. The results showed: 1) During 21–42 days of age, compared with the control group, the average daily gain (ADG) of yellow-feathered broilers in Group increased by 8.61% (P<0.05), and the feed-to-gain ratio (F/G) decreased by 7.69% (P<0.05); there were no significant differences in ADG, average daily feed intake (ADFI), and F/G in Group (P>0.05). Compared with Group , the ADG of Group increased by 4.36% (P<0.05). During 43–64 days of age, compared with the control group, the ADG of Group increased by 12.32% (P<0.05); there were no significant differences in ADG, ADFI, and F/G in Group (P>0.05). Compared with Group , the ADG of Group increased by 7.58% (P<0.05). During 21–64 days of age, there were no significant differences in ADG, ADFI, and F/G among all groups (P>0.05). 2) At 42 days of age, compared with the control group, the dressing percentage, semi-eviscerated yield, and eviscerated yield of yellow-feathered broilers in Group increased by 2.99%, 7.55%, and 12.33%, respectively (P<0.05); there were no significant differences in slaughter

performance indices in Group (P>0.05). Compared with Group , the dressing percentage and semi-eviscerated yield of Group increased by 3.43% and 6.38%, respectively (P<0.05). At 64 days of age, compared with the control group, the dressing percentage, breast muscle percentage, and leg muscle percentage of Group increased by 5.27%, 8.92%, and 7.05%, respectively (P<0.05), and the semi-eviscerated yield increased by 6.84% (P<0.01); there were no significant differences in slaughter performance indices in Group (P>0.05). Compared with Group , the dressing percentage, semi-eviscerated yield, and breast muscle percentage of Group increased by 4.08%, 4.35%, and 5.16%, respectively (P<0.05). 3) At 42 days of age, compared with the control group, the serum total protein (TP) and albumin (ALB) contents of yellow-feathered broilers in Group increased by 10.06% and 12.60%, respectively (P<0.05), and the serum urea nitrogen (UN) content decreased by 46.76% (P<0.01); there were no significant differences in serum biochemical indices in Group (P>0.05). Compared with Group , the serum TP and ALB contents of Group increased by 9.58% and 13.10%, respectively (P<0.05), and the serum UN content decreased by 47.88% (P<0.01). At 64 days of age, compared with the control group, the serum calcium (Ca) content of Group increased by 11.74% (P<0.05), and the serum UN content decreased by 37.40% (P<0.01); there were no significant differences in serum biochemical indices in Group (P>0.05). Compared with Group , the serum UN content of Group decreased by 40.58% (P<0.01). In conclusion, dietary supplementation with fermented cottonseed meal can improve the growth performance and slaughter performance of yellow-feathered broilers, and enhance their digestion and absorption of nutrients such as protein, fat, and Ca.

Full Text

Effects of Cottonseed Meal Fermented by *Lactobacillus acidophilus* on Growth Performance, Slaughter Performance and Serum Biochemical Indices of Yellow-Feathered Broilers

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Abstract: This study investigated the effects of cottonseed meal fermented by *Lactobacillus acidophilus* on growth performance, slaughter performance, and serum biochemical indices in yellow-feathered broilers. A total of 180 healthy 21-day-old male yellow-feathered broilers were randomly allocated into three groups: Group (control), Group , and Group , with six replicates per group and ten broilers per replicate. The control group received a basal diet containing 6% cottonseed meal, Group received an experimental diet containing 6% fermented cottonseed meal, and Group received a basal diet containing 6% cottonseed meal supplemented with 7$×10^{4}$ CFU/g of *Lactobacillus aci-*

dophilus. The experiment consisted of two phases: 21–42 days and 43–64 days of age. The results showed: (1) During 21–42 days, compared with the control group, Group exhibited significantly increased average daily gain (ADG) by 8.61% (P<0.05) and decreased feed-to-gain ratio (F/G) by 7.69% (P<0.05), while Group showed no significant differences in ADG, average daily feed intake (ADFI), or F/G (P>0.05). Group ' s ADG was 4.36% higher than Group ' s (P<0.05). During 43–64 days, Group ' s ADG was significantly increased by 12.32% compared to the control (P<0.05), while Group showed no significant differences in ADG, ADFI, or F/G (P>0.05). Group ' s ADG was 7.58% higher than Group ' s (P<0.05). Over the entire 21–64 day period, no significant differences were observed among groups for ADG, ADFI, or F/G (P>0.05). (2) At 42 days, Group showed significantly increased dressing percentage, half-eviscerated yield, and eviscerated yield by 2.99%, 7.55%, and 12.33% respectively (P<0.05) compared to the control, while Group showed no significant differences in slaughter performance indices (P>0.05). Group ' s dressing percentage and half-eviscerated yield were 3.43% and 6.38% higher than Group ' s respectively (P<0.05). At 64 days, Group exhibited significantly increased dressing percentage, breast muscle percentage, and leg muscle percentage by 5.27%, 8.92%, and 7.05% respectively (P<0.05), and half-eviscerated yield was significantly increased by 6.84% (P<0.01), while Group showed no significant differences in slaughter performance indices (P>0.05). Group ' s dressing percentage, half-eviscerated yield, and breast muscle percentage were 4.08%, 4.35%, and 5.16% higher than Group ' s respectively (P<0.05). (3) At 42 days, Group showed significantly increased serum total protein (TP) and albumin (ALB) by 10.06% and 12.60% respectively (P<0.05), and significantly decreased serum urea nitrogen (UN) by 46.76% (P<0.01) compared to the control, while Group showed no significant differences in serum biochemical indices (P>0.05). Group ' s serum TP and ALB were 9.58% and 13.10% higher than Group ' s respectively (P<0.05), and UN was 47.88% lower (P<0.01). At 64 days, Group exhibited significantly increased serum calcium (Ca) by 11.74% (P<0.05) and significantly decreased UN by 37.40% (P<0.01), while Group showed no significant differences (P>0.05). Group ' s UN was 40.58% lower than Group ' s (P<0.01). These results demonstrate that dietary fermented cottonseed meal can improve growth and slaughter performance while enhancing nutrient digestion and absorption of protein, fat, and calcium in yellow-feathered broilers.

Keywords: fermented cottonseed meal; yellow-feathered broilers; growth performance; slaughter performance; serum biochemical indices

Introduction

Animal health is the prerequisite for efficient production, yet it faces constant threats from various adverse factors in livestock and poultry production. The mechanism by which animals resist these factors involves the immune system and immune responses. Consequently, improving poultry immunity, enhancing

disease resistance, maintaining health, and increasing production performance have long been major concerns for researchers. While antibiotics can improve poultry performance and have been widely used, their disadvantages have become increasingly apparent, leading many countries to strictly limit their use. Probiotics serve as an ideal antibiotic alternative, effectively increasing beneficial microorganisms in the animal intestine, enhancing intestinal epithelial barrier function, maintaining microecological balance in the digestive tract, improving production performance and immune function, and maintaining animal health.

Cottonseed meal, a byproduct of cottonseed pre-pressing and extraction, is rich in protein and metabolizable energy, making it a valuable feed resource. However, its application in livestock and poultry production is limited by antinutritional factors such as gossypol, cyclopropenoid fatty acids, and tannins. Fermenting cottonseed meal with probiotics can effectively degrade these antinutritional factors (e.g., free gossypol, fiber), improve nutritional value, and the enzymes secreted by these probiotics can degrade proteins into small molecular peptides [1-2]. Research has shown that fermentation can effectively reduce free gossypol content, improve essential amino acid utilization, enhance protein quality, and increase functional nutrients such as enzymes [3-5], thereby improving nutritional quality and enabling large-scale application in poultry production. Lactic acid bacteria are commonly used probiotics for fermenting cottonseed meal, which can reduce dietary pH, increase viable bacterial counts, improve intestinal pH, increase dominant microflora, inhibit harmful bacteria growth, prevent intestinal diseases, and enhance immune function [6].

Lactobacillus acidophilus is an important intestinal microorganism in humans and animals that maintains intestinal flora balance, improves production performance and immune function, exhibits strong acid tolerance, and can ferment glucose, fructose, lactose, and sucrose to produce organic acids such as lactic acid [7]. Lactic acid regulates intestinal pH and inhibits harmful microorganisms [8], playing a significant role in preventing pathogen proliferation. This study utilized *Lactobacillus acidophilus* to ferment cottonseed meal and investigated the effects of dietary fermented cottonseed meal on growth performance, slaughter performance, and serum biochemical indices in yellow-feathered broilers to provide a basis for its application in animal production.

Materials and Methods

1.1 Experimental Materials The fermentation substrate consisted of cottonseed meal and wheat bran provided by Xinjiang Tiankang Feed Technology Co., Ltd. The fermentation strain, *Lactobacillus acidophilus*, was provided by the Animal Nutrition Graduate Laboratory of Shihezi University. MRS solid and liquid culture media were purchased from Hangzhou Microbial Reagent Co., Ltd.

1.2 Preparation of Fermented Cottonseed Meal The fermentation substrate consisted of cottonseed meal and wheat bran at a 9:1 ratio. Fermentation conditions were as follows: 6 mL of *Lactobacillus acidophilus* inoculum (concentration: 2.52$×10^{6}$ CFU/mL) per 100 g substrate, initial moisture content of 40%, anaerobic fermentation at 37°C for 48 h in a digital electric incubator (HPX-9162MBE). After fermentation, the product was dried at 40°C, crushed, and passed through a 60-mesh sieve. Nutritional composition changes before and after fermentation are shown in .

1.3 Experimental Animals and Diets Three hundred 1-day-old male yellow-feathered broilers were fed commercial diets for 17 days, then fed the control diet for 3 days to adapt to the experimental powdered diet. From these, 180 healthy 21-day-old broilers with similar body weight were selected and randomly divided into three groups using a single-factor experimental design: Group (control), Group , and Group , with six replicates per group and ten broilers per replicate. Experimental diets were formulated according to NRC (1994) [9] standards. Diet composition and nutrient levels are presented in . The control group received a basal diet containing 6% cottonseed meal, Group received an experimental diet containing 6% fermented cottonseed meal (actual viable count: 7.00$×10^{4}$ CFU/g), and Group received a basal diet containing 6% cottonseed meal supplemented with 7.00$×10^{4}$ CFU/g *Lactobacillus acidophilus*, freshly prepared before feeding. The experimental period consisted of two phases: 21–42 days and 43–64 days of age. Broilers were housed in metabolic cages with free access to feed and water, and managed according to conventional procedures. The experiment was conducted at Xinjiang Tiankang Experimental Chicken Farm, which also provided the experimental broilers.

1.4.1 Growth Performance Body weight was measured at 21, 42, and 64 days of age (after overnight fasting), and feed consumption was recorded to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G) per replicate.

1.4.2 Slaughter Performance At 42 and 64 days of age, after 12 h of fasting (with free access to water), one broiler was randomly selected from each replicate, weighed, and slaughtered. Slaughter procedures followed methods described in *Poultry Production Science* [10]. Live weight, carcass weight, eviscerated weight, breast muscle weight, leg muscle weight, and abdominal fat weight were measured to calculate slaughter performance indices using the following formulas:

- Dressing percentage (%) = (carcass weight/live weight) × 100
- Half-eviscerated yield (%) = (half-eviscerated weight/live weight) × 100
- Eviscerated yield (%) = (eviscerated weight/live weight) × 100
- Breast muscle percentage (%) = (breast muscle weight/eviscerated weight) × 100

- Leg muscle percentage (%) = (leg muscle weight/eviscerated weight) × 100
- Abdominal fat percentage (%) = (abdominal fat weight + fat outside gizzard)/eviscerated weight × 100

1.4.3 Serum Biochemical Indices At 42 and 64 days of age, after 12 h of fasting (with free access to water), one broiler was randomly selected from each replicate, and 5 mL of blood was collected from the wing vein. Serum was separated by centrifugation at 2,500 rpm for 15 min, frozen in liquid nitrogen, and stored at -80°C for analysis. All biochemical indices were measured using kits from Nanjing Jiancheng Bioengineering Institute.

1.5 Statistical Analysis Experimental data were analyzed using one-way ANOVA in SPSS 13.0 software. Multiple comparisons among group means were performed using the LSD method. Results are expressed as mean ± standard deviation. $P<0.05$ was considered statistically significant, and $P<0.01$ was considered highly significant.

Results

2.1 Effects of Fermented Cottonseed Meal on Growth Performance As shown in , during 21–42 days, Group exhibited significantly increased ADG by 8.61% ($P<0.05$) and decreased F/G by 7.69% ($P<0.05$) compared to the control group, while ADFI increased by 0.58% ($P>0.05$). Group showed no significant differences in growth performance indices compared to the control ($P>0.05$), though ADG increased by 3.87% while ADFI and F/G decreased by 2.23% and 6.07% respectively. Compared with Group , Group showed significantly increased ADG by 4.36% ($P<0.05$) and increased ADFI by 2.80% ($P>0.05$), while F/G decreased by 1.75% ($P>0.05$).

During 43–64 days, Group exhibited significantly increased ADG by 12.32% ($P<0.05$) and increased ADFI by 2.64% ($P>0.05$) compared to the control, while F/G decreased by 8.62% ($P>0.05$). Group showed no significant differences in growth performance indices compared to the control ($P>0.05$), though ADG and ADFI increased by 3.81% and 1.21% respectively while F/G decreased by 2.07%. Compared with Group , Group showed significantly increased ADG by 7.58% ($P<0.05$) and increased ADFI by 1.40% ($P>0.05$), while F/G decreased by 7.17% ($P>0.05$).

Over the entire 21–64 day period, Group showed increased ADG by 6.24% and ADFI by 1.81% compared to the control, while F/G decreased by 4.20%. Group showed increased ADG by 2.33% while ADFI and F/G decreased by 0.19% and 2.29% respectively. Compared with Group , Group showed increased ADG by 3.68% and ADFI by 1.96%, while F/G decreased by 1.99%. However, no

significant differences were observed among groups for any growth performance indices during this period (P>0.05).

2.2 Effects of Fermented Cottonseed Meal on Slaughter Performance As shown in , at 42 days of age, Group exhibited significantly increased dressing percentage, half-eviscerated yield, and eviscerated yield by 2.99%, 7.55%, and 12.33% respectively (P<0.05) compared to the control group, while breast muscle percentage and leg muscle percentage increased by 4.05% and 7.57% respectively (P>0.05) and abdominal fat percentage decreased by 17.86% (P>0.05). Group showed no significant differences in slaughter performance indices compared to the control (P>0.05), though half-eviscerated yield, eviscerated yield, breast muscle percentage, and leg muscle percentage increased by 1.09%, 2.80%, 2.05%, and 4.91% respectively while abdominal fat percentage decreased by 3.95%. Compared with Group , Group showed significantly increased dressing percentage, half-eviscerated yield, and eviscerated yield by 3.43% (P<0.05), 6.38% (P<0.05), and 9.27% (P>0.05) respectively, while breast muscle percentage and leg muscle percentage increased by 1.97% and 2.54% respectively (P>0.05) and abdominal fat percentage decreased by 14.48% (P>0.05).

At 64 days of age, Group exhibited significantly increased dressing percentage, half-eviscerated yield, breast muscle percentage, and leg muscle percentage by 5.27% (P<0.05), 6.84% (P<0.01), 8.92% (P<0.05), and 7.05% (P<0.05) respectively compared to the control, while eviscerated yield increased by 6.97% (P>0.05) and abdominal fat percentage decreased by 17.37% (P>0.05). Group showed no significant differences in slaughter performance indices compared to the control (P>0.05), though dressing percentage, half-eviscerated yield, eviscerated yield, breast muscle percentage, and leg muscle percentage increased by 1.15%, 2.39%, 1.63%, 3.57%, and 2.27% respectively while abdominal fat percentage decreased by 19.74%. Compared with Group , Group showed significantly increased dressing percentage, half-eviscerated yield, and breast muscle percentage by 4.08% (P<0.05), 4.35% (P<0.05), and 5.16% (P<0.05) respectively, while eviscerated yield and leg muscle percentage increased by 5.26% and 4.67% respectively (P>0.05).

2.3 Effects of Fermented Cottonseed Meal on Serum Biochemical Indices As shown in , at 42 days of age, Group exhibited significantly increased serum total protein (TP), albumin (ALB), and calcium (Ca) by 10.06% (P<0.05), 12.60% (P<0.05), and 13.33% (P>0.05) respectively compared to the control, while serum phosphorus (P), total cholesterol (T-CHO), urea nitrogen (UN), and triglycerides (TG) decreased by 9.47% (P>0.05), 6.05% (P>0.05), 46.76% (P<0.01), and 15.87% (P>0.05) respectively. Group showed no significant differences in serum biochemical indices compared to the control (P>0.05), though serum TP, Ca, P, and UN increased by 0.45%, 5.24%, 1.58%, and 2.16% respectively while ALB, T-CHO, and TG decreased by 0.44%, 3.03%, and 20.63% respectively. Compared with Group , Group showed significantly increased serum TP and ALB by 9.58% (P<0.05) and 13.10% (P<0.05) respec-

tively, while serum P, T-CHO, and UN decreased by 10.88% (P>0.05), 3.12% (P>0.05), and 47.88% (P<0.01) respectively.

At 64 days of age, Group exhibited increased serum TP, ALB, and Ca by 8.74% (P>0.05), 12.20% (P>0.05), and 11.74% (P<0.05) respectively compared to the control, while serum P, T-CHO, UN, and TG decreased by 14.84% (P>0.05), 13.69% (P>0.05), 37.40% (P<0.01), and 15.09% (P>0.05) respectively. Group showed no significant differences in serum biochemical indices compared to the control (P>0.05), though serum TP, ALB, Ca, and UN increased by 1.45%, 2.36%, 1.30%, and 5.34% respectively while P, T-CHO, and TG decreased by 2.75%, 6.78%, and 7.55% respectively. Compared with Group , Group showed increased serum TP, ALB, and Ca by 7.18% (P>0.05), 9.60% (P>0.05), and 10.30% (P>0.05) respectively, while serum P, T-CHO, UN, and TG decreased by 12.43% (P>0.05), 7.41% (P>0.05), 40.58% (P<0.01), and 8.16% (P>0.05) respectively.

Discussion

3.1 Effects on Growth Performance Cottonseed meal contains antinutritional factors such as gossypol, cyclopropenoid fatty acids, and tannins that can reduce animal performance when used directly. Fermentation effectively degrades these factors. Zhang et al. [3] reported that fermentation can degrade up to 94.6% of free gossypol in cottonseed meal. Previous studies have shown that dietary inclusion of 15% fermented cottonseed meal had no significant effect on ADFI or F/G in broilers, likely due to effective gossypol degradation [11]. Our results demonstrate that dietary inclusion of 6% fermented cottonseed meal improved ADG and reduced F/G in yellow-feathered broilers, consistent with findings by Yan et al. [12]. The improved growth performance may be attributed to reduced free gossypol content, though the specific mechanisms require further investigation. Additionally, fermented cottonseed meal showed increased crude protein content, possibly due to carbohydrate consumption during fermentation and the presence of bacterial protein, which could also influence growth performance. Probiotics are dominant microorganisms in the intestinal tract that form a biological barrier with the intestinal mucosa, preventing pathogen invasion and controlling opportunistic pathogens through competitive exclusion, nutrient competition, and production of metabolites and bacteriocins, thereby maintaining microecological balance [13]. Fermenting cottonseed meal with probiotics not only improves its quality but also introduces viable probiotics into the diet. Our results showed that direct supplementation of *Lactobacillus acidophilus* in the 6% cottonseed meal diet also slightly improved growth performance, indicating that dietary *Lactobacillus acidophilus* promotes growth. However, the supplementation level was based on the concentration in the 6% fermented cottonseed meal diet and did not reach 10^5 CFU/g, which may explain the non-significant effect. Additionally, enzymes produced by *Lactobacillus acidophilus* in the diet may play an important role in nutrient

digestion and absorption.

3.2 Effects on Slaughter Performance Previous studies have reported effects of fermented cottonseed meal on slaughter performance in yellow-feathered broilers. Yan et al. [12] found that dietary inclusion of 3–6% fermented cottonseed meal significantly increased half-eviscerated yield, dressing percentage, breast muscle percentage, and leg muscle percentage. Liao et al. [14] reported that probiotics significantly increased dressing percentage, eviscerated yield, and breast muscle percentage while tending to reduce abdominal fat percentage, thereby improving slaughter performance. Our results show that dietary inclusion of 6% fermented cottonseed meal increased dressing percentage, half-eviscerated yield, eviscerated yield, breast muscle percentage, and leg muscle percentage while reducing abdominal fat percentage. Direct supplementation of *Lactobacillus acidophilus* in the 6% cottonseed meal diet also slightly improved slaughter performance, consistent with previous studies. These findings indicate that fermentation reduces antinutritional factor content and that dietary *Lactobacillus acidophilus* promotes growth, though fermented cottonseed meal has a greater effect on slaughter performance than *Lactobacillus acidophilus* alone. Furthermore, *Lactobacillus acidophilus* in fermented cottonseed meal may contribute to nutrient digestion and absorption, potentially affecting animal growth.

3.3 Effects on Serum Biochemical Indices Protein is one of the most important biological macromolecules in organisms, distributed throughout cells and performing diverse biological functions, with each cellular activity depending on specific proteins. Serum total protein (TP) is a protein source for the body and a complex of various proteins primarily used for tissue repair and energy supply, with its level serving as an indicator of protein metabolism intensity. Serum albumin (ALB) level reflects immune status [15]. Yan et al. [16] and Nie et al. [17] reported that fermented cottonseed meal increased TP and ALB levels in broiler serum. Our results show that dietary fermented cottonseed meal significantly increased serum TP and ALB at 42 days and tended to increase them at 64 days, consistent with previous studies, indicating enhanced protein metabolism and improved immunity. Urea nitrogen (UN) is a primary product of protein catabolism, and its level directly reflects protein metabolism status; reduced UN indicates enhanced protein synthesis, reduced catabolism, and improved nitrogen utilization [18]. Our results demonstrate that fermented cottonseed meal significantly reduced serum UN, indicating improved dietary protein utilization and nutrient absorption. Calcium and phosphorus are the two most abundant mineral elements in animals, with levels varying slightly by species, age, and nutritional status. Wu et al. [19] reported that organic acids produced during cottonseed meal fermentation reduce intestinal pH, facilitating Ca and P absorption in ionic form and increasing their serum levels. Our results show that fermented cottonseed meal increased serum Ca but did not increase P, which actually decreased slightly, consistent with Nie et al. [17], though the

mechanism requires further investigation. Reduced serum P may contribute to increased Ca levels [20]. Probiotics also affect nutrient metabolism. Our results show that direct *Lactobacillus acidophilus* supplementation slightly increased serum Ca, indicating that *Lactobacillus acidophilus* in fermented cottonseed meal influences Ca metabolism. Probiotics can increase serum lipase activity, which degrades excess fat into fatty acids and glycerol for energy consumption, inhibiting fat synthesis and deposition [15]. Our results show that probiotics in fermented cottonseed meal reduced serum T-CHO and TG levels, consistent with Hu et al. [21].

Conclusion

1. Dietary inclusion of 6% fermented cottonseed meal can improve growth performance and slaughter performance in yellow-feathered broilers while enhancing digestion and absorption of nutrients such as protein, fat, and calcium.
2. Direct supplementation of *Lactobacillus acidophilus* in a diet containing 6% cottonseed meal also has some promoting effects on growth and slaughter performance in yellow-feathered broilers.

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