

Effects of *Lactobacillus acidophilus*-Fermented Cottonseed Meal on Muscle Nutritional Composition and Flavor Characteristics of Yellow-Feathered Broilers (Postprint)

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Abstract

This study aimed to investigate the effects of *Lactobacillus acidophilus*-fermented cottonseed meal on the nutritional components and flavor characteristics of yellow-feathered broiler muscle. A total of 360 healthy 21-day-old yellow-feathered broiler roosters (449.66 ± 6.01 g) were randomly allocated into 6 groups with 6 replicates per group. The control groups (Groups 1, 2, and 3) were fed diets supplemented with 3%, 6%, and 9% cottonseed meal, respectively, while the experimental groups (Groups 4, 5, and 6) were fed diets supplemented with 3%, 6%, and 9% fermented cottonseed meal, respectively. After a 42-day feeding trial, 6 chickens were randomly selected from each group for slaughter and sample collection, and indices including proximate nutrients, fatty acids, amino acids, and inosine monophosphate content were measured and analyzed. The results showed: 1) Compared with the control groups, dietary supplementation with fermented cottonseed meal had no significant effect on the proximate nutrient content in yellow-feathered broiler muscle ($P > 0.05$); 2) The linoleic acid content in muscle of Group 4 was significantly higher than that of Group 1 by 13.91% ($P < 0.05$), the linolenic acid content in muscle of Group 4 was significantly higher than that of Group 1 by 21.51% ($P < 0.05$), and the polyunsaturated fatty acid content in muscle of Groups 4 and 5 was significantly higher than that of Groups 1 and 2 by 14.22% and 9.04%, respectively ($P < 0.05$); 3) The total free amino acid content in muscle of Group 4 was significantly higher than that of Group 1 by 13.85% ($P < 0.05$); the flavor amino acid (FAA) content in muscle of Groups 4 and 5 was significantly higher than that of Groups 1 and 2 by 6.04% and 9.27%, respectively ($P < 0.05$), and the FAA in muscle of Groups 4 and 5 was higher than that of Group 1 by 5.55% and 5.39%, respectively ($P < 0.05$); 4) The inosine monophosphate content in

muscle of Group was significantly higher than that of Group by 10.81% ($P < 0.05$), while no significant differences were observed among other groups ($P > 0.05$). In conclusion, dietary supplementation with fermented cottonseed meal can improve the flavor characteristics of yellow-feathered broiler muscle, with supplementation at 6% or 9% showing better effects.

Full Text

Effects of Cottonseed Meal Fermented by *Lactobacillus acidophilus* on Muscle Nutrients and Flavor Characteristics of Yellow-Feathered Broilers

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Abstract

This study investigated the effects of cottonseed meal fermented by *Lactobacillus acidophilus* on muscle nutrients and flavor characteristics of yellow-feathered broilers. Three hundred sixty 21-day-old healthy male yellow-feathered broilers with an average body weight of (449.66±\$6.01) g were randomly allocated to six groups with six replicates per group and ten broilers per replicate. Broilers in control groups (Groups , , and) were fed diets supplemented with 3%, 6%, and 9% cottonseed meal, respectively, while those in experimental groups (Groups , , and) received diets supplemented with 3%, 6%, and 9% fermented cottonseed meal, respectively. After a 42-day feeding trial, six broilers from each group were randomly selected for slaughter and sampling. Conventional nutrient content, fatty acids, amino acids, and inosinic acid in muscle samples were measured and analyzed. The results showed: (1) Compared with control groups, dietary supplementation with fermented cottonseed meal had no significant effect on conventional nutrient content in broiler muscle ($P > 0.05$). (2) The linoleic acid content in muscle of Group was significantly increased by 13.91% compared with Group ($P < 0.05$). The linolenic acid content in muscle of Group was significantly increased by 21.51% compared with Group ($P < 0.05$). The polyunsaturated fatty acid (PUFA) content in muscle of Groups and was significantly increased by 14.22% and 9.04%, respectively, compared with Groups and ($P < 0.05$). (3) The total free amino acid (TAA) content in muscle of Group was significantly increased by 13.85% compared with Group

($P < 0.05$). The flavor amino acid (FAA) content in muscle of Groups and was significantly increased by 6.04% and 9.27%, respectively, compared with Groups and ($P < 0.05$). The FAA content in Groups and was significantly increased by 5.55% and 5.39%, respectively, compared with Group ($P < 0.05$). (4) The inosinic acid content in muscle of Group was significantly increased by 10.81% compared with Group ($P < 0.05$), while no significant differences were observed among other groups ($P > 0.05$). These results indicate that dietary supplementation with fermented cottonseed meal can improve the flavor characteristics of yellow-feathered broiler muscle, with better effects observed at 6% or 9% inclusion levels.

Keywords: *Lactobacillus acidophilus*; fermented cottonseed meal; nutrient; flavor; yellow-feathered broilers

Introduction

With the rapid development of animal husbandry and continuous improvement of living standards, the proportion of animal-derived foods such as meat, eggs, and milk in human diets has increased substantially. However, the promotion of large-scale intensive farming has indirectly led to declines in the nutritional quality and flavor of animal products, while food safety issues have become increasingly prominent. Consequently, producing safe and high-quality livestock products has become a major research focus. Numerous studies have demonstrated that probiotics and probiotic-fermented feeds possess unique nutritional and physiological properties, and their application in livestock production can improve growth performance, slaughter characteristics, digestion and metabolism, meat quality, and immunity to varying degrees, serving as effective antibiotic alternatives and providing new solutions to these challenges. Fermented cottonseed meal is a novel biological protein feed produced through solid-state fermentation of cottonseed meal using probiotics. Currently, research on the effects of dietary fermented cottonseed meal supplementation on muscle nutrient content and flavor characteristics of yellow-feathered broilers remains limited. Both domestic and international studies have identified intramuscular fat, fatty acids, amino acids, and inosinic acid as important indicators closely related to muscle nutritional properties. Therefore, this study focused on these indicators to investigate the effects of *Lactobacillus acidophilus*-fermented cottonseed meal on muscle nutrients and flavor characteristics of yellow-feathered broilers, providing an important reference for developing new functional protein feeds and producing high-quality livestock products.

Materials and Methods

1.1 Experimental Materials

Fermentation strain: High acid-producing *Lactobacillus acidophilus* (J-5-1409) was selected and preserved by the Animal Nutrition Laboratory, College of Animal Science and Technology, Shihezi University.

Feed ingredients: Cottonseed meal, wheat bran, and other feed ingredients were provided by Xinjiang Tiangkang Animal Husbandry Biotechnology Co., Ltd.

1.2 Preparation of Fermented Cottonseed Meal

The preparation process for *Lactobacillus acidophilus*-fermented cottonseed meal followed the optimized fermentation parameters from Wu et al. with minor modifications. The fermentation substrate was prepared by mixing cottonseed meal and wheat bran at a 9:1 ratio. The substrate moisture content was adjusted to 40% and thoroughly mixed before high-temperature sterilization (0.056 MPa, 121°C, 30 min). After cooling to room temperature, the substrate was inoculated with J-5-1409 secondary culture (viable count 2.52×10^8 CFU/mL) at 6% inoculation rate. The mixture was stirred evenly, compacted, sealed with plastic wrap, and subjected to solid-state fermentation in a water-jacketed electric thermostatic incubator (HPX-962MBE) at 37.5°C for 48 hours. After fermentation, the fermented cottonseed meal was removed, dried in an oven at 40°C, bagged, and stored for later use. The main nutrient changes in cottonseed meal before and after fermentation are shown in Table 1 .

1.3 Experimental Design and Diets

1.3.1 Experimental Design The yellow-feathered broiler breed used was Guangxi Sanhuang chicken, purchased from Xinjiang Tiangkang Animal Husbandry Biotechnology Co., Ltd. A total of 360 healthy 21-day-old male yellow-feathered broilers with similar body weight (449.66 ± 6.01 g) were selected and randomly allocated to six groups according to a single-factor completely randomized design, with six replicates per group and ten broilers per replicate. Groups , , and served as control groups with dietary inclusion of 3%, 6%, and 9% non-fermented cottonseed meal, respectively. Groups , , and served as experimental groups with dietary inclusion of 3%, 6%, and 9% fermented cottonseed meal, respectively.

1.3.2 Experimental Diets The basal diets were formulated according to the NY/T 33-2004 “Feeding Standard of Chicken” and NRC (1994) nutrient requirements for broilers, including starter diets (21-42 days) and finisher diets (43-63 days). The diet composition and nutrient levels of each group are shown in Table 2 .

1.3.3 Broiler Management All experimental broilers were housed in the same chicken house and raised in double-deck cages with six broilers per cage. Standardized broiler feeding and management procedures were followed for a 42-day feeding period. Feed was added three times daily, with free access to feed and water.

1.4 Sample Collection and Measurements

At 64 days of age, six yellow-feathered broilers were randomly selected from each group. Left breast muscle samples (50-80 g) were collected from each broiler according to GB/T 9695.19-2008. All samples were frozen in liquid nitrogen and stored at -80°C until analysis.

1.4.1 Determination of Muscle Conventional Nutrient Content Muscle moisture content was determined by direct drying method according to GB/T 9695.15-2008 “Determination of Moisture Content in Meat and Meat Products.” Crude protein content was measured using an automatic Kjeldahl analyzer (FOSS Kjeltex 8400) according to GB/T 5009.5-2010. Crude fat content was determined by Soxhlet extraction method according to GB/T 5009.6-2003. Crude ash content was measured according to GB/T 9695.15-2008.

1.4.2 Determination of Muscle Fatty Acid Content Appropriate amounts of samples were freeze-dried (Telstar/LyoQuest, -40°C, 7 h) and ground finely in a mortar. The remaining procedures followed GB/T 9695.2-2008 “Determination of Fatty Acids in Meat and Meat Products.” Analysis was performed using an Agilent 7890A GC-5975 instrument. Fatty acids were identified by comparing retention times with standard samples, and results were expressed as the percentage of individual fatty acid peak area to total methylated fatty acid peak area. Saturated and unsaturated fatty acid contents were calculated from fatty acid composition.

1.4.3 Determination of Muscle Amino Acid Content Muscle amino acid content was determined according to GB/T 5009.124-2010 “Determination of Amino Acids in Foods” using an automatic amino acid analyzer (Hitachi L-8900).

1.4.4 Determination of Muscle Inosinic Acid Content Muscle inosinic acid content was determined by high-performance liquid chromatography (HPLC) (Agilent 1200) according to the method of Zhao et al. [9].

1.5 Statistical Analysis

Data were analyzed using one-way ANOVA in SPSS 22.0 software. Duncan's multiple comparison test was used for comparisons among groups with different inclusion levels, while T-tests were used for comparisons between fermented and non-fermented cottonseed meal groups at the same inclusion level. All data were expressed as means and standard errors, with $P < 0.05$ as the significance level.

Results

2.1 Effects of Fermented Cottonseed Meal on Muscle Conventional Nutrient Content

As shown in Table 3 , dietary supplementation with different proportions of fermented cottonseed meal had no significant effects on conventional nutrient content in yellow-feathered broiler muscle ($P>0.05$). The moisture and crude fat contents in the three experimental groups supplemented with 3%, 6%, and 9% fermented cottonseed meal showed an upward trend compared with their respective control groups at the same inclusion levels, but the differences were not significant ($P>0.05$).

2.2 Effects of Fermented Cottonseed Meal on Muscle Fatty Acid Content

As shown in Table 4 , fermented cottonseed meal had the most significant effect on polyunsaturated fatty acids (PUFA) in yellow-feathered broiler muscle. The PUFA content in muscle of the three experimental groups supplemented with 3%, 6%, and 9% fermented cottonseed meal increased by 14.22% ($P<0.05$), 9.04% ($P<0.05$), and 3.48% ($P>0.05$), respectively, compared with their respective control groups. Additionally, significant differences in PUFA content existed among the three cottonseed meal groups ($P<0.05$), with the 9% inclusion group showing a 17.95% increase compared with the 3% group ($P<0.05$). Among the three fermented cottonseed meal groups, the 9% group had the highest PUFA content, followed by the 6% group, with the 3% group being the lowest, though no significant differences were detected among them ($P>0.05$).

2.3 Effects of Fermented Cottonseed Meal on Muscle Amino Acid Content

As shown in Table 5 , dietary supplementation with different proportions of fermented cottonseed meal significantly improved several amino acids in 64-day-old yellow-feathered broiler muscle, including aspartic acid (Asp), glutamic acid (Glu), lysine (Lys), and arginine (Arg), with Asp and Glu being the primary flavor amino acids (FAA). For Asp content, the 3%, 6%, and 9% fermented cottonseed meal groups showed increases of 2.86% ($P>0.05$), 5.14% ($P>0.05$), and 10.68% ($P<0.05$), respectively, compared with their respective control groups. For Glu content, the corresponding increases were 1.83% ($P>0.05$), 7.36% ($P<0.05$), and 7.34% ($P<0.05$). Significant differences were also observed among the three fermented cottonseed meal groups ($P<0.05$), with the 6% and 9% groups showing 4.79% and 5.09% increases, respectively, compared with the 3% group, though no significant difference existed between the 6% and 9% groups ($P>0.05$). For glycine (Gly) content, increases of 2.06%, 3.77%, and 12.90% were observed in the three fermented groups, respectively, but these differences were not significant ($P>0.05$). Among the three cottonseed meal groups, the 6% group was significantly higher than

the 9% group ($P < 0.05$) but did not differ significantly from the 3% group ($P > 0.05$). For Lys content, the fermented groups showed increases of 5.39% ($P > 0.05$), 9.76% ($P < 0.05$), and 7.54% ($P < 0.05$), respectively, with the 6% group having the highest Lys content, though not significantly different from the 3% and 9% fermented groups ($P > 0.05$). For Arg content, the fermented groups showed increases of 5.30% ($P > 0.05$), 4.79% ($P > 0.05$), and 13.10% ($P < 0.05$), respectively, with the 9% group having the highest Arg content, though not significantly different from the 3% and 6% groups ($P < 0.05$). For total free amino acid (TAA) content, the fermented groups showed increases of 3.91% ($P > 0.05$), 7.60% ($P > 0.05$), and 13.85% ($P < 0.05$), respectively. For FAA content, the 3% fermented group showed a 2.04% increase compared with the 3% cottonseed meal group ($P > 0.05$), while the 6% and 9% fermented groups showed significant increases of 6.04% and 9.27%, respectively ($P < 0.05$). Among the three fermented groups, the 6% and 9% groups were 5.55% and 5.39% higher than the 3% group ($P < 0.05$), with no significant difference between the 6% and 9% groups ($P > 0.05$).

2.4 Effects of Fermented Cottonseed Meal on Muscle Inosinic Acid Content

As shown in Table 6, dietary supplementation with fermented cottonseed meal affected inosinic acid content in yellow-feathered broiler muscle to varying degrees. The 9% fermented cottonseed meal group showed a significant 10.81% increase in muscle inosinic acid content compared with the 9% cottonseed meal group ($P < 0.05$), while the 6% fermented group showed a 7.45% increase that was not significant ($P > 0.05$). No significant differences were observed among the three fermented cottonseed meal groups ($P > 0.05$), though the 9% group had the highest inosinic acid content, followed by the 6% group, with the 3% group being the lowest.

Discussion

3.1 Effects of Fermented Cottonseed Meal on Muscle Conventional Nutrient Content

Conventional nutrients in meat are closely related to meat quality and flavor, with crude fat content receiving particular attention from researchers worldwide. Studies have shown that intramuscular fat directly affects meat quality attributes, including tenderness, flavor, and juiciness. Intramuscular fat is not only a physical factor contributing to meat moisture and juiciness but also an important precursor for flavor compound formation, making it a key indicator for meat quality evaluation. Previous research has demonstrated that *Lactobacillus* can significantly affect fatty acid metabolism in vivo, thereby enhancing fat deposition and distribution while reducing fat oxidation in pork and increasing intramuscular fat content. Li et al. found that dietary supplementation with *Lactobacillus* in broilers had no significant effects on breast muscle nutrients but showed an upward trend in muscle moisture and fat content. Mi et

al. reported that feeding fermented diets to finishing pigs resulted in higher intramuscular fat content compared with the control group, though the difference was not significant. Studies by Ma and Zhang et al. also confirmed that feeding probiotic-fermented diets could significantly promote intramuscular fat deposition and improve pork flavor. Huang et al. found that compound probiotics significantly increased crude protein and ash contents in broiler muscle while significantly decreasing crude fat and cholesterol contents. The present study showed that dietary fermented cottonseed meal supplementation had no significant effects on conventional nutrient content in yellow-feathered broiler muscle, with moisture and crude fat contents showing an upward trend and crude protein content showing a downward trend. These results are consistent with findings by Li et al. and Mi et al. but differ from those of Huang et al., possibly due to variations in broiler breed, grouping, diet formulation, and complex metabolites produced after feed fermentation.

3.2 Effects of Fermented Cottonseed Meal on Muscle Fatty Acid Content

Fatty acids are important indicators for evaluating meat quality and flavor, and their composition in muscle plays a decisive role in fat tissue hardness and muscle oxidative stability, thereby affecting muscle taste, flavor, and color. Fatty acids include saturated fatty acids (SFA) and unsaturated fatty acids (UFA), with UFA further divided into monounsaturated fatty acids (MUFA) and PUFA. PUFA is one of the important precursors determining the intensity of meat aroma, making its detection essential in meat quality and flavor research. This study focused on six conventional fatty acids: stearic acid (C18:0), palmitic acid (C16:0), oleic acid (C18:1), palmitoleic acid (C16:1), linoleic acid (C18:2), and linolenic acid (C18:3), as these constitute approximately 90-95% of total fatty acids in poultry meat. Research has shown that probiotics and fermented diets can regulate fatty acids in animal muscle and improve meat flavor. Endo et al. found that adding a mixed probiotic containing *Lactobacillus*, *Bacillus*, and yeast at 3 g/kg to broiler diets significantly increased SFA and UFA contents and significantly improved linolenic acid content in broiler muscle. Li et al. fed finishing pigs with fermented diets containing *Lactobacillus* and *Bacillus subtilis* and found that fermented diets significantly increased linoleic acid and PUFA contents in pork without significantly affecting stearic acid, palmitic acid, or oleic acid contents. Ma et al. studied the effects of compound bacteria-fermented complete feed on fatty acids in pig muscle and found significantly higher linoleic acid, arachidonic acid, eicosapentaenoic acid, PUFA content, and PUFA/SFA ratio compared with the control group. Su et al. reported that microbial fermented diets significantly increased PUFA content in Qingjia Ma chicken muscle, thereby improving meat quality. Lin et al. also found that adding 4% probiotic-fermented feed to pig diets significantly increased MUFA and PUFA contents in pork and improved amino acid content without antibiotic or heavy metal residues, indicating that probiotic-fermented feed can significantly improve pork flavor and aroma, providing safer, healthier, and more

nutritious pork. The present study found that dietary fermented cottonseed meal supplementation improved major fatty acid contents in yellow-feathered broiler muscle, with the most significant improvement observed for PUFA, consistent with the aforementioned studies. These results suggest that *Lactobacillus acidophilus* and its fermentation metabolites in fermented cottonseed meal can directly or indirectly participate in fatty acid synthesis and metabolic regulation in broilers, thereby affecting fatty acid composition in muscle, which is important for improving broiler muscle flavor. The regulatory mechanism of fermented cottonseed meal on fatty acids in broiler muscle has not been reported and warrants further investigation. Additionally, the results indicate that 3% and 6% fermented cottonseed meal supplementation effectively improved PUFA content in yellow-feathered broiler breast muscle, with the 6% group showing better results than the 3% group.

3.3 Effects of Fermented Cottonseed Meal on Muscle Amino Acid Content

The types, contents, and composition ratios of amino acids in muscle importantly influence nutritional value and flavor characteristics, as amino acids are important taste compounds and flavor precursors. Numerous studies have shown that Glu, Asp, and Gly contribute most to the umami taste of livestock and poultry muscle and are fundamental components of muscle flavor, with Glu being the most prominent—its umami taste is more than 50 times stronger than monosodium glutamate. Asp and Glu, classified as acidic amino acids, play important roles in meat umami taste. Gly has a unique sweet taste, can improve meat color, reduce meat pH, and increase the content of flavor compounds (1,2-ethanedithiol, 2-ethylpyrazine, 2-acetylfuran, and 2,4,5-trimethylthiazole). Therefore, these three amino acids were examined as the primary flavor amino acids in this study. Previous studies have reported on the effects of fermented feed on meat amino acid content, with some showing that dietary supplementation with compound bacterial fermentation could increase Glu, Gly, alanine, and Lys contents in finishing pig longissimus dorsi muscle, thereby improving pork quality. Su et al. found that Qingjia Ma chickens fed microbial fermented diets had advantages in total amino acid, essential amino acid, and FAA contents. This study detected 17 free amino acids in yellow-feathered broiler muscle, with TAA content ranging from 20.14% to 21.57% and FAA content ranging from 6.49% to 6.85%. The results showed that dietary fermented cottonseed meal supplementation improved amino acid composition in yellow-feathered broiler muscle, particularly by increasing FAA content, consistent with the aforementioned studies. The regulation of amino acid composition and flavor improvement by fermented cottonseed meal may be related to its nutritional characteristics and the ability of beneficial microorganisms and active metabolites to promote efficient digestion, absorption, and utilization of nutrients. Additionally, this study found that 9% fermented cottonseed meal supplementation significantly increased TAA content in yellow-feathered broiler muscle compared with the same inclusion level of cottonseed meal, while 6% and 9% fermented cottonseed

meal supplementation significantly increased FAA content compared with 3% fermented cottonseed meal.

3.4 Effects of Fermented Cottonseed Meal on Muscle Inosinic Acid Content

Inosinic acid is internationally recognized as an important aromatic compound with meat umami characteristics and is significant for evaluating meat flavor quality. Inosinic acid in muscle can synergize with various flavor compounds such as monosodium glutamate to enhance meat umami taste while buffering taste perception to inhibit sour and bitter flavors. Studies by Li et al. and Huang et al. confirmed that dietary probiotic supplementation significantly increased inosinic acid content in broiler muscle, thereby improving meat flavor. Lü et al. added *Lactobacillus* and *Bacillus*-fermented sesame meal to duck diets and found that fermented sesame meal significantly increased muscle inosinic acid content. The present study showed that dietary fermented cottonseed meal supplementation promoted increased inosinic acid content in yellow-feathered broiler muscle, consistent with the findings of Li et al. and Lü et al. Additionally, this study found that 9% fermented cottonseed meal supplementation significantly improved muscle inosinic acid content, indicating that feeding fermented cottonseed meal can improve flavor characteristics of yellow-feathered broiler muscle. The mechanism by which probiotic-fermented feed improves animal meat flavor may be related to beneficial metabolites produced during fermentation and the regulatory effects of beneficial microorganisms in fermented feed on animal gut microbiota.

Conclusions

1. Dietary supplementation with different proportions of fermented cottonseed meal had no significant effects on conventional nutrient content (moisture, crude protein, crude fat, and crude ash) in yellow-feathered broiler muscle.
2. Dietary fermented cottonseed meal supplementation increased PUFA content in yellow-feathered broiler muscle, with significant improvements observed in the 3% and 6% inclusion groups.
3. Dietary fermented cottonseed meal supplementation increased TAA and FAA contents in yellow-feathered broiler muscle, particularly FAA content, with better effects observed at 6% and 9% inclusion levels.
4. Dietary fermented cottonseed meal supplementation increased inosinic acid content in yellow-feathered broiler muscle.

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