

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

Authors: Wang Yongqiang, Zhang Xiaoyang, Liu Jiancheng, Nie Cunxi, Zhang Fanfan, Jiang Lixin, Ma Guijun, Zhang Wenju

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Abstract

This study aimed to investigate the effects of *Lactobacillus acidophilus*-fermented cottonseed meal on muscle nutritional components and flavor characteristics of yellow-feathered broilers. 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; control groups (Groups I, III, and V) received diets supplemented with 3%, 6%, and 9% cottonseed meal, respectively, while experimental groups (Groups II, IV, and VI) received diets supplemented with 3%, 6%, and 9% fermented cottonseed meal, respectively. Following a 42-day feeding trial, 6 chickens were randomly selected from each group for slaughter and sample collection, and indices including conventional nutritional components, fatty acids, amino acids, and inosine monophosphate content were determined and analyzed. The results showed that: 1) Compared with control groups, dietary supplementation with fermented cottonseed meal exerted no significant effects on the contents of conventional nutritional components in yellow-feathered broiler muscle ($P > 0.05$); 2) The linoleic acid content in muscle of Group II was significantly elevated by 13.91% compared with Group I ($P < 0.05$), the linolenic acid content in muscle of Group IV was significantly elevated by 21.51% compared with Group III ($P < 0.05$), and the polyunsaturated fatty acid content in muscle of Groups II and IV was significantly elevated by 14.22% and 9.04% compared with Groups I and III, respectively ($P < 0.05$); 3) The total free amino acid content in muscle of Group VI was significantly increased by 13.85% compared with Group V ($P < 0.05$); the flavor amino acid (FAA) content in muscle of Groups IV and VI was significantly increased by 6.04% and 9.27% compared with Groups III and V, respectively ($P < 0.05$), and the FAA content in muscle of Groups IV and

VI was increased by 5.55% and 5.39% compared with Group II ($P < 0.05$); 4) The inosine monophosphate content in muscle of Group VI was significantly increased by 10.81% compared with Group V ($P < 0.05$), while no significant differences were observed among other groups ($P > 0.05$). Based on these results, dietary supplementation with fermented cottonseed meal can improve the flavor characteristics of yellow-feathered broiler muscle, with 6% or 9% supplementation levels showing superior effects.

Full Text

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

WANG Yongqiang^{1,2}, ZHANG Xiaoyang¹, LIU Jiancheng^{1,3}, NIE Cunxi¹, ZHANG Fanfan¹, JIANG Lixin³, MA Guijun³, ZHANG Wenju^{1*}

¹College of Animal Science and Technology, Shihezi University, Shihezi 832000, China

²College of Animal Science and Technology, Henan Institute of Science and Technology, Xinxiang 453003, China

³Biological Additive Branch, Xinjiang Tiankang Animal Husbandry Biotechnology Co., Ltd., Urumqi 830011, China

Abstract: This experiment was conducted to investigate the effects of cottonseed meal fermented by *Lactobacillus acidophilus* on the muscle nutrient composition and flavor characteristics of yellow-feathered broilers. A total of 360 healthy 21-day-old male yellow-feathered broilers with an average body weight of (449.66 ± 6.01) g were randomly divided into 6 groups with 6 replicates per group and 10 broilers per replicate. The control groups (groups , , and) were fed diets supplemented with 3%, 6%, and 9% cottonseed meal, respectively, while the experimental groups (groups , , and) were fed diets supplemented with 3%, 6%, and 9% fermented cottonseed meal, respectively. After a 42-day feeding trial, 6 broilers were randomly selected from each group for slaughter and sampling. The conventional nutrient composition, fatty acids, amino acids, and inosinic acid content of the samples were measured and analyzed. The results showed that: 1) Compared with the control groups, dietary supplementation with fermented cottonseed meal had no significant effect on the conventional nutrient content of broiler muscle ($P > 0.05$). 2) The linoleic acid content in muscle of group was significantly higher than that of group by 13.91% ($P < 0.05$), the linolenic acid content in muscle of group was significantly higher than that of group by 21.51% ($P < 0.05$), and the polyunsaturated fatty acid content in muscle of groups and was significantly higher than that of groups and by 14.22% and 9.04%, respectively ($P < 0.05$). 3) The total free amino acid content in muscle of group was significantly higher than that of group by 13.85% ($P < 0.05$). The flavor amino acid (FAA) content in muscle of

groups and was significantly higher than that of groups and by 6.04% and 9.27%, respectively ($P < 0.05$), and the FAA content in muscle of groups and was significantly higher than that of group by 5.55% and 5.39%, respectively ($P < 0.05$). 4) The inosinic acid content in muscle of group was significantly higher than that of group by 10.81% ($P < 0.05$), with no significant differences 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 supplementation levels of 6% or 9%.

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

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 daily diets has been increasing. 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. Therefore, producing safe and high-quality livestock products has become a hot research topic. Numerous studies have shown that probiotics and probiotic-fermented feeds have unique nutritional and physiological characteristics that can improve growth performance, slaughter performance, digestion and metabolism, meat quality, and immunity in livestock and poultry to varying degrees, making them effective alternatives to antibiotics and providing new solutions to these problems. Fermented cottonseed meal is a novel biological protein feed produced through solid-state fermentation of cottonseed meal using probiotics. Currently, there are few reports on the effects of dietary fermented cottonseed meal supplementation on muscle nutrient composition and flavor characteristics of yellow-feathered broilers. Many studies have demonstrated that intramuscular fat, fatty acids, amino acids, and inosinic acid are important evaluation indicators closely related to muscle nutritional properties. Therefore, this study focused on these indicators to investigate the effects of cottonseed meal fermented by *Lactobacillus acidophilus* on muscle nutrient composition and flavor characteristics of yellow-feathered broilers, providing an important reference basis for the development of new functional protein feeds and the production of high-quality livestock meat products.

1.1 Experimental Materials

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

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

1.2 Preparation of Fermented Cottonseed Meal

The preparation process of *Lactobacillus acidophilus*-fermented cottonseed meal was based on the optimized fermentation process from Wu et al. with slight modifications. The fermentation substrate was prepared by mixing cottonseed meal and wheat bran at a ratio of 9:1. The moisture content of the substrate was adjusted to 40% and mixed thoroughly 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 strain culture (viable count 2.52×10^8 CFU/mL, inoculation amount 6%). The feed was mixed 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 h. After fermentation, the fermented cottonseed meal was removed, dried in an oven at 40°C, bagged, and stored for later use. The changes in main nutrient components of cottonseed meal before and after fermentation are shown in .

1.3.1 Experimental Design

The yellow-feathered broiler breed selected was Guangxi Sanhuang chicken, purchased from Xinjiang Tiankang Animal Husbandry Biotechnology Co., Ltd. According to the principle of similar body weight, 360 healthy 21-day-old male yellow-feathered broilers with an average weight of (449.66 ± 6.01) g were selected and randomly divided into 6 groups using a single-factor completely randomized design, with 6 replicates per group and 10 broilers per replicate. Groups , , and were control groups with 3%, 6%, and 9% unfermented cottonseed meal added to the diets, respectively. Groups , , and were experimental groups with 3%, 6%, and 9% fermented cottonseed meal added to the diets, respectively.

1.3.2 Experimental Diets

The basal experimental diets were formulated according to the NY/T 33-2004 “Feeding Standard of Chickens” and NRC (1994) nutrient requirements for broilers, including early-stage feed (21-42 days) and late-stage feed (43-63 days). The diet composition and nutrient levels of each group are shown in .

1.3.3 Broiler Husbandry and Management

All experimental broilers were raised in the same chicken house using a two-tier cage system with 6 broilers per cage. The broilers were raised for 42 days according to standardized broiler feeding and management procedures, with feed added three times daily and free access to feed.

1.4 Sample Collection and Index Determination

At 64 days of age, 6 yellow-feathered broilers were randomly selected from each group. Left breast muscle samples (50-80 g) were collected from each experimental broiler according to GB/T 9695.19-2008. All samples were frozen in

liquid nitrogen and stored at -80°C for later analysis.

1.4.1 Determination of Conventional Nutrient Content in Muscle

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 determined using an automatic Kjeldahl nitrogen analyzer (FOSS Kjeltac 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 determined according to GB/T 9695.15-2008.

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

1.4.3 Determination of Amino Acid Content in Muscle Muscle samples were analyzed 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 Inosinic Acid Content in Muscle The inosinic acid content in muscle was determined by high-performance liquid chromatography (HPLC) (Agilent 1200) according to the method of Zhao et al.

1.5 Data Analysis

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

2.1 Effects of Fermented Cottonseed Meal on Conventional Nutrient Content in Muscle of Yellow-Feathered Broilers

As shown in , dietary supplementation with different proportions of fermented cottonseed meal had no significant effect on conventional nutrient content in muscle of yellow-feathered broilers ($P > 0.05$). 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 groups supplemented with the same proportions of cottonseed meal, but the differences were not significant ($P>0.05$).

2.2 Effects of Fermented Cottonseed Meal on Fatty Acid Content in Muscle of Yellow-Feathered Broilers

As shown in , fermented cottonseed meal had the most significant effect on polyunsaturated fatty acids (PUFA) in 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 groups supplemented with the same proportions of cottonseed meal. Additionally, significant differences in PUFA content existed among the three cottonseed meal groups ($P<0.05$), with the 9% supplementation group showing significantly higher PUFA content than the 3% supplementation group ($P<0.05$). Among the three experimental groups supplemented with different proportions of fermented cottonseed meal, the 9% group had the highest PUFA content, followed by the 6% group, with the 3% group being the lowest, but no significant differences were observed among the three groups ($P>0.05$).

2.3 Effects of Fermented Cottonseed Meal on Amino Acid Content in Muscle of Yellow-Feathered Broilers

As shown in , dietary supplementation with different proportions of fermented cottonseed meal significantly improved the contents of aspartic acid (Asp), glutamic acid (Glu), lysine (Lys), and arginine (Arg) in muscle of 64-day-old yellow-feathered broilers, with Asp and Glu being the main flavor amino acids (FAA). Regarding 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 groups supplemented with the same proportions of cottonseed meal. For Glu content, the 3%, 6%, and 9% fermented cottonseed meal groups showed increases of 1.83% ($P>0.05$), 7.36% ($P<0.05$), and 7.34% ($P<0.05$), respectively, compared with same-proportion cottonseed meal groups. Additionally, significant differences existed among the three fermented cottonseed meal groups ($P<0.05$), with the 6% and 9% groups showing 4.79% and 5.09% higher Glu content than the 3% group ($P<0.05$), though no significant difference was observed between the 6% and 9% groups ($P>0.05$). For glycine (Gly) content, the 3%, 6%, and 9% fermented cottonseed meal groups showed increases of 2.06%, 3.77%, and 12.90%, respectively, compared with same-proportion cottonseed meal groups, but the 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 not significantly different from the 3% group ($P>0.05$). For Lys content, the 3%, 6%, and 9% fermented cottonseed meal groups showed increases of 5.39% ($P>0.05$), 9.76% ($P<0.05$), and 7.54% ($P<0.05$), respectively, compared with same-proportion cottonseed

meal groups, with the 6% fermented cottonseed meal group showing the highest Lys content, though not significantly different from the 3% and 9% fermented cottonseed meal groups ($P>0.05$). For Arg content, the 3%, 6%, and 9% fermented cottonseed meal groups showed increases of 5.30% ($P>0.05$), 4.79% ($P>0.05$), and 13.10% ($P<0.05$), respectively, compared with same-proportion cottonseed meal groups. Among the three fermented cottonseed meal groups, the 9% group had the highest Arg content, but it was not significantly different from the 3% and 6% groups ($P>0.05$). For total free amino acid (TAA) content, the 3%, 6%, and 9% fermented cottonseed meal groups showed increases of 3.91% ($P>0.05$), 7.60% ($P>0.05$), and 13.85% ($P<0.05$), respectively, compared with same-proportion cottonseed meal groups. For FAA content, the 3% fermented cottonseed meal group showed a 2.04% increase compared with the 3% cottonseed meal group, but the difference was not significant ($P>0.05$). However, the 6% and 9% fermented cottonseed meal groups showed 6.04% and 9.27% higher muscle FAA content than same-proportion cottonseed meal groups ($P<0.05$). Among the three fermented cottonseed meal groups, the 6% and 9% groups showed 5.55% and 5.39% higher FAA content than the 3% group ($P<0.05$), but no significant difference was observed between the 6% and 9% groups ($P>0.05$).

2.4 Effects of Fermented Cottonseed Meal on Inosinic Acid Content in Muscle of Yellow-Feathered Broilers

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

3.1 Effects of Fermented Cottonseed Meal on Conventional Nutrient Content in Muscle of Yellow-Feathered Broilers

Conventional nutrients in meat are closely related to meat quality and flavor, with crude fat content in meat receiving increasing attention from researchers worldwide. Studies have shown that intramuscular fat directly affects meat quality, including tenderness, flavor, and juiciness. Intramuscular fat is not only one of the physical factors contributing to meat juiciness but also an important precursor for flavor compounds, making it a crucial meat quality evaluation indicator. Current research has demonstrated that lactobacilli can significantly affect fatty acid metabolism in the body, thereby increasing fat deposition and distribution while reducing fat oxidation in pork and increasing intramuscular fat con-

tent. Li et al. found that dietary supplementation with lactic acid bacteria had no significant effect on breast muscle nutrient composition in broilers but showed an increasing trend in muscle moisture and fat content. Mi et al. reported that feeding fermented diets to finishing pigs resulted in higher intramuscular fat content than the control group, though the difference was not significant. Ma and Zhang et al. 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 content in broiler muscle but significantly decreased crude fat and cholesterol content. This experiment showed that dietary supplementation with fermented cottonseed meal had no significant effect on conventional nutrient content in yellow-feathered broiler muscle, with moisture and crude fat content showing an upward trend while crude protein content showed a downward trend. These results are basically consistent with Li et al. and Mi et al. but differ from Huang et al., possibly due to differences in broiler breed, grouping, diet formulation, and complex metabolites from feed fermentation.

3.2 Effects of Fermented Cottonseed Meal on Fatty Acid Content in Muscle of Yellow-Feathered Broilers

Fatty acids are important indicators for evaluating meat quality and flavor. The fatty acid composition in muscle plays a decisive role in the hardness of adipose tissue and the oxidative stability of muscle, 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. Studies have 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 lactic acid bacteria, bacillus, and yeast at 3 g/kg to broiler diets significantly increased SFA and UFA content in broiler muscle and significantly increased linolenic acid content. 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 content in pork but had no significant effect on stearic acid, palmitic acid, or oleic acid content. Ma et al. studied the effects of compound bacteria-fermented complete feed on fatty acids in pig muscle and found that linoleic acid, arachidonic acid, eicosapentaenoic acid, PUFA content, and PUFA/SFA ratio in pig muscle were significantly higher than in the control group. Su et al. found that microbial fermented diets significantly increased PUFA content in muscle of Qingjiaoma chickens, thereby improving meat quality. Lin et al. also found that adding 4% probiotic-fermented feed to pig diets significantly increased MUFA and PUFA content in pork and increased amino

acid content to varying degrees without antibiotic or heavy metal residues, indicating that feeding probiotic-fermented diets significantly improved pork flavor and aroma and provided safer, healthier, and more nutritious pork. This study found that dietary supplementation with fermented cottonseed meal improved the content of major fatty acids in yellow-feathered broiler muscle, with the most significant improvement observed for PUFA, consistent with the above research results. These findings 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 broiler muscle, which is significant for improving broiler muscle flavor. The regulatory mechanism of fermented cottonseed meal on fatty acids in broiler muscle has not been reported and requires further in-depth study. Additionally, this study showed that supplementation with 3% and 6% fermented cottonseed meal 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 Amino Acid Content in Muscle of Yellow-Feathered Broilers

The types, content, and composition ratio of amino acids in muscle are important factors affecting muscle nutritional value and flavor characteristics. Amino acids are important taste compounds and flavor precursors in muscle. Many studies have shown that Glu, Asp, and Gly contribute most to the umami taste of livestock and poultry muscle and are basic components of muscle umami, with Glu being the most significant—its umami intensity is more than 50 times that of monosodium glutamate. Asp and Glu are both acidic amino acids that play important roles in meat umami taste. Gly has a unique sweetness that 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) in meat. Therefore, these three amino acids were investigated as main flavor amino acids. Current studies have reported on the effects of fermented diets on amino acid content in meat. Research has shown that dietary supplementation with compound bacterial fermentation can increase Glu, Gly, alanine, and Lys content in pig longissimus dorsi muscle, thereby improving pork quality. Su et al. found that feeding microbial fermented diets to Qingjiaoma chickens resulted in advantages in total amino acid, essential amino acid, and FAA content. This study detected 17 free amino acids in yellow-feathered broiler muscle samples, 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 supplementation with fermented cottonseed meal improved amino acid composition in yellow-feathered broiler muscle, particularly by increasing FAA content, consistent with the above research reports. The regulation of amino acid composition and flavor improvement in yellow-feathered broiler muscle by fermented cottonseed meal may be related to the nutritional characteristics of fermented cottonseed meal and the promotion of efficient digestion, absorption, and uti-

lization of nutrients by the probiotics and active metabolites contained therein. Additionally, this study found that dietary supplementation with 9% fermented cottonseed meal significantly increased TAA content in yellow-feathered broiler muscle compared with the same proportion of cottonseed meal, while supplementation with 6% and 9% fermented cottonseed meal significantly increased FAA content compared with 3% fermented cottonseed meal.

3.4 Effects of Fermented Cottonseed Meal on Inosinic Acid Content in Muscle of Yellow-Feathered Broilers

Inosinic acid is internationally recognized as an important aromatic compound with meat umami characteristics and is significant for measuring meat umami quality. Inosinic acid in muscle can synergize with various flavor substances such as monosodium glutamate to enhance the umami of meat products while buffering taste to inhibit sourness and bitterness. Li et al. and Huang et al. confirmed that dietary supplementation with probiotic preparations 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 in ducks. This study showed that dietary supplementation with fermented cottonseed meal promoted increased inosinic acid content in yellow-feathered broiler muscle, consistent with the results of Li et al. and Lü et al. Additionally, this study found that dietary supplementation with 9% fermented cottonseed meal 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 by probiotic fermentation and the regulatory effects of beneficial microorganisms in fermented feed on animal digestive tract microbiota.

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 muscle of yellow-feathered broilers.
2. Dietary supplementation with fermented cottonseed meal increased PUFA content in yellow-feathered broiler muscle, with significant improvement observed in the 3% and 6% supplementation groups.
3. Dietary supplementation with fermented cottonseed meal increased TAA and FAA content in yellow-feathered broiler muscle, particularly FAA content, with better effects observed at 6% and 9% supplementation levels.
4. Dietary supplementation with fermented cottonseed meal increased inosinic acid content in yellow-feathered broiler muscle.

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