Effects of Daidzein on Carcass Performance and Meat Quality of Finishing Xiangzhong Black Cattle (Postprint)

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Abstract

This study aimed to investigate the effects of dietary soybean isoflavone supplementation on carcass performance and meat quality in Xiangzhong Black finishing cattle. Fourteen healthy castrated Xiangzhong Black cattle with similar body weight [(450$±$20) kg] and approximately 2 years of age were randomly allocated into 2 groups (n=7). The control group received a basal diet, while the treatment group received the basal diet supplemented with 500 mg/kg soybean isoflavones. The experimental period was 120 d. The results showed that, compared with the control group: 1) soybean isoflavone supplementation had no significant effect on pre-slaughter live weight, hot carcass weight, or dressing percentage (P>0.05), and exhibited a non-significant decreasing trend in carcass fat content and backfat thickness (P>0.05); 2) soybean isoflavone supplementation significantly increased the crude fat content and marbling score of the longissimus dorsi muscle (P<0.05) by 8.34% and 1.93 points, respectively, significantly decreased the pH and moisture content of muscle after 24-hour chilling (P<0.05), and significantly increased the muscle redness value (P<0.05); 3) soybean isoflavone supplementation significantly decreased serum concentrations of glucose, urea nitrogen, triglycerides, total cholesterol, non-esterified fatty acids, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol (P<0.05); 4) soybean isoflavone supplementation significantly increased isocitrate dehydrogenase activity while decreasing glucose-6-phosphate dehydrogenase activity in the longissimus dorsi muscle (P<0.05). These results indicate that dietary soybean isoflavone supplementation can influence lipid metabolism, promote intramuscular fat deposition, and improve marbling and meat quality in finishing cattle.

Full Text

Effects of Daidzein on Carcass Performance and Meat Quality of Fattening Xiangzhong Black Cattle

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Abstract

This study investigated the effects of dietary daidzein supplementation on carcass performance and meat quality in fattening Xiangzhong Black cattle. Fourteen healthy castrated Xiangzhong Black cattle, approximately 2 years old with similar body weight [(450$±$20) kg], were randomly divided into two groups (n=7). The control group received a basal diet, while the test group received the basal diet supplemented with 500 mg/kg daidzein. The experimental period lasted 120 days. The results showed that, compared with the control group: (1) daidzein supplementation had no significant effects on live weight before slaughter, hot carcass weight, or dressing percentage (P>0.05), while carcass fat content and backfat thickness showed a decreasing trend without significant difference (P>0.05); (2) daidzein significantly increased ether extract content and marbling score in the longissimus dorsi muscle by 8.34% and 1.93 points, respectively (P<0.05), significantly reduced pH and moisture content after 24-hour aging (P<0.05), and significantly increased muscle redness value (P<0.05); (3) daidzein significantly reduced serum concentrations of glucose, urea nitrogen, triglyceride, total cholesterol, non-esterified fatty acid, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol (P<0.05); and (4) daidzein significantly increased isocitrate dehydrogenase activity while decreasing glucose-6-phosphate dehydrogenase activity in the longissimus dorsi muscle (P<0.05). These findings indicate that dietary daidzein supplementation can influence lipid metabolism, promote intramuscular fat deposition, and improve beef marbling and meat quality in fattening cattle.

Keywords: daidzein; Xiangzhong Black cattle; meat quality; intramuscular fat; carcass performance

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With rising living standards, consumer demands for beef quality have increased substantially. Intramuscular fat (IMF) content is a critical factor affecting meat quality, significantly influencing flavor, juiciness, and tenderness, and determining the degree of beef marbling [1]. Enhancing IMF content and meat quality in beef has become a major research focus. Daidzein (Dai), a phytoestrogen found in soybeans, alfalfa, and other feedstuffs [2-3], exhibits estrogen-like, antioxidant,

immunomodulatory, and health-promoting functions. Its practical advantages include low dosage requirements, rapid efficacy, and low toxicity, making it a promising novel feed additive with significant development potential. Daidzein may affect lipid metabolism, marbling, and meat quality in beef cattle. However, no studies have yet reported on its effects on carcass performance and meat quality. Therefore, this research was conducted to explore these effects and provide technical support for improving beef quality.

1.1 Experimental Animals, Grouping, and Basal Diet Composition

Fourteen healthy castrated Xiangzhong Black cattle, approximately 2 years old with similar body weight [(450$±$20) kg], were randomly assigned to control and test groups. The control group received a basal diet, while the test group received the basal diet supplemented with 500 mg/kg daidzein (purchased from Shaanxi Ciyuan Biotechnology Co., Ltd., daidzein content >98%). The daidzein was mixed thoroughly into the concentrate during feed preparation. The composition and nutrient levels of the basal diet are presented in Table 1 .

Table 1 Basal diet composition and nutrient levels (air-dry basis), %

Items	Content
Ingredients	
Rice straw	
Corn	
Roasted barley	
Wheat bran	
Roasted soybean	
Dried distillers grains	
Limestone	
Premix[1]	
Total	
Nutrient levels[2]	
Dry matter	
Ash	
Crude protein	
Neutral detergent fiber	
Acid detergent fiber	
Ether extract	
NEmf (MJ/kg)	

[1]One kg of premix contained: VA 250,000 IU, VD_3 30,000 IU, VE 800 IU, Cu 1 g, Fe 5 g, Mn 4 g, Zn 3 g, Se 10 mg, I 50 mg, Co 10 mg.
[2]Calculated values.

1.2 Feeding Management

The feeding trial was conducted at the Lianyuan Experimental Station of the National Beef Cattle Industry Technology System. The adaptation period lasted 10 days, followed by a 120-day formal experimental period. Cattle were housed in small pens with consistent ventilation and lighting, with free access to feed and water. Feed was provided twice daily at 07:00 and 16:00.

1.3 Slaughter, Aging, Dissection, and Sample Collection

After the feeding trial, all cattle were weighed and slaughtered at the slaughterhouse of Hunan Tianhua Animal Husbandry Co., Ltd. Cattle were fasted for 24 hours before slaughter while ensuring adequate water and rest. Slaughter procedures followed GB/T 19477-2004 (Operating Procedures of Cattle Slaughter). Immediately after slaughter, 20 mL of carotid arterial blood was collected, allowed to clot at room temperature, and centrifuged at 3,000 r/min for 20 min at 4 °C to separate serum, which was stored at -80 °C for subsequent serum biochemical analysis.

Within 30 minutes post-slaughter, carcasses were weighed, and longissimus dorsi muscle samples were collected from the left carcass between the 12th and 13th ribs. Each sample was divided into two 2.54 cm-thick portions: one portion was sealed in plastic bags and stored at -20 °C for chemical composition analysis, while the other was placed in a 4 °C aging room for 24 hours before measuring meat color, shear force, drip loss, pH, and other meat quality parameters.

After 24 hours of aging at 4 °C, the left half of each carcass was dissected into meat, bone, fat (subcutaneous fat), and other tissues (e.g., connective tissue) to calculate dressing percentage and determine fat, muscle, and bone contents.

1.4.1 Backfat Thickness and Loin Eye Area Measurement

Backfat thickness was measured on both sides of the carcass at the 12th-13th rib using vernier calipers, and the average was calculated from four measurement points. The longissimus dorsi muscle at the 12th-13th rib of the left carcass was traced onto transparent sulfuric acid paper, and the loin eye area was calculated using Leica QWIN software.

1.4.2 Meat pH Measurement

Longissimus dorsi muscle samples from the 12th-13th ribs of the left carcass were used for pH measurement. Muscle pH was measured directly at the center of each sample using a Mettler Toledo Delta 320 pH meter with a metal probe at two time points: 45 minutes and 24 hours post-slaughter. Three measurements were taken at different positions on each sample, and the average was used for statistical analysis. After measurement, samples were stored at 0-4 °C for subsequent analyses.

1.4.3 Meat Color and Marbling Score

Longissimus dorsi muscle samples from the 12th-13th ribs were used to measure meat color at 24 hours post-slaughter using a WSC-S colorimeter, including lightness (L*), redness (a*)*, and yellowness (b*) values. Three measurements were taken at different positions on each sample, and the average was calculated. Marbling score was assessed using the Japanese marbling scoreboard, which has 12 grading levels: 8-12 indicates abundant, 5-7 moderate, 3-4 average, 2 slight, and 1 minimal marbling.

1.4.4 Muscle Shear Force Measurement

Longissimus dorsi muscle blocks (3 cm × 4 cm × 5 cm) aged for 24 hours at 0-4 °C were placed at room temperature for 1 hour, wrapped in plastic film, and fitted with a thermometer at the center. Samples were heated in an 80 °C water bath until the core temperature reached 70 °C, then immediately removed and cooled to room temperature until the core temperature reached approximately 20 °C. Standard strips (1 cm × 3 cm) were cut along the muscle fiber direction, and shear force was measured using a Warner-Bratzler shear device (C-LM4). Six standard strips were measured per sample, and the average value was calculated.

1.4.5 Muscle Conventional Nutrient Determination

Approximately 50 g of longissimus dorsi muscle from the 12th-13th ribs of the left carcass was trimmed of visible fat, tendons, and surface connective tissue, minced, and dried in a 65 °C oven to prepare air-dried samples. Moisture and ether extract contents were determined according to AOAC (1990) methods [4], while crude protein content was measured using the Kjeldahl method. All values were expressed on a fresh weight basis.

1.4.6 Serum Biochemical Index Determination

Serum triglyceride, total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and urea nitrogen were measured using assay kits from Kyowa Medex (Japan). Serum glucose was determined using kits from HemoCue (Germany), glycated serum protein using kits from Sichuan Mike Biological Technology, and non-esterified fatty acid (NEFA) using kits from Nanjing Jiancheng Bioengineering Institute. All analyses were performed using a Beckman AU5421 automatic biochemical analyzer at the First Affiliated Hospital of Nanchang University according to the manufacturers' instructions.

1.4.7 Muscle Lipid Synthesis Enzyme Activity Determination

Longissimus dorsi muscle samples stored at -80 °C were thawed at 37 °C. A 100 mg tissue sample was mixed with 1,000 mL physiological saline, homoge-

nized, and centrifuged; the supernatant was removed. Activities of glucose-6-phosphate dehydrogenase (G6PDH), malate dehydrogenase (MDH), and isocitrate dehydrogenase (ICDH) were measured using colorimetric assay kits from Suzhou Keming Biological Technology. G6PDH and ICDH activities were defined as 1 unit per mg protein producing 1 nmol NADPH per minute. MDH activity was defined as 1 unit per mg protein consuming 1 nmol NADH per minute.

1.5 Data Processing and Statistical Analysis

All data were initially organized using Excel and analyzed using SPSS 17.0 software via one-way ANOVA. Results are expressed as means, with $P<0.05$ considered statistically significant.

2.1 Carcass Performance Indices

As shown in Table 2 , compared with the control group, the test group showed decreasing trends in carcass fat content (32.04% vs. 28.62%, P=0.054) and backfat thickness (3.39 cm vs. 2.85 cm, P=0.055), though these differences were not significant ($P>0.05$). Bone content was significantly increased ($P<0.05$). Daidzein supplementation had no significant effects on live weight before slaughter, hot carcass weight, dressing percentage, carcass muscle content, or loin eye area ($P>0.05$).

Table 2 Effects of daidzein on carcass performance indexes of fattening cattle

Items	Control group	Test group	P-value
Live weight before slaughter (kg)			
Hot carcass weight (kg)			
Dressing percentage (%)			
Carcass composition (%)			
Fat			
Lean			
Bone			
Backfat thickness (cm)			
Longissimus dorsi area (cm^2)			

2.2 Meat Quality Indices

As shown in Table 3 , compared with the control group, the test group exhibited significantly higher redness values ($P<0.05$) and significantly lower pH after 24-hour aging ($P<0.05$) in the longissimus dorsi muscle. Daidzein supplementation significantly increased ether extract content and marbling score by 8.34% and 1.93 points, respectively ($P<0.05$), while significantly reducing moisture content ($P<0.05$). Shear force showed a decreasing trend but without significant difference ($P>0.05$).

Table 3 Effects of daidzein on meat quality indexes of longissimus dorsi of fattening cattle

Items	Control group	Test group	P-value
45 min			
Meat color			
Lightness (L*)			
Redness (a*)			
Yellowness (b*)			
Chemical composition			
Moisture (%)			
Ether Extract (%)			
Crude protein (%)			
Shear force (kgf)			
Marbling score			

2.3 Serum Biochemical Indices

As shown in Table 4 , daidzein supplementation significantly reduced serum concentrations of glucose, urea nitrogen, triglyceride, total cholesterol, non-esterified fatty acid, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol ($P<0.05$), while having no significant effect on glycated serum protein concentration ($P>0.05$).

Table 4 Effects of daidzein on serum biochemical indexes of fattening cattle (mmol/L)

Items	Control group	Test group	P-value
Glucose			
Triglyceride			
Non-esterified fatty acid			
Glycated serum protein			
Total cholesterol			
HDL-C			
LDL-C			
Urea nitrogen			

2.4 Muscle Lipid Synthesis Enzyme Activity

As shown in Table 5 , daidzein supplementation significantly increased isocitrate dehydrogenase activity while significantly decreasing glucose-6-phosphate dehydrogenase activity in the longissimus dorsi muscle ($P<0.05$). Malate dehydrogenase activity was not significantly affected ($P>0.05$).

Table 5 Effects of supplemental daidzein on lipogenic enzyme activity of longissimus dorsi muscle of fattening cattle

Items	Control group	Test group	P-value
ICDH			
G6PDH			
MDH			

3.1 Effects of Daidzein on Carcass Performance Indices in Beef Cattle

This study found that daidzein increased bone content and decreased carcass fat content and backfat thickness in fattening Xiangzhong Black cattle, consistent with findings from studies on rodents by Kaludjerovic et al. [5], Fujioka et al. [6], and Ohtomo et al. [7]. This may be attributed to daidzein' s ability to inhibit adipocyte differentiation in adipose tissue [8] while promoting osteoblast growth [9-10], thereby enhancing bone formation and development while suppressing body fat synthesis. Research indicates that daidzein can be partially metabolized into equol in the rumen [11], which inhibits body fat accumulation (excluding intramuscular fat) and promotes skeletal development [6-7].

3.2 Effects of Daidzein on Meat Quality Indices in Beef Cattle

Muscle pH significantly affects shear force, flavor, and water-holding capacity—critical factors influencing consumer acceptance [12-13]. When muscle pH after 24-hour aging increases from 5.5 to 6.1, shear force tends to decrease [13]. A pH above 5.5 after 24-hour aging generally indicates low muscle glycogen content, preventing adequate lactic acid accumulation [12]. This study found that daidzein supplementation reduced 24-hour pH in the longissimus dorsi muscle with a concomitant decreasing trend in shear force, likely due to increased muscle glycogen content. Malardé et al. [14] reported that soy isoflavone supplementation (containing daidzein and genistein) increased muscle glycogen content in rats, supporting our findings.

Meat color is an important quality trait that directly influences consumer purchase intent. Color variation relates to the proportions of myoglobin forms: deoxymyoglobin (bright red), myoglobin (dark red), and metmyoglobin (gray-brown) [15]. When meat color shifts from bright red to dark brown, consumers perceive nutrient loss or spoilage and avoid such products [16]. Therefore, myoglobin should remain in its ferrous form. This study found that daidzein supplementation increased redness values in the longissimus dorsi muscle, suggesting that daidzein inhibits muscle oxidation in air and improves color stability, possibly due to the inherent antioxidant properties of daidzein and equol [17].

3.3 Effects of Daidzein on Muscle Fat and Marbling Score in Beef Cattle

This study demonstrated that daidzein significantly reduced subcutaneous fat content while promoting intramuscular fat content and marbling score in Xiangzhong Black cattle. Crespillo et al. [18] found that daidzein supplementation increased fat content in skeletal muscle while decreasing liver fat content in rodents. Additional in vivo and in vitro studies have shown that daidzein and equol enhance preadipocyte differentiation, lipid droplet formation, and fat accumulation in humans and rodents [19-21]. However, Rehfeldt et al. [22] reported that maternal daidzein supplementation during gestation did not affect subcutaneous fat content but significantly increased total body fat content in piglets. These results indicate that daidzein possesses lipid-regulating properties that selectively promote fat deposition in muscle.

3.3 Effects of Daidzein on Serum Biochemical Indices and Muscle Lipid Synthesis Enzyme Activity in Beef Cattle

This study found that daidzein supplementation reduced serum concentrations of lipid metabolites, including non-esterified fatty acids, triglycerides, and total cholesterol, in Xiangzhong Black cattle. These results align with previous rodent studies demonstrating daidzein' s cholesterol-lowering effects [18,23-24]. Daidzein also reduced serum glucose concentration in this study, consistent with findings by Cao et al. [24] and Choi et al. [25]. Daidzein may lower serum glucose by increasing expression of glucose transporter 4 (GLUT4) and insulin receptor substrate-1 (IRS-1), enhancing insulin concentration and stimulating glucose uptake [19].

Glucose-6-phosphate dehydrogenase, malate dehydrogenase, and isocitrate dehydrogenase are involved in NADPH production during de novo fatty acid synthesis. This study found that daidzein supplementation increased isocitrate dehydrogenase activity in the longissimus dorsi muscle, which may be associated with enhanced intramuscular fat content. Glucose-6-phosphate dehydrogenase has been considered related to intramuscular fat deposition in beef cattle and a potential predictor of marbling [26]. However, this study found that daidzein significantly decreased glucose-6-phosphate dehydrogenase activity compared with the control group. The reason for this contradictory result remains unclear and requires further investigation.

Daidzein supplementation can influence lipid metabolism, promote intramuscular fat deposition, and improve beef marbling and quality in fattening Xiangzhong Black cattle. Daidzein represents a green additive suitable for high-quality beef production.

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