Original Research

Daidzein enhances intramuscular fat deposition and improves meat quality in finishing steers

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Abstract

An experiment was conducted to determine the effects of soy isoflavone daidzein on carcass characteristics, fat deposition, meat quality, and blood metabolites in finishing steers. Fourteen crossbred steers were used in a 120-d finishing study. These steers were stratified by weight into groups and randomly allotted by group to one of two dietary treatments: (1) control and (2) daidzein (500 mg/kg concentrate). The steers were fed a 90% concentrate diet. Supplemental daidzein did not affect slaughter weight, hot carcass weight, and dressing percentage, but tended to reduce fat proportion (not including intramuscular fat) in carcass and backfat thickness of steers. The carcass bone proportion was greater in steers fed daidzein diets than those fed control diets. Daidzein supplementation reduced pH at 24 h after slaughtered and moisture content and increased isocitrate dehydrogenase activity, fat content (16.28% and 7.94%), marbling score (5.29 and 3.36), redness (a^*), and chroma (C^*) values in longissimus muscle relative to control treatment. The concentrations of blood metabolites including glucose, blood urea nitrogen, triglyceride, total cholesterol, non-esterified fatty acid, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were all lower in steers fed daidzein diets than those fed control diets. Current results suggest that supplemental daidzein can affect lipid metabolism, increase intramuscular fat content and marbling score, and improve meat quality in finishing steers. Daidzein should be a promising feed additive for production of high-quality beef meat.

Keywords: Daidzein, finishing steers, meat quality, intramuscular fat, lipid metabolism

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Introduction

High-quality beef have grown in popularity along with the rapid development of economy in China. Marbling, i.e. intramuscular fat, is one of the main factors used to determine beef quality grade in many countries due to its beneficial effect on juiciness, tenderness, and palatability of beef.[1] Some nutritional methods can be used to regulate fat deposition in intramuscular adipose tissue by affecting glucolipid metabolism of adipose cells, such as adding zinc and some vitamins to feedlot diets.[2] Daidzein is a natural isoflavone widely existed in plants, mostly legumes such as soybeans.[3,4] Therefore, daidzein is present in virtually all natural-ingredient cattle diets that use legumes as feedstuff. Because of its strikingly similar chemical structure to the mammalian estrogen and weak estrogenic activity in animals, daidzein is known as a phytoestrogen and has been studied extensively for possible beneficial biological activities.[5] The results show that daidzein possess a variety of characteristics such as antioxidant, antiproliferative, differentiation-inducing abilities and can thus affect many biologic and physiological processes,[6] one of which is glucolipid metabolism. In murine 3T3-L1 preadipocytes and C3H10T1/2 cells, daidzein enhanced insulin-stimulated glucose uptake, adipocyte differentiation, and consequently lipid accumulation.[7] Similarly, daidzein also increased cytoplasmic lipid droplets compared to control in human preadipocyte cell line.[8] Supplemental daidzein in the maternal diet during late gestation significantly elevated the concentration of fat in the whole piglet body.[9] Daidzein can be partly metabolized to equol by ruminal microorganisms.[10] Previous studies found that equol also enhanced the conversion of C3H10T1/2 cells to adipocytes and increased peroxisome proliferators-activated receptor gamma (PPARγ) expression and lipid accumulation.[7] Supplemental soy isoflavones (containing 10.2% daidzein, 6.34% genistein, and 0.03% equol) up-regulated the mRNA expression of lipogenic genes in adipose, longissimus muscle and liver tissues, and enhanced the fat depositions in Chinese mini-pigs.[11,12]

Table 1 Basic diet and nutrient composition (daidzein was included according to treatment group)

Item	Inclusion levels (% as fed)
Diet ingredient	
Rice straw	10.0
Corn	41.4
Roasted barley	11.2
Wheat bran	19.8
Roasted soybean	5.4
Dried distillers grains	10.3
Limestone	0.9
Mineral–vitamin premix	1.0
Chemical composition	
Dry matter	88.4
Ash	3.5
Crude protein	12.8
Neutral detergent fiber	25.3
Acid detergent fiber	10.8
Crude fat	4.2

Mineral–vitamin premix (per kg): vitamin A, 250,000 IU; vitamin D3, 30,000 IU; vitamin E, 800 IU; Cu, 1 g; Fe, 5 g; Mn, 4 g; Zn, 3 g; Se, 10 mg; I, 50 mg; Co, 10 mg.

Based on the above results, we hypothesized that supplemental daidzein may affect lipid metabolism, marbling and consequently meat quality in beef cattle. However, little relevant information is available regarding the speculation. Therefore, the objective of the present study was to evaluate the effects of daidzein on carcass characteristics, meat quality, and lipid metabolism in finishing steers.

Material and methods

Animals, diets, and experimental design

All procedures were approved by Jiangxi agricultural university.

Daidzein was purchased from Ci Yuan Biotechnology Co., Ltd. (purity > 98%, Shaanxi, China). Fourteen crossbred steers (Chinese Yellow × Augus), with an average initial body weight (BW) of 565.2 ± 31.2 kg, were used in a 120-d feeding study during the finishing stage. These steers were stratified by weight into groups and randomly allotted by group to one of two dietary treatments: (1) control and (2) daidzein (500 mg/kg concentrate). The steers were tethered in individual stalls and fed a 90% concentrate diet in quantities sufficient to provide *ad libitum* consumption (Table 1). For daidzein treatment, daidzein was mixed into the mineral premix and added to the concentrate. Diets were supplied to the steers twice daily at 07:00 and 16:00 h. Fresh water was available for *ad libitum* consumption throughout the study.

Carcass characteristics and meat quality

Feed was removed from the steers and a blood sample was collected via jugular venipuncture at 07:00, 24 h before slaughter. Blood samples were allowed to clot at room temperature for 30 min, after which serum was harvested by centrifugation at $3000 \times g$ for 15 min at 4°C and stored frozen until required for analysis.

Following the final weighing, steers were slaughtered in a commercial meat plant according to normal procedures. After slaughtered, the carcasses were weighed hot. Hot dressing percentage was calculated as the ratio of hot carcass weight to final live weight. At this moment samples of longissimus muscles (between the 12th and 13th ribs) were removed and immediately frozen in liquid nitrogen for subsequent measurements of lipogenic enzyme assays. The carcasses were then cooled for 24 h at 2°C and subsequently weighed (cold carcass weight). The fat thickness over the longissimus muscles was obtained by means (triplicate in each carcass) of a vernier caliper. The left side of each carcass was then deboned, and lean, bone, fat, and other tissues including tendon and fascia data were collected, so there was a small loss because of the deboning process. Longissimus muscle samples were taken by cross-section between the 12th and 13th ribs to determine the chemical composition, pH, color, ribeye area, marbling, and shear force.

Analytical methods

The contents of moisture, crude protein, and fat in longissimus muscle samples were determined according to Association of Official Analytical Chemists (AOAC).[13] Briefly, moisture content was determined by oven-drying a wet sample at 105°C, until constant weight. Crude protein was determined using the Kjeldahl method. For the determination of lipid content, samples were extracted with diethyl ether for 16 h using a conventional Soxhlet apparatus. Lipid percentages were used to determine the amount of intramuscular fat (marbling) for the longissimus muscles.

Postmortem pH was obtained at 45 min ($pH_{45\,min}$) and 24 h ($pH_{24\,h}$) after slaughtered by a digital pH-meter (Mettler Toledo, Greifensee, Switzerland). The probe was inserted into the center of longissimus muscles between the 12th and 13th ribs.

Meat color of longissimus muscle samples was measured using a spectrocolorimeter (WSC-S, Shanghai, China) with a 10° observer and illuminant D65, and expressed in CIE lightness (L^*), redness (a^*), and yellowness (b^*). The chroma (C^*) and hue angle (H°) were estimated as $C^* = [(a^*)^2 + (b^*)^2]^{1/2}$, and $H^\circ = \arctan(b^*/a^*)$. Samples were allowed to bloom for 1 h before measuring directly in contact with air.

Ribeye area of longissimus muscle samples was traced on a sulfate paper, and then calculated by using Leica QWIN software. Marbling score of longissimus muscle samples was measured according to the Japan scoring system (8–12: abundant, 5–7: moderate, 3–4: mean, 2: small and 1: traces).

To obtain shear force values, steaks (3 cm × 4 cm × 5 cm) were dissected from each longissimus muscle samples and subsequently were cooked placing package bags in a water bath with automatic temperature control until they reached an internal temperature of 70°C. After cooking, samples were cooled to room temperature. Seven meat pieces of 1 × 1 × 3 cm (height × width × length) were removed

parallel to the muscle fiber direction and were completely cut using a Warner-Braztler shear device (C-LM4, Haerbin, China) to determine the shear force.

Diagnostic kits of Determiner-L low-density lipoprotein cholesterol (LDL-C), Determiner-L high-density lipoprotein cholesterol (HDL-C), Determiner-L TCII, and Determiner-C triglycerides (Kyowa Medex, Tokyo, Japan) were used to measure LDL-C, HDL-C, total cholesterol, and triglycerides levels in serum samples, respectively, following the manufacturer's instructions. The Fructosamine Assay kit (Sichuan Maker, Chengdu, China) was used to determine the concentration of glycation serum protein. A routine glucose oxidase method was used to measure the glucose concentration.[14] All measurements were performed in an AU5421 Automatic Biochemistry Analyzer (Backman-Kelt, USA) at the First Affiliated Hospital of Nanchang University. The concentration of non-esterified fatty acid was measured by a colorimetric assay kit (Jiangcheng, Nanjing, China).

To determine the activities of lipogenic enzyme including isocitrate dehydrogenase (ICDH), glucose-6-phosphate dehydrogenase (G6PDH), and malate dehydrogenase (MDH), samples of longissimus muscle samples were removed from −80°C storage, and thawed at 37°C. One hundred milligram tissue samples with added 1000 μL physiological saline was homogenized and centrifuged. The centrifugal extracts were prepared, and the enzyme activities were measured using colorimetric assay kits (Comin BIO-TEK, Suzhou, China). Enzyme activities were expressed as nmol of NADPH produced (G6PDH, ICDH) or NADH spent (MDH) per minute and per milligram of protein, after measurement of soluble protein content in enzyme homogenates using bovine serum albumin as the standard.[15]

Statistical analyses

Differences between means were tested for statistical significance with one-way analysis of variance (ANOVA). All statistical procedures were performed with SPSS 15.0 for Windows (SPSS Inc., Chicago, IL). Significance was declared at $P \leq 0.05$, and trends were discussed at $P \leq 0.10$.

Results

Carcass characteristics

Supplemental daidzein did not affect slaughter weight, hot carcass weight, and dressing percentage, but tended to reduce fat proportion ($P = 0.054$) in carcass and backfat thickness ($P = 0.055$) by 3.4% and 0.54 cm, respectively, compared with control steers (Table 2). The carcass bone proportion was greater in steers fed daidzein diets than those fed control diets ($P = 0.020$). No treatment differences were noted for longissimus muscle area and carcass lean proportion.

Meat quality of longissimus muscles

Daidzein supplementation did not affect $pH_{45\,min}$ but reduced $pH_{24\,h}$ of longissimus muscle ($P = 0.029$; Table 3). The color parameters affected by treatment were a^* ($P = 0.031$) and C^* ($P = 0.027$) values that were both greater for steers fed daidzein diets than those fed control diets. Supplemental daidzein significantly reduced moisture content (68.5% and 63.08%; $P = 0.033$) and increased intramuscular fat content (7.94% and 16.28%; $P = 0.012$) of longissimus muscle. No difference was detected between the two groups in shear force, but marbling score was significantly improved by added daidzein relative to control (3.36 and 5.29; $P = 0.004$).

Table 2 Effects of supplemental daidzein on carcass characteristics in finishing steers

Item	Control	Daidzein	SEM	*P*
Slaughter wt. (kg)	653.1	677.1	11.881	0.332
Hot carcass wt. (kg)	400.0	402.7	7.664	0.868
Carcass composition (%)				
Fat	32.04	28.62	0.904	0.054
Lean	49.57	51.85	0.849	0.189
Bone	13.31	14.22	0.205	0.020
Other tissues	5.08	5.31	0.318	0.737
Dressing percentage (%)	61.24	59.44	0.661	0.183
Backfat thickness (cm)	3.39	2.85	0.144	0.055
Longissimus area (cm^2)	90.21	86.97	2.213	0.486

SEM: standard error of the means, $n = 7$ steers per treatment.

Table 3 Effects of supplemental daidzein on meat quality parameters of longissimus muscle in finishing steers

Item	Control	Daidzein	SEM	*P*
pH				
$pH_{45\,min}$	6.63	6.54	0.091	0.663
$pH_{24\,h}$	5.57	5.45	0.028	0.029
Color parameters				
L^*	38.90	38.91	0.499	0.993
a^*	17.22	20.27	0.736	0.031
b^*	3.14	3.06	0.306	0.898
C^*	17.55	20.54	0.705	0.027
H°	1.38	1.42	0.019	0.391
Chemical composition (%)				
Moisture	68.50	63.08	1.316	0.033
Crude fat	7.94	16.28	1.782	0.012
Crude protein	21.10	19.87	0.382	0.113
Shear force	3.83	3.03	0.271	0.149
Marbling score	3.36	5.29	0.377	0.004

SEM: standard error of the means, $n = 7$ steers per treatment; $pH_{45\,min}$: pH at 45 min after slaughtered; $pH_{24\,h}$: pH at 24 h after slaughtered; L^*: lightness; a^*: redness; b^*: yellowness; C^*: chroma; H°: hue angle.

Serum metabolites

Daidzein supplementation reduced the concentrations of glucose ($P = 0.002$), blood urea nitrogen ($P = 0.019$), and all lipid metabolites including triglyceride ($P = 0.024$), total cholesterol ($P = 0.025$), HDL-C ($P = 0.024$), and LDL-C ($P = 0.019$) compared with control (Table 4).

Table 4 Effects of supplemental daidzein on serum metabolites in finishing steers

Item	Control	Daidzein	SEM	*P*
Glucose	13.36	7.85	1.050	0.002
Triglyceride	0.138	0.087	0.0118	0.024
Non-esterified fatty acid	145.49	89.40	13.749	0.037
Glycated serum protein	1.79	1.70	0.042	0.314
Total cholesterol	4.68	3.57	0.259	0.025
HDL-C	2.79	2.34	0.105	0.024
LDL-C	1.26	0.84	0.095	0.019
Blood urea nitrogen	4.84	3.86	0.222	0.019

SEM: standard error of the means, $n = 7$ steers per treatment; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.

Table 5 Effects of supplemental daidzein on lipogenic related enzyme activity of longissimus muscle in finishing steers

Item	Control	Daidzein	SEM	*P*
ICDH	31.10	51.10	3.598	0.001
G6PDH	10.36	5.96	1.091	0.038
MDH	188.82	151.19	17.793	0.309

SEM: standard error of the means, $n = 7$ steers per treatment; ICDH: isocitrate dehydrogenase; G6PDH: glucose-6-phosphate dehydrogenase; MDH: malate dehydrogenase; enzyme activities were expressed as nmol of NADPH produced (G6PDH, ICDH) or NADH spent (MDH) per minute and per milligram of protein.

The concentrations of glucose and non-esterified fatty acid ($P = 0.037$) were reduced by 5.51 and 56.09 mmol/L with added daidzein, respectively.

Lipogenic enzyme activities of longissimus muscles

The ICDH activity of longissimus muscles was greater for steers fed daidzein diets than those fed control diets ($P = 0.038$; Table 5). However, supplemental daidzein reduced G6PDH activity of muscle relative to control ($P = 0.038$). No difference was found between the two groups in MDH activity.

Discussion

It is well-known that daidzein can modulate the lipid metabolism of animals,[16] but little information is available regarding its effects on meat quality of beef cattle. This study was conducted to examine, whether daidzein, as it occurs in commonly used soy-based feed formulations for cattle, affects carcass characteristics and meat quality of beef cattle, when it has been supplemented to the finishing diet. Current results showed that supplemental daidzein reduced carcass fat proportion and backfat thickness of beef cattle. Considering few references about the relation between daidzein and beef cattle, this paper only compared our results with those obtained from mice or other animals. Similar to our results, Guo *et al*.[16] and Cao *et al*.[17] reported reduced epididymal and subcutaneous fat-pad weights and visceral fat content in mice in response to daidzein supplementation. Daidzein supplementation also increased carcass bone proportion of beef cattle in the present study. This is consistent with the study by Kaludjerovic and Ward,[18] in which daidzein exhibited a positive effect on the skeleton of mice. Daidzein could repress adipogenic differentiation of adipose tissue[19] and enhance osteoblast growth,[20,21] which maybe resulted in its positive effect on osteogenesis and negative effects on adipogenesis. In addition, daidzein can be metabolized to equol in the rumen by ruminal microorganisms.[10] Some studies found that equol could inhibit body fat not including intramuscular fat accumulation and improve bone formation.[22,23]

The differences in pH values of beef meat could affect some quality parameters including tenderness, flavor, water holding capacity, and liking that are important for the consumer.[24,25] With an increase in $pH_{24\,h}$, the tenderness of beef increased in some studies,[26] but tended to decrease in others.[24] A curvilinear relationship between pH and tenderness has also been reported by some authors.[27] These results suggested that the effect of meat pH on tenderness remains inconclusive. Current results showed that supplemental daidzein reduced the $pH_{24\,h}$ of longissimus muscle of beef cattle along with numerical decrease in shear force. Beef meat with $pH_{24\,h}$ greater than 5.5 is thought to be the result of less glycogen in muscle, and the consequent inability of muscle to accumulate adequate lactic acid concentration.[25] We hypothesized that decreased pH in current study might be due to the increased glycogen content in muscle caused by daidzein supplementation. Corresponding with our hypothesis, supplemental soy isoflavones including daidzein and genistein increased the muscle glycogen content of rats in the study by Malardé *et al*.[28]

The color of meat is the most important quality attribute influencing the consumer decision to purchase. Different colors observed in meat are largely related to the relative proportions of oxymyoglobin (bright red, the ferrous oxygenated form of myoglobin), myoglobin (dark red), and metmyoglobin (grey-brown).[29] When the meat color changes from bright red to brown, consumers associate these changes with a loss of quality or deterioration and consequently discriminate against the product.[30] Therefore, the stability of meat color needs to maintain the myoglobin in its ferrous oxygenated form rather than further oxygenated ferric metmyoglobin. Current results showed that supplementation with daidzein increased the redness (a^*) and chroma (C^*) of longissimus muscle, which meant that daidzein repressed the oxidation of meat in air and improved the stability of meat color. These results may be related to the intrinsic antioxidant activity of daidzein and equol.[31]

Daidzein has been shown to have a direct effect on lipid metabolism in our study. Concentrations of lipid metabolites in serum including non-esterified fatty acid, triglyceride, total cholesterol, HDL-C, and LDL-C were reduced by supplemental daidzein. The hypocholesterolemic effects of daidzein were also showed in previous studies using mice.[17,32,33] Daidzein inhibited subcutaneous fat accumulation of beef cattle in the current study. Theoretically, agents that interfere with the efficient deposition of fat into the adipose tissue or increase lipolysis of adipose tissue could

elevate the levels of non-esterified fatty acid and triglyceride in plasma or other tissues.[16] This theory was sustained by our study, in which triglyceride concentration in serum was reduced but the fat content (intramuscular fat) in longissimus muscle was increased greatly with supplementation of daidzein. As a result, marbling score of longissimus muscle was improved significantly by supplemental daidzein. These results suggested that daidzein can promote selectively deposition of fat into muscle tissues. Though many studies mentioned above showed the negative effect of daidzein on fat deposition in animals, some studies of *in vitro* and *in vivo* also found that supplementation with daidzein benefited synthesis of lipid. Daidzein and equol enhanced adipocyte differentiation, formation of lipid droplets, and lipid accumulation in murine and human preadipocytes.[7,8,34] Rehfeldt *et al*.[9] investigate the effects of dietary daidzein during late gestation on neonatal body composition and found that daidzein supplementation did not affect subcutaneous fat content but significantly elevated the concentration of fat in the whole piglet body. Similar to our results, Crespillo *et al*.[33] observed that supplemental daidzein tended to increase the fat content in skeletal muscle and reduced that in liver of male Wistar rats. A decrease in blood glucose concentration was observed in daidzein-supplied beef cattle, which was consistent with the results obtained from mice by Choi *et al*.[35] and Cao *et al*.[17] Daidzein could enhance insulin-stimulated glucose uptake in adipocytes by increasing the expression of insulin-sensitive glucose transporter 4 (GLUT4) and insulin receptor substrate 1 (IRS-1),[7] which may partly result in the lower blood glucose concentration in current study.

The enzymes including G6PDH, MDH, and ICDH are involved in the synthesis of NADPH for de novo fatty acid synthesis. Supplementation of daidzein increased ICDH activity in longissimus muscle in this study, which might be an explanation of the corresponding elevated muscular fat content. The activity of G6PDH is closely positively related to the deposition of intramuscular adipose tissue in beef cattle, and thus could be good indexes of marbling.[36] However, daidzein reduced the G6PDH activities compared to the control group in the present study. At this moment, we are not able to explain why the activity of G6PDH did not support the fat content in daidzein groups. These need to be elucidated in further studies. Such an inconsistency between G6PDH activity and fat accumulation has also been observed in other studies.[35]

In conclusion, supplemental daidzein reduced the backfat thickness, carcass fat content, concentrations of glucose and lipid metabolites in serum, and pH_{24}, but increased the ICDH activity, color, intramuscular fat content, and marbling score of longissimus muscle in finishing beef cattle. These results indicated that supplementation with daidzein in diets can reduce the subcutaneous fat content, promote the accumulation of intramuscular fat, and improve marbling and quality of beef meat. Daidzein should be a promising feed additive for production of high-quality beef meat.

Author contributions: X-HZ was responsible for the experimental design, writing the manuscript, and statistical analyses; Z-QY, L-BB, C-YW, S-Z, J-MG, C-BF, L-JX, and C-JL carried out the feeding study, responsible for all the analyses; M-RQ, project leader, was responsible for the administrative part, reviewed the manuscript. The authors state that there is no conflict of interest.

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