Effects of Daidzein on Production Performance, Antioxidant Capacity, and Immune Function in Growing Jinjiang Yellow Cattle (Postprint)

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Abstract

This experiment aimed to investigate the effects of daidzein on production performance, antioxidant capacity, and immune performance of growing Jinjiang cattle. Twenty growing Jinjiang cattle with good health status and body weight of $(140\$\pm5)kgwereselectedandrandomlydividedinto4groupswith5cattlepergroup.Thecontrolgroupwasfedabasal$
$to-foragerationof4:6),andtheexperimentalgroupswerefedexperimentaldietssupplementedwith100,200,and$
$1)Theaveragedailygainofeachexperimentalgroupwassignificantlyhigherthanthatofthecontrolgroup(P<$
$0.05),andthe400mg/kggroupwas126.6g/dhigherthanthecontrolgroup.2)Theapparentcrudeproteindigestibilit$
$0.05).3)Comparedwiththecontrolgroup,theserumtotalsuperoxidedismutase(T-$
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$0.05);theserumglutathioneperoxidase(GSH-Px)activityofeachexperimentalgroupshowedanincreasingtren$
the serum malondialdehyde (MDA) content of each experimental group was lower than that of the control group, but the difference was not significant (P>0.05). 4) The serum immunoglobulin (Ig) M content of each experimental group was significantly higher than that of the control group (P<0.05), and the 400 mg/kg group was significantly higher than the 100 mg/kg group (P<0.05); the serum IgG content of each experimental group was increased to a certain extent compared with the control group, but the differences were not significant (P>0.05). From the above results, it can be seen that dietary supplementation with an appropriate amount of daidzein can improve nutrient digestibility, promote growth, and enhance immune and antioxidant performance in growing Jinjiang cattle.

Full Text

Effects of Daidzein on Performance, Antioxidant Capacity and Immune Function of Growing Jinjiang Cattle

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Abstract

This experiment aimed to investigate the effects of daidzein on performance, antioxidant capacity, and immune function of growing Jinjiang cattle. Twenty healthy growing Jinjiang cattle with an initial body weight of $(140\$\pm5)kgwererandomlyallocatedintofourgroupswithfivecattlepergroup.Thecontrolgroupwasfedabasaldie$ 6), $whiletheexperimentalgroupswerefedthebasaldietsupplementedwith100, 200, and400mg/kgdaidzein, respe$ $daypre-trialperiodfollowedbya60-dayformaltrialperiod.Theresultsshowedthat:$ $1)Averagedailygaininallexperimentalgroupswassignificantlyhigherthanthatinthecontrolgroup(P <$ $0.05), withthe400mg/kggroupshowinganincreaseof126.6g/dcomparedtothecontrol.2)Apparentdigestibilityof$ $0.05).3)Comparedwiththecontrolgroup, serumtotalsuperoxidedismutase(T -$ $SOD)activityinallexperimentalgroupswassignificantlyincreased(P <$ $0.05); serumglutathioneperoxidase(GSH-Px)activityshowedanincreasingtrend(0.05 P \$0.10);$ and serum malondialdehyde (MDA) content was lower in experimental groups, though the difference was not significant (P>0.05). 4) Serum immunoglobulin (Ig) M content in all experimental groups was significantly higher than in the control group (P<0.05), with the 400 mg/kg group being significantly higher than the 100 mg/kg group (P<0.05). Serum IgG content in experimental groups increased to some extent compared with the control, but the differences were not significant (P>0.05). These results indicate that dietary supplementation with appropriate levels of daidzein can improve nutrient digestibility, promote growth, and enhance antioxidant capacity and immune function in growing Jinjiang cattle.

Keywords: beef cattle; daidzein; performance; antioxidant capacity; immune function

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Introduction

With China's continuous economic development and improving living standards, dietary patterns among the population are undergoing constant changes. Beef has gained popularity due to its low fat content and high protein content. Calf rearing represents a critical stage in beef cattle production, as this period is

associated with high mortality risk, and calf development significantly affects subsequent production performance. Therefore, during the calf rearing stage, it is essential not only to ensure that calves obtain all necessary nutrients for growth but also to enhance their immunity to guarantee healthy development. This approach ensures both economic benefits for producers and provides safe, high-quality beef for consumers.

Daidzein (DA), also known as daidzin or soybean flavone, is one of the major free aglycones among soybean isoflavone compounds. Its molecular formula is $C_{15}H_{10}O_4$ with a relative molecular mass of 254.24. Daidzein typically appears as a white, odorless, non-toxic powder that is insoluble in water but readily soluble in alcohols and ketones, and highly soluble in dimethyl sulfoxide. It was first isolated from soybeans in 1931. The structure of daidzein is similar to that of mammalian estrogens, conferring weak estrogenic biological activity. Additionally, research by Kaldas et al. has demonstrated that daidzein also exhibits anti-estrogenic effects.

As an estrogen-like natural bioactive substance, daidzein can regulate neuroendocrine function and influence hormone secretion levels, thereby improving feed utilization in male broilers and castrated male piglets, enhancing daily weight gain in male broilers, castrated male piglets, and Northeast fine-wool sheep. However, it shows no significant effect on the growth performance of female broilers and even inhibits growth in castrated female piglets. Daidzein has also been shown to improve nutrient digestibility in weaned piglets. Previous studies have demonstrated that daidzein possesses significant antioxidant capacity, and practical applications in animal production have shown that dietary supplementation with daidzein can substantially improve antioxidant capacity in Small-tailed Han sheep, aging laying hens, finishing pigs, and dairy cows. Research has also indicated that daidzein can significantly increase maternal antibody levels in newborn piglets, elevate the proportion of T lymphocyte $CD8^+$ and $CD4^+$ subpopulations, enhance humoral immune function in mammary tissue and systemically in sows, increase immunoglobulin (Ig) A and IgG content in plasma and milk of dairy cows, and boost lymphocyte responsiveness to phytohemagglutinin while significantly increasing the relative weight of immune organs in young roosters.

Previous applications of daidzein in animal production have primarily focused on poultry, dairy cows, and pigs, investigating the effects of dietary supplementation at different growth stages on performance and physiological-biochemical parameters to provide evidence for reducing production costs and improving efficiency. Numerous studies have shown that dietary daidzein supplementation can enhance antioxidant capacity and immune function, thereby promoting animal growth. Therefore, this experiment was designed to investigate the effects of dietary daidzein supplementation on performance, antioxidant capacity, and immune function in growing Jinjiang cattle, providing a scientific basis for the application of daidzein in beef cattle production.

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1. Materials and Methods

1.1 Experimental Materials, Duration and Location Daidzein (purity >98%) was purchased from Shaanxi Ciyuan Biotech Co., Ltd. The experiment was conducted from April 25 to July 3, 2015, at the cattle farm of Gao'an Yufeng Agricultural and Livestock Co., Ltd. in Jiangxi Province. The trial consisted of a 10-day pre-trial period and a 60-day formal trial period, totaling 70 days.

1.2 Experimental Design and Diets Twenty healthy 6-month-old Jinjiang cattle bull calves with a body weight of (140$±$5) kg were randomly divided into four groups with five cattle per group. The control group received the basal diet, while the experimental groups received the basal diet supplemented with 100, 200, and 400 mg/kg daidzein (air-dry basis), respectively. The basal diet had a concentrate to forage ratio of 4:6, and dry matter (DM) intake was restricted to 3.98 kg/d for all groups. The nutrient levels of the diet for growing Jinjiang cattle during the formal trial period were formulated according to China' s "Feeding Standard of Beef Cattle" (NY/T 815-2004). The concentrate consisted of corn, soybean meal, sodium bicarbonate, salt, and premix, while the roughage was rice straw. The composition and nutrient levels of the basal diet are presented in Table 1 .

Table 1 Composition and nutrient levels of the basal diet (air-dry basis) %

Item	Content
Ingredients	
Corn	
Soybean meal	
NaCl	
$NaHCO_3$	
Premix[1])	
Rice straw	
Total	
Nutrient levels[2])	
NEmf/(MJ/kg)	
DM	
CP	
NDF	
ADF	
Ash	
Ca	

[1]) Each kilogram of premix contained: VA 150,000 IU, VD_3 20,000 IU, VE 3,000 IU, Fe 3,200 mg, Mn 1,500 mg, Zn 2,000 mg, Cu 650 mg, I 35 mg, Se 10 mg, Co 10 mg, Ca 130 g, P 30 g.

[2]) NEmf, Ca, and P were calculated values, while others were measured values.

1.3 Measurement Indicators and Methods

1.3.1 Growth Performance Measurement On days 1 and 60 of the formal trial period at 08:30, calves were weighed after fasting to record initial and final body weights for calculating average daily gain (ADG) during the trial period. ADG (g/d) = (final weight - initial weight) / number of trial days.

1.3.2 Fecal Sample Collection, Nutrient Analysis and Digestibility Calculation The digestibility trial was conducted using the internal indicator method during the last 3 days of the experiment. Three cattle with similar body weight were selected from each group. Fresh fecal samples were collected at regular intervals three times daily, mixed and weighed for each group at 18:00. Using the quartering method, two 500 g samples of fresh feces were taken as analytical samples. One sample was mixed with 20 mL of 10% dilute sulfuric acid to prevent ammonia nitrogen loss and stored at 4 °C. After collection, samples from each replicate over three consecutive days were mixed and dried in a 65 °C oven. Dried samples were ground using a sealed sample grinder, passed through a 40-mesh sieve, and stored in sealed bags for later analysis. The sample with dilute sulfuric acid was used for nitrogen analysis, while the other sample was used for nutrient content determination.

Nutrient content in diets and feces was determined according to "Feed Analysis and Feed Quality Detection Technology" [19]. Dry matter, crude protein (CP), organic matter (OM), neutral detergent fiber (NDF), and acid detergent fiber (ADF) were measured. Acid-insoluble ash (AIA) content in diets and feces was determined using the 4 mol/L HCl-insoluble ash method.

Apparent nutrient digestibility (%) = 1 - (b×c)/(a×d), where: a represents the nutrient content (%) in the diet sample; b represents the nutrient content (%) in the fecal sample; c represents the indicator (AIA) content (%) in the diet sample; and d represents the indicator (AIA) content (%) in the fecal sample.

1.3.3 Antioxidant Capacity and Immune Function Measurement On day 60 of the formal trial period at 08:30, 15 mL of blood was collected from the jugular vein of each calf using vacuum tubes. The blood was placed in tubes without anticoagulant, allowed to stand at an angle for 30 minutes, then centrifuged at 3,500 r/min for 10 minutes. The supernatant was collected to prepare serum, which was stored at -20 °C. Serum malondialdehyde (MDA) content and activities of glutathione peroxidase (GSH-Px) and total superoxide dismutase (T-SOD) were measured according to kit instructions (Nanjing Jiancheng Bioengineering Institute). Serum IgA, IgM, and IgG were measured by immunoturbidimetry.

1.4 Data Processing and Analysis All experimental data were initially processed using Excel 2003. One-way ANOVA in SPSS 17.0 was used for significance analysis. Duncan' s multiple comparison test was applied when signif-

icant differences were detected. P<0.05 indicated significant difference, while 0.05≤P$ $0.10 indicated a trend toward significance.

2. Results

2.1 Effects of Dietary Daidzein Supplementation on Growth Performance of Growing Jinjiang Cattle As shown in Table 2 , average daily gain (ADG) in all experimental groups differed significantly from the control group (P<0.05). Compared with the control group, ADG increased by 19.05%, 21.05%, and 29.19% in the 100, 200, and 400 mg/kg groups, respectively. The 400 mg/kg group was significantly higher than the 100 mg/kg group (P<0.05), while no significant differences were observed between the 200 mg/kg group and other experimental groups (P>0.05).

Table 2 Effects of dietary supplementation of daidzein on average daily gain of growing Jinjiang cattle (g/d)

Item	Daidzein supplemental levels (mg/kg)	P-value
	0	100
ADG	433.7	516.3

In the same row, values with the same or no letter superscripts mean no significant difference (P>0.05), while different small letter superscripts mean significant difference (P<0.05). The same as below.

2.2 Effects of Dietary Daidzein Supplementation on Nutrient Digestibility of Growing Jinjiang Cattle As shown in Table 3 , dietary daidzein supplementation significantly improved the apparent digestibility of crude protein in growing Jinjiang cattle compared with the control group (P<0.05). The 100, 200, and 400 mg/kg groups showed improvements of 4.79%, 5.52%, and 5.11%, respectively, though no significant differences were observed among experimental groups (P>0.05). Apparent digestibility of organic matter, neutral detergent fiber, and acid detergent fiber did not differ significantly between experimental and control groups (P>0.05).

Table 3 Effects of dietary supplementation of daidzein on apparent digestibility of nutrients of growing Jinjiang cattle (%)

Item	Daidzein supplemental levels (mg/kg)	P-value
	0	100
OM	65.78	68.93
CP	65.78	68.93
NDF		

Item	Daidzein supplemental levels (mg/kg)	P-value
ADF		

2.3 Effects of Dietary Daidzein Supplementation on Serum Antioxidant Indices of Growing Jinjiang Cattle As shown in Table 4 , serum MDA content in experimental groups was somewhat lower than in the control group, but the difference was not significant (P>0.05). Serum T-SOD activity increased continuously with increasing dietary daidzein levels, with all experimental groups being significantly higher than the control group (P<0.05), peaking in the 400 mg/kg group. Serum GSH-Px activity in experimental groups showed an increasing trend compared with the control group (0.05≤P$ $0.10).

Table 4 Effects of dietary supplementation of daidzein on serum antioxidant indices of growing Jinjiang cattle

Item	Daidzein supplemental levels (mg/kg)	P-value
	0	100
MDA (nmol/mL)		
T-SOD (U/mL)	146.01	167.75
GSH-Px (U/mL)		

2.4 Effects of Dietary Daidzein Supplementation on Serum Immunological Indices of Growing Jinjiang Cattle As shown in Table 5 , compared with the control group, serum IgG content in the 100, 200, and 400 mg/kg groups increased by 8.60%, 6.38%, and 16.43%, respectively, though no significant differences were observed among groups (P>0.05). Serum IgM content in the 200 and 400 mg/kg groups (2.67 and 2.82 g/L, respectively) was significantly higher than in the control group (P<0.05), but did not differ significantly between these two groups (P>0.05). The 100 mg/kg group had higher serum IgM content than the control, but the difference was not significant (P>0.05). No significant differences in serum IgA content were observed among any groups (P>0.05).

Table 5 Effects of dietary supplementation of daidzein on serum immunological indexes of growing Jinjiang cattle

Item	Daidzein supplemental levels (mg/kg)	P-value
	0	100
IgG		
IgM	2.41	2.55
IgA		

3. Discussion

3.1 Effects of Dietary Daidzein Supplementation on Growth Performance of Growing Jinjiang Cattle Numerous studies have demonstrated that daidzein promotes growth in male animals, though its effects on female and castrated animals are inconsistent. Guo et al. [5] investigated the effects of adding 5, 10, and 15 mg/kg daidzein to basal diets of 1-day-old Avian broilers, finding that daidzein significantly increased average body weight gain, feed utilization, and average daily feed intake in male broilers but had no significant effect on female broilers. Guo et al. [6] fed weaned piglets (both sexes castrated) a basal diet supplemented with 5 mg/kg daidzein for 30 days, revealing obvious gender differences in weight gain: castrated male piglets showed a 59.15% increase in weight gain, while castrated female piglets showed a 26.39% decrease. The results of the present study are generally consistent with previous findings, as dietary daidzein supplementation significantly improved ADG in bull calves across all experimental groups. Han [20] systematically investigated the mechanism by which daidzein promotes male animal growth, demonstrating that its weak estrogenic activity allows it to bind with estradiol receptors in the hypothalamus and pituitary, influencing the gonadal and growth axes of the neuroendocrine system. This binding promotes testosterone secretion in male animals while increasing growth hormone production and release from the pituitary, stimulating hepatic growth hormone receptor development and insulin-like growth factor-I production, thereby promoting animal growth and development.

3.2 Effects of Dietary Daidzein Supplementation on Nutrient Apparent Digestibility of Growing Jinjiang Cattle Li et al. [9] investigated the effects of supplementing weaned piglet diets with 0 (control), 5, 10, and 15 mg/kg soybean isoflavones on nutrient apparent digestibility, reporting that crude protein apparent digestibility in all experimental groups was higher than in the control group, with the 10 mg/kg group showing a significant difference. The present study yielded consistent results, demonstrating that daidzein supplementation significantly improved crude protein apparent digestibility in growing Jinjiang cattle. Research has reported that soybean isoflavones can increase serum insulin content and decrease serum glucose levels, thereby promoting the decomposition of nutrients in feed to provide energy for the body and improving dietary protein and fat utilization [19].

Liu et al. [21] found that supplementing dairy cow diets with 200 mg/d daidzein significantly increased rumen xylanase, carboxymethyl cellulase, salicinase, and microcrystalline cellulase activities, indicating that daidzein at appropriate levels can promote the degradation of fibrous feed materials. However, the present study' s results were inconsistent with these findings, as the 100 mg/kg group showed a decreasing trend in neutral detergent fiber apparent digestibility compared with the control. The authors speculate that this may be due to reduced abundance of fiber-degrading bacteria in the rumen at this supplementation

level, leading to decreased neutral detergent fiber digestibility.

3.3 Effects of Dietary Daidzein Supplementation on Antioxidant Capacity of Growing Jinjiang Cattle Under normal physiological conditions, the production and clearance of free radicals in animal bodies maintain a dynamic balance, keeping free radical levels within an appropriate range [22]. This defense system consists primarily of enzymatic and non-enzymatic components. The enzymatic system includes antioxidant enzymes such as superoxide dismutase (SOD), GSH-Px, and catalase (CAT), while the non-enzymatic system comprises vitamin E, vitamin C, β-carotene, metalloproteins, and cysteine. Generally, high activities of CAT, SOD, and GSH-Px combined with low MDA content indicate good antioxidant capacity [23]. Xie et al. [24] reported that soybean products possess antioxidant properties and can scavenge hydrogen peroxide and superoxide anions, attributing these effects to daidzein. Liu et al. [11] supplemented dairy cow diets with daidzein and found that serum CAT, SOD, and GSH-Px activities in the experimental groups were significantly increased while serum MDA content was significantly decreased compared with the control group. Cheng et al. [13] found that daidzein significantly decreased serum MDA content and significantly increased serum SOD and GSH-Px activities in finishing pigs. The present study demonstrated that dietary daidzein supplementation significantly increased serum T-SOD activity in growing Jinjiang cattle, while GSH-Px activity showed an increasing trend and serum MDA content was lower in all experimental groups compared with the control. These findings suggest that dietary supplementation with appropriate levels of daidzein can improve antioxidant capacity in livestock and poultry.

3.4 Effects of Dietary Daidzein Supplementation on Immune Function of Growing Jinjiang Cattle Daidzein belongs to the class of plant-derived immunomodulators that can act on estrogen receptors in target tissues or cells, often exerting immunomodulatory effects through neuro-endocrine pathways that regulate estrogen receptors in immune organs or cells [25-26]. Appropriate dietary supplementation with daidzein can promote lymphocyte proliferation and enhance immune function, while excessive supplementation may inhibit lymphocyte proliferation and suppress immune function [27]. Zheng et al. [16] and Yang et al. [17] found that daidzein increased IgG and IgA content in plasma and milk of dairy cows. Liu et al. [28] reported that supplementing diets of heat-stressed dairy cows in late lactation with 300 and 400 mg/d daidzein resulted in significantly higher blood IgG content compared with the control and 200 mg/d groups. The present study showed that dietary daidzein supplementation significantly increased serum IgM content in growing Jinjiang cattle, with a tendency to increase serum IgG content, though it had no significant effect on serum IgA content. These results indicate that daidzein can improve immune function in calves.

Conclusions

1. Dietary supplementation with daidzein can significantly improve average daily gain in growing Jinjiang cattle, with 400 mg/kg showing the best growth-promoting effect.
2. Dietary supplementation with daidzein can significantly improve apparent digestibility of crude protein in growing Jinjiang cattle.
3. Dietary supplementation with daidzein can improve serum antioxidant capacity in growing Jinjiang cattle.
4. Dietary supplementation with daidzein can enhance immune function in growing Jinjiang cattle, with 200 and 400 mg/kg supplementation significantly increasing serum IgM content.

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