Effects of Dietary Flaxseed Supplementation on Fatty Acid Composition and Related Gene Expression in Muscle Tissue and Subcutaneous Fat of Yanbian Cattle Postprint

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Abstract

This experiment aimed to investigate the effects of dietary flaxseed supplementation on fatty acid composition and related gene expression in muscle tissue and subcutaneous adipose tissue of Yanbian cattle. Thirty Yanbian cattle steers with an average body weight of approximately 480 kg were randomly divided into 3 groups (n=10): the control group (CON group) was fed a basal diet, while the experimental groups were fed experimental diets supplemented with 8% whole flaxseed (WPS group) and 8% ground flaxseed (PS group) in the basal diet, respectively. After the experiment, the cattle were slaughtered, and longissimus dorsi muscle and subcutaneous adipose tissue were collected to determine fatty acid composition and related gene expression. The pre-trial period was 10 d, and the formal trial period was 180 d. The results showed: 1) Compared with the CON group, the average daily gain and average daily feed intake of the WPS and PS groups were significantly increased ($P<0.05$), but feed conversion efficiency showed no significant difference ($P>0.05$). 2) Compared with the CON group, in muscle tissue, the contents of saturated fatty acids C16:0 and C18:0 in the WPS and PS groups were significantly decreased ($P<0.05$), while the contents of monounsaturated fatty acids C18:1n-9, C18:2cis-9,trans-11, and C18:2trans-10,cis-12 were significantly increased ($P<0.05$); in subcutaneous adipose tissue, the contents of monounsaturated fatty acids C18:1n-9, C18:2n-6, C18:2cis-9,trans-11, and C18:3n-3 in the WPS and PS groups were significantly increased ($P<0.05$), while the contents of saturated fatty acids C10:0, C12:0, C16:0, and C18:0 were significantly decreased ($P<0.05$). 3) Compared with the CON group, in muscle tissue, the expression levels of G protein-coupled receptor (GPR43), sterol regulatory element-binding protein (SREBP), CCAAT/enhancer-binding protein (CEBP), and CCAAT/enhancer-binding protein (CEBP) genes in the WPS and PS groups were significantly decreased ($P<0.05$); in subcutaneous adipose

tissue, the expression levels of the four genes in the PS and WPS groups were significantly increased (P<0.05). In conclusion, dietary flaxseed supplementation can regulate fatty acid composition and related gene expression in muscle tissue and subcutaneous adipose tissue of Yanbian cattle. Under the conditions of this experiment, supplementation with 8% ground flaxseed (PS group) showed better effects, which is beneficial for improving growth performance and regulating lipid metabolism in Yanbian cattle.

Full Text

Effects of Dietary Flax Seed Supplementation on Fatty Acid Composition and Related Gene Expression in Muscle Tissue and Subcutaneous Fat of Yanbian Yellow Cattle

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Abstract

This study investigated the effects of dietary flax seed supplementation on fatty acid composition and related gene expression in muscle tissue and subcutaneous fat of Yanbian yellow cattle. Thirty healthy Yanbian yellow cattle steers with an average body weight of approximately 480 kg were randomly divided into three groups (n=10). The control group (CON) received a basal diet, while the experimental groups received the basal diet supplemented with 8% whole flax seed (WPS group) and 8% broken flax seed (PS group), respectively. The pre-trial period lasted 10 days, followed by a formal experimental period of 180 days. The results demonstrated that: (1) Compared with the CON group, both WPS and PS groups exhibited significantly increased average daily gain and average daily feed intake (P<0.05), though feed efficiency remained unchanged (P>0.05). (2) In muscle tissue, saturated fatty acids (C16:0 and C18:0) decreased significantly (P<0.05), while monounsaturated fatty acids (C18:1n-9, C18:2cis-9,trans-11, and C18:2trans-10,cis-12) increased significantly (P<0.05). In subcutaneous fat, monounsaturated fatty acids (C18:1n-9, C18:2n-6, C18:2cis-9,trans-11, and C18:3n-3) increased significantly (P<0.05), whereas saturated fatty acids (C10:0, C12:0, C16:0, and C18:0) decreased significantly (P<0.05). (3) In muscle tissue, the expression of G protein-coupled receptor 43 (GPR43), sterol regulatory element-binding protein (SREBP), CCAAT enhancer-binding protein (CEBP), and CCAAT enhancer-binding protein (CEBP) decreased significantly (P<0.05). Conversely, in subcutaneous fat, expression of these four genes increased significantly (P<0.05). These findings indicate that dietary flax seed supplementation can effectively regulate fatty acid composition and related gene expression in both muscle tissue and subcutaneous fat of Yanbian yellow cattle. Under the conditions of this study, supplementation with 8% broken

flax seed (PS group) demonstrated superior effects on growth performance and lipid metabolism regulation.

Keywords: Yanbian yellow cattle; fatty acids; gene expression; flax seed

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1 Materials and Methods

1.1 Experimental Animals

Thirty healthy Yanbian yellow cattle steers with an average body weight of 480 kg and similar body condition were selected for the experiment. All animals were provided by the Yanbian Yellow Cattle Science and Technology Demonstration Park of Yanbian University.

1.2 Experimental Design

The thirty steers were randomly divided into three groups of ten animals each. The control group (CON) received a basal diet, while the experimental groups received the basal diet supplemented with 8% whole flax seed (WPS group) and 8% broken flax seed (PS group), respectively. All other conditions remained consistent across groups to control for single variables. The experiment consisted of a 10-day pre-trial period followed by a 180-day formal trial period. The composition and nutrient levels of the basal diet are presented in Table 1 .

1.3 Feeding Management

The barn was maintained in a clean condition with adequate lighting and appropriate temperature. Relative humidity was maintained at 55%. Animals had free access to water at all times and were fed a mixed concentrate-forage diet at 05:00 and 17:00 daily, with feed provided ad libitum.

1.4 Sample Collection and Processing

Upon completion of the trial, three steers from each group with similar body condition and body weight close to their group average were selected for slaughter after a 24-hour fast. Following slaughter, the longissimus dorsi muscle (between the 12th and 13th ribs) and subcutaneous fat tissue were collected. One portion was sealed in bags and stored at -20°C for fatty acid analysis, while another portion was preserved in liquid nitrogen for gene expression analysis.

1.5.1 Growth Performance Measurement

Body weight was measured at the beginning and end of the trial period. Animals were weighed on two consecutive days before morning feeding, and the average values were used to calculate average daily gain. Feed intake was measured daily

by precisely recording the amount of feed offered and refused for each group, with dry matter content determined to calculate average daily feed intake per animal. Feed efficiency was calculated using the formula: Feed efficiency (%) = (Average daily gain / Average daily feed intake) × 100.

1.5.2 Determination of Fatty Acid Content in Muscle and Subcutaneous Fat

Lipid extraction was performed by grinding the sample and placing 1 g in a 50 mL centrifuge tube. One milliliter of internal standard was added, followed by 15 mL of extraction solution (chloroform:methanol = 2:1). The mixture was thoroughly stirred using a vortex mixer, rinsed with hot water, and then rinsed again with chloroform:methanol to ensure complete extraction. After one hour of oscillation, 10 mL of 0.88% sodium chloride was added, followed by vortex oscillation and centrifugation. The supernatant was collected for esterification.

For the esterification process, the supernatant in the esterification tube was dried under nitrogen at 55°C. After cooling to room temperature, 4 mL of hydrochloric acid-methanol was added and catalyzed in a 75°C water bath. The mixture was cooled to room temperature again, and 2 mL of n-hexane was added. After vortex oscillation and standing for 0.5 hours, 4 mL of saturated sodium chloride was added. The mixture was oscillated, allowed to stand for layering, centrifuged, and the supernatant was collected for fatty acid content determination using an Agilent GC-7890A gas chromatograph.

1.5.3 Determination of Related Gene Expression in Muscle and Subcutaneous Fat

Total RNA was extracted from muscle and fat tissues using the Promega Eastep total RNA extraction kit. According to the instructions of the TaKaRa RR047A (Prime Script™ RT reagent Kit with gDNA Eraser), a 20 L reverse transcription system was established to reverse transcribe the extracted total RNA into cDNA, which was stored at 4°C.

Real-time fluorescent quantitative PCR (RT-PCR) was performed according to the instructions of the TaKaRa RR420 (SYBR® Premix Ex Taq™) kit on an ABI Veriti® 96-Well Thermal Cycler. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as the housekeeping gene, with primer sequences listed in Table 2 . The reaction system (20 L) contained 10×Buffer (10 L), forward and reverse primers (0.5 L each), ROX (0.5 L), Reference Dye II (0.5 L), cDNA template (1 L), and dH2O (7.5 L). The PCR conditions were: pre-denaturation at 95°C for 15 s, followed by 40 cycles of denaturation at 95°C for 5 s, annealing at 54-60°C for 30 s, and extension at 72°C for 30 s. Relative quantification was calculated using the 2^(-ΔΔCt) method [10].

1.6 Data Processing

All experimental data were analyzed using SPSS 19.0 statistical software with one-way ANOVA. Results are expressed as mean ± standard error. Differences were considered significant at $P<0.05$ and non-significant at $P>0.05$.

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2 Results

2.1 Effects of Dietary Flax Seed on Daily Fatty Acid Intake in Yanbian Yellow Cattle

As shown in Table 3 , supplementation with flax seed significantly increased the daily intake of various fatty acids compared with the CON group. Notably, the contents of C6:0, C16:0, C18:0, C18:2n-6, C18:3n-3, and C18:3n-6 increased significantly ($P<0.05$).

2.2 Effects of Dietary Flax Seed on Growth Performance of Yanbian Yellow Cattle

Table 4 presents the effects on growth performance. Compared with the CON group, the average daily gain in the WPS and PS groups increased by 6.25% and 16.67%, respectively ($P<0.05$), though the difference between WPS and PS groups was not significant ($P>0.05$). Similarly, average daily feed intake increased by 6.02% and 15.05% in the WPS and PS groups, respectively ($P<0.05$), with no significant difference between the two experimental groups ($P>0.05$). Feed efficiency did not differ significantly among groups ($P>0.05$).

2.3 Effects of Dietary Flax Seed on Fatty Acid Composition in Muscle Tissue of Yanbian Yellow Cattle

As shown in Table 5 , compared with the CON group, the WPS and PS groups exhibited significantly decreased saturated fatty acid contents, primarily C16:0 and C18:0 ($P<0.05$). Conversely, monounsaturated fatty acid contents, particularly C18:1n-9, C18:2cis-9,trans-11, and C18:2trans-10,cis-12, increased significantly ($P<0.05$). Consequently, the ratio of monounsaturated to saturated fatty acids (MUFA/SFA) increased significantly ($P<0.05$).

2.4 Effects of Dietary Flax Seed on Fatty Acid Composition in Subcutaneous Fat of Yanbian Yellow Cattle

Table 6 shows that compared with the CON group, the WPS and PS groups had significantly increased monounsaturated fatty acids including C18:1n-9, C18:2n-6, C18:2cis-9,trans-11, and C18:3n-3 ($P<0.05$). In contrast, saturated fatty acids C10:0, C12:0, C16:0, and C18:0 decreased significantly ($P<0.05$).

2.5 Effects of Dietary Flax Seed on Related Gene Expression in Muscle Tissue of Yanbian Yellow Cattle

As presented in Table 7 , compared with the CON group, expression of all four target genes decreased significantly in the WPS and PS groups (P<0.05). Specifically, GPR43 and CEBP expression decreased significantly in the PS group (P<0.05), while SREBP and CEBP expression decreased significantly in the WPS group (P<0.05).

2.6 Effects of Dietary Flax Seed on Related Gene Expression in Subcutaneous Fat of Yanbian Yellow Cattle

Table 8 demonstrates that compared with the CON group, expression of all four genes increased significantly in the PS and WPS groups (P<0.05). Notably, GPR43 and CEBP expression increased significantly in the WPS group (P<0.05), while SREBP and CEBP expression increased significantly in the PS group (P<0.05).

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3 Discussion

3.1 Effects of Dietary Flax Seed on Growth Performance of Yanbian Yellow Cattle

In this study, different processing methods of flax seed supplementation increased the average daily gain of Yanbian yellow cattle, consistent with Raes et al. [10] who reported improved daily weight gain in beef cattle fed various processed flax seed diets. Landblom et al. [11] also demonstrated that dietary inclusion of 12.5% flax seed significantly increased average daily gain in beef cattle. Additionally, Li et al. [12] showed that flax seed supplementation could enhance energy utilization efficiency, thereby increasing daily weight gain. The present results also indicated that flax seed supplementation increased average daily feed intake, which aligns with the findings of Xu [13]. The increased content of unsaturated fatty acids from flax seed may enhance feed intake and daily gain without significantly affecting feed efficiency. However, current literature presents inconsistent results regarding the effects of flax seed supplementation on feed intake. For instance, Cooper et al. [14] reported minimal impact of oilseed crops on animal feed intake and growth performance. Therefore, further investigation is warranted to clarify the relationship between flax seed supplementation and feed intake in beef cattle.

3.2 Effects of Dietary Flax Seed on Fatty Acid Composition in Muscle Tissue and Subcutaneous Fat of Yanbian Yellow Cattle

Flax seed contains substantial oil content, with unsaturated fatty acids comprising up to 80% of total lipids, predominantly -linolenic acid and linoleic acid [15]. In the rumen of ruminants, linolenic acid undergoes incomplete microbial

hydrogenation to produce conjugated fatty acids (CLA and CLNA), which are biologically important and represent promising research targets. Conjugated fatty acids can be synthesized through two pathways: endogenous synthesis and ruminal biohydrogenation. Linolenic acid serves as a precursor for endogenous synthesis of conjugated linolenic acid in ruminants. Both -linolenic acid and linoleic acid from flax seed are precursors for n-3 and n-6 polyunsaturated fatty acids, respectively, and are considered essential fatty acids as animals cannot synthesize them de novo [16]. Luo et al. [17] reported that dietary n-3 polyunsaturated fatty acids could regulate the expression of genes related to lipid metabolism, thereby influencing body fat deposition.

The present study revealed that compared with the CON group, the WPS and PS groups exhibited significantly decreased saturated fatty acids (C16:0 and C18:0) and increased monounsaturated fatty acids (C18:1n-9, C18:2cis-9,trans-11, and C18:2trans-10,cis-12) in muscle tissue. Similarly, in subcutaneous fat, monounsaturated fatty acids (C18:1n-9, C18:2n-6, C18:2cis-9,trans-11, and C18:3n-3) increased significantly while saturated fatty acids (C16:0 and C18:0) decreased significantly. These findings are consistent with Zhang et al. [18] who reported that dietary oilseed supplementation in dairy cows reduced medium-chain fatty acids (C10:0, C12:0, C14:0, and C16:0) while increasing C18:1 and t11C18:1 content in milk fat. The results demonstrate that flax seed supplementation effectively altered fatty acid composition by enhancing the ruminal biohydrogenation of linolenic acid, thereby increasing unsaturated fatty acid content. This suggests that flax seed supplementation promotes dietary lipid uptake while controlling fatty acid biosynthesis in adipose tissue, corroborating the findings of Yu et al. [19].

3.3 Effects of Dietary Flax Seed on Related Gene Expression in Muscle Tissue and Subcutaneous Fat of Yanbian Yellow Cattle

The expression of GPR43, SREBP, and CEBP genes can be influenced by fatty acid composition, as these genes participate in the regulatory mechanisms of adipocyte synthesis and differentiation and are closely associated with lipid droplet accumulation [20]. GPR43, a short-chain fatty acid receptor, is widely expressed in various tissues including adipose tissue, gastrointestinal tract, and immune cells, and can be activated by short-chain fatty acids [21]. Studies in mice have shown that GPR43 is predominantly expressed in white adipose tissue, with higher expression levels in adipocytes compared to vascular stromal cells [22]. GPR43 belongs to the G protein-coupled receptor family and plays a crucial regulatory role in mediating intercellular signal transduction. Due to its specific chromosomal location, it is considered a specific receptor, also known as a free fatty acid receptor [23]. Its expression in multiple tissues including adipose tissue, immune cells, and lung endows it with diverse biological functions, participating in cellular lipid metabolism and immune cell differentiation. Short-chain fatty acids (SCFAs) not only provide energy but also exert important physiological regulatory functions, including modulation of cell prolif-

eration, differentiation, apoptosis, immune response, and lipid metabolism [24]. Research has demonstrated that GPR43 serves as a receptor for SCFAs, and the binding of SCFAs to GPR43 elicits important physiological functions. GPR43 is closely correlated with adipocyte differentiation.

The present results showed that compared with the CON group, GPR43 expression decreased in muscle tissue but increased in subcutaneous fat of the WPS and PS groups. The concurrent increase in monounsaturated fatty acids and decrease in saturated fatty acids, along with altered GPR43 expression, suggest that flax seed supplementation modifies fatty acid composition and regulates lipid metabolism in Yanbian yellow cattle. The higher GPR43 expression in adipose tissue and lower expression in muscle tissue indicate tissue-specific regulatory effects, which are consistent with the findings of Wang [25].

SREBP is a crucial nuclear transcription factor closely associated with the regulation of lipogenic genes. Numerous studies have shown that SREBP can directly activate more than 30 genes involved in fatty acid, cholesterol, triglyceride, and phospholipid biosynthesis and uptake, as well as NADPH expression [26]. Increased expression of SREBP1 or SREBP2 significantly promotes cellular lipid droplet accumulation [27]. Li et al. [28] reported that SREBP intron 5 was significantly correlated with C16:1, C18:0, SFA, triglycerides, and the C16 fatty acid unsaturation index. In this study, dietary flax seed supplementation significantly increased SREBP expression while decreasing saturated fatty acids (C16:1 and C18:0) and increasing monounsaturated fatty acids (C18:1n-9, C18:2n-6, C18:2cis-9,trans-11, and C18:3n-3), indicating a correlation between gene expression changes and fatty acid profile alterations.

CEBPs are transcription factors that play important regulatory roles in adipocyte generation and differentiation [29]. Both CEBP and CEBP belong to the CEBP family and are essential for growth, differentiation, and metabolism in adipose and other tissues [30]. CEBP regulates transcription through both activation and repression, and plays important roles in cell proliferation, differentiation, energy metabolism, and signal transduction [31]. The present results showed that dietary flax seed supplementation significantly increased CEBP and CEBP expression, concurrent with significantly increased monounsaturated fatty acid content, suggesting that flax seed supplementation can regulate both fatty acid composition and related gene expression.

4 Conclusions

Based on the results of this study, dietary flax seed supplementation in Yanbian yellow cattle not only increased average feed intake and average daily gain but also altered fatty acid composition in muscle and adipose tissues while regulating the expression of GPR43, SREBP, CEBP , and CEBP genes. Under the experimental conditions employed, supplementation with 8% broken flax seed (PS group) demonstrated superior effects compared to whole flax seed, proving

beneficial for improving growth performance and regulating lipid metabolism in Yanbian yellow cattle.

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