

## Effect of Replacing Distiller' s Grains with Sweet Potato Residue on Intramuscular Fat Deposition-Related Gene Expression in Finishing Cattle: Postprint

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### Abstract

This experiment aimed to investigate the effects of substituting sweet potato residue for distiller' s grains on the expression of genes related to intramuscular fat (IMF) deposition in fattening cattle. Thirty crossbred castrated male fattening cattle (Simmental  $\times$  local yellow cattle) were randomly divided into 3 groups with 10 cattle per group, with one cattle per replicate. The control group (Group A) was fed a basal diet, while experimental Groups B and C were fed diets in which 50% and 100% of the distiller' s grains in the basal diet were replaced by sweet potato residue, respectively, for an experimental period of 8 weeks. The results showed: 1) The average daily gain and IMF content in Group C were significantly lower than those in Groups A and B ( $P < 0.05$ ). 2) The serum contents of triglycerides, total cholesterol, high-density lipoprotein, and low-density lipoprotein in Group C were significantly lower than those in Group A ( $P < 0.05$ ). 3) The activities of fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) in the longissimus dorsi muscle of Group C were significantly lower than those in Groups A and B ( $P < 0.05$ ), while the activities of hormone-sensitive lipase (HSL) and carnitine palmitoyltransferase-1 (CPT-1) were significantly higher than those in Groups A and B ( $P < 0.05$ ). The activities of FAS and ACC in the longissimus dorsi muscle of Group B were significantly lower than those in Group A ( $P < 0.05$ ), while the activities of HSL and CPT-1 were significantly higher than those in Group A ( $P < 0.05$ ). 4) The gene expression levels of sterol regulatory element-binding protein-1 (SREBP-1), FAS, ACC, and peroxisome proliferator-activated receptor (PPAR) in the longissimus dorsi muscle of Group C were significantly lower than those in Groups A and B ( $P < 0.05$ ), while the gene expression levels of HSL and CPT-1 were significantly higher than those in Groups A and B ( $P < 0.05$ ). The gene expression levels of SREBP-1, FAS, ACC, and PPAR in the longissimus dorsi muscle of

Group B were significantly lower than those in Group A ( $P < 0.05$ ), while the gene expression levels of HSL and CPT-1 were significantly higher than those in Group A ( $P < 0.05$ ). These results indicate that increasing the proportion of sweet potato residue substituting for distiller's grains can downregulate the expression of fatty acid synthesis-related genes (SREBP-1, FAS, ACC, and PPAR) and upregulate the expression of lipolysis-related genes (HSL and CPT-1) in the longissimus dorsi muscle of fattening cattle, thereby reducing IMF deposition in the longissimus dorsi muscle.

## Full Text

### Effects of Sweet Potato Residue Replacing Distiller's Grains on Intramuscular Fat Deposition-Related Gene Expression in Fattening Cattle

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#### Abstract

This experiment investigated the effects of replacing distiller's grains with sweet potato residue on intramuscular fat (IMF) deposition-related gene expression in fattening cattle. Thirty crossbred fattening steers (Simmental  $\times$  local yellow cattle) were randomly divided into three groups of ten head each, with individual animals serving as replicates. The control group (Group A) received a basal diet, while experimental Groups B and C had 50% and 100% of distiller's grains replaced with sweet potato residue, respectively. The trial lasted for 9 weeks (including a 1-week pre-trial period and an 8-week formal trial period). The results showed: (1) Average daily gain and IMF content in Group C were significantly lower than in Groups A and B ( $P < 0.05$ ). (2) Serum triglycerides, total cholesterol, high-density lipoprotein, and low-density lipoprotein in Group C were significantly lower than in Group A ( $P < 0.05$ ). (3) Activities of fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) in the longissimus dorsi muscle of Group C were significantly lower than in Groups A and B ( $P < 0.05$ ), while hormone-sensitive lipase (HSL) and carnitine palmitoyltransferase-1 (CPT-1) activities were significantly higher ( $P < 0.05$ ). Group B also showed significantly lower FAS and ACC activities and higher HSL and CPT-1 activities compared to Group A ( $P < 0.05$ ). (4) Expression levels of sterol regulatory element-binding protein-1 (SREBP-1), FAS, ACC, and peroxisome proliferator-activated receptor (PPAR) genes in Group C were significantly lower than in Groups A and B ( $P < 0.05$ ), while HSL and CPT-1 gene expression was significantly higher ( $P < 0.05$ ). Group B exhibited significantly lower SREBP-1, FAS, ACC, and PPAR expression and higher HSL and CPT-1 expression compared to Group A ( $P < 0.05$ ). These findings indicate that increasing the replacement ratio of sweet potato residue for distiller's grains downregulates expression of fatty acid

synthesis-related genes (SREBP-1, FAS, ACC, and PPAR ) and upregulates expression of lipolysis-related genes (HSL and CPT-1) in the longissimus dorsi muscle, thereby reducing IMF deposition.

**Keywords:** fattening cattle; sweet potato residue; intramuscular fat; gene expression; distiller' s grains

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Sweet potato residue, a byproduct of starch extraction from sweet potatoes, is characterized by high crude fiber and low crude protein and fat content. Ruminant rumen microbes can effectively utilize crude fiber, making sweet potato residue a viable feed resource for ruminants. Distiller' s grains, a byproduct of liquor production, are produced at approximately 15 million tons annually in China and serve as a major residue feed in beef cattle farming. Their use helps alleviate feed resource shortages and plays an important role in grain-saving animal husbandry. Intramuscular fat (IMF) content is closely associated with meat flavor, tenderness, and juiciness, directly influencing beef grading, product development, and market competitiveness. IMF deposition results from competition between fatty acid synthesis and degradation, regulated by lipogenic genes such as sterol regulatory element-binding protein-1 (SREBP-1), acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), and peroxisome proliferator-activated receptor (PPAR ), as well as lipolytic genes including hormone-sensitive lipase (HSL) and carnitine palmitoyltransferase-1 (CPT-1). Previous studies have focused on dietary supplementation with distiller' s grains in beef cattle, but research on replacing distiller' s grains with sweet potato residue and its effects on IMF deposition-related gene expression remains limited. Therefore, this study investigated the effects of such replacement on growth performance, slaughter performance, serum biochemical indices, and IMF deposition-related gene expression to provide theoretical basis and parameters for rational use of sweet potato residue and distiller' s grains in fattening cattle diets.

### 1.1 Experimental Design, Diets, and Management

Thirty healthy 16-month-old crossbred fattening steers (Simmental  $\times$  local yellow cattle) weighing approximately 400 kg were randomly allocated to three groups of ten head each, with individual animals as replicates. The control group (Group A) received a basal diet, while Groups B and C had 50% and 100% of distiller' s grains replaced with sweet potato residue (containing 10% and 20% sweet potato residue in total diet, respectively). The trial lasted 9 weeks, including a 1-week pre-trial period and an 8-week formal trial period. Sweet potato residue was sourced from a single sweet potato starch factory, dehydrated, and stored in a cellar for the entire trial. Distiller' s grains were fresh sorghum distiller' s grains from a single liquor factory. Both were mixed with concentrate supplements during feeding. The concentrate-to-forage ratio was 40:60. Basal diet formulation referenced beef cattle nutritional requirements, with diet composition and nutrient levels shown in Table 1 . Nutrient composi-

tion on a dry matter basis was: for distiller's grains—crude protein 24.83%, crude fat 13.21%, acid detergent fiber 41.12%, neutral detergent fiber 47.79%, calcium 0.31%, phosphorus 0.42%; for sweet potato residue—crude protein 3.90%, crude fat 0.60%, acid detergent fiber 14.63%, neutral detergent fiber 24.10%, calcium 0.19%, phosphorus 0.02%. Cattle were managed according to conventional beef cattle practices, individually tethered and fed twice daily at fixed times (08:00 and 16:00) with ad libitum access to feed and water.

### **1.2 Sample Collection**

At the end of the trial, five cattle per group with body weights close to the group average were selected for slaughter. Immediately post-slaughter, longissimus dorsi muscle samples were collected. One portion was snap-frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  for RNA extraction and gene expression analysis, while another portion was stored at  $-20^{\circ}\text{C}$  for enzyme activity determination. Blood samples (15 mL) were collected from the jugular vein at trial conclusion, centrifuged at 3,000 r/min for 10 minutes, and serum was stored at  $-20^{\circ}\text{C}$  for biochemical analysis.

### **1.3 Growth and Slaughter Performance Measurement**

Body weight was measured at trial initiation and conclusion to calculate average daily gain (ADG) using the formula:  $\text{ADG} = (\text{final weight} - \text{initial weight})/\text{days}$ . Daily feed intake of concentrate and roughage was recorded to calculate average daily feed intake and feed-to-gain ratio. After the feeding trial, five cattle per group with body weights close to the group average were slaughtered to determine carcass yield and slaughter percentage.

### **1.4 Serum Biochemical Index Determination**

Serum cholesterol, triglyceride, low-density lipoprotein, and high-density lipoprotein concentrations were measured using an automatic biochemical analyzer.

### **1.5 Longissimus Dorsi Muscle IMF Content and Enzyme Activity Determination**

IMF content in longissimus dorsi muscle was determined according to the method of Zhang et al. Activities of FAS, ACC, CPT-1, and HSL in longissimus dorsi muscle were measured using assay kits from Nanjing Jiancheng Bioengineering Institute following the manufacturer's instructions.

### **1.6 Gene Expression Determination**

Total RNA was extracted from longissimus dorsi muscle using an RNAiso kit. RNA concentration was measured using NanoDrop 2000 (Thermo Scientific, USA) and integrity was checked via agarose gel electrophoresis. Total RNA

was reverse-transcribed to cDNA using a reverse transcription kit. Primers were designed and gene expression was determined following Zhang et al., with  $\beta$ -actin as the internal reference gene to calculate relative expression levels of target genes. Primer sequences are shown in Table 2 .

### 1.7 Statistical Analysis

Data were processed using Excel 2003 and subjected to one-way ANOVA using SPSS 19.0 software. Duncan' s multiple range test was used for inter-group mean comparisons. Results are expressed as mean  $\pm$  standard deviation, with  $P < 0.05$  considered statistically significant.

### 2.1 Effects of Sweet Potato Residue Replacement on Growth and Slaughter Performance

As shown in Table 3 , no significant differences were observed among groups in average daily feed intake, initial body weight, slaughter percentage, or carcass meat production rate ( $P > 0.05$ ). However, ADG and IMF content in Group C were significantly lower than in Groups A and B ( $P < 0.05$ ), while feed-to-gain ratio was significantly higher ( $P < 0.05$ ). No significant differences were found between Groups A and B in final body weight, ADG, feed-to-gain ratio, or IMF content ( $P > 0.05$ ).

### 2.2 Effects of Sweet Potato Residue Replacement on Serum Biochemical Indices

Table 4 shows that serum triglyceride, total cholesterol, high-density lipoprotein, and low-density lipoprotein concentrations in Group C were significantly lower than in Group A ( $P < 0.05$ ). No significant differences were observed between Groups A and B in these parameters ( $P > 0.05$ ).

### 2.3 Effects of Sweet Potato Residue Replacement on Longissimus Dorsi Muscle Enzyme Activities

As presented in Table 5 , FAS and ACC activities in Group C were significantly lower than in Groups A and B ( $P < 0.05$ ), while HSL and CPT-1 activities were significantly higher ( $P < 0.05$ ). Group B also exhibited significantly lower FAS and ACC activities and higher HSL and CPT-1 activities compared to Group A ( $P < 0.05$ ).

### 2.4 Effects of Sweet Potato Residue Replacement on IMF Deposition-Related Gene Expression in Longissimus Dorsi Muscle

Figure 1 [Figure 1: see original paper] demonstrates that SREBP-1, FAS, ACC, and PPAR  $\alpha$  gene expression levels in Group C were significantly lower than in Groups A and B ( $P < 0.05$ ), while HSL and CPT-1 expression was significantly higher ( $P < 0.05$ ). Group B showed significantly lower SREBP-1, FAS, ACC, and

PPAR expression and higher HSL and CPT-1 expression compared to Group A ( $P < 0.05$ ).

### **3.1 Effects of Sweet Potato Residue Replacement on Growth and Slaughter Performance in Fattening Cattle**

The results indicate that increasing the replacement ratio of sweet potato residue for distiller's grains decreased final body weight and ADG while increasing feed-to-gain ratio, consistent with findings from Peng et al. and Zhang et al. Peng et al. reported that 20% sweet potato residue in the diet reduced growth performance in fattening cattle, while Zhang et al. found that high-level replacement decreased final body weight and ADG by regulating dietary energy levels. This may be attributed to reduced dietary energy and crude protein content with higher replacement ratios, which negatively impacts growth performance. Additionally, increased replacement reduced IMF content in the longissimus dorsi muscle, aligning with results from Peng et al. and Chen et al. Peng et al. demonstrated that sweet potato residue replacement lowered dietary energy and consequently decreased IMF content. In summary, replacement at 50% (10% of total diet) did not significantly affect growth performance or IMF content, whereas 100% replacement (20% of total diet) significantly reduced both parameters, suggesting that replacement ratio should not be excessive and sweet potato residue should be limited to 10% of the total diet.

### **3.2 Effects of Sweet Potato Residue Replacement on Serum Biochemical Indices in Fattening Cattle**

Serum triglyceride, total cholesterol, high-density lipoprotein, and low-density lipoprotein concentrations reflect the activity level of fat metabolism, with higher values within a certain range indicating more vigorous fat anabolism. The current study showed that increasing replacement ratio decreased these serum lipid parameters, consistent with Zhang et al. and Chen et al. This likely resulted from reduced dietary energy and crude protein content with higher replacement levels, thereby decreasing serum lipid concentrations. Zhang et al. reported that increased replacement ratio lowered dietary energy and consequently reduced serum triglyceride, total cholesterol, high-density lipoprotein, and low-density lipoprotein in fattening cattle.

### **3.3 Effects of Sweet Potato Residue Replacement on IMF Deposition-Related Gene Expression in Longissimus Dorsi Muscle**

IMF deposition results from competition between fatty acid synthesis and degradation, with net accumulation occurring when anabolism exceeds catabolism. First, IMF deposition is closely related to fatty acid synthesis capacity, as enhanced synthesis promotes IMF accumulation and increases IMF content. The PPAR gene positively regulates expression of lipid metabolism-related genes to control fatty acid synthesis and release. SREBP-1 regulates expression of lipogenic genes including ACC and FAS to control fatty acid synthesis and release.

FAS catalyzes fatty acid synthesis and represents a key enzyme in the pathway, while ACC promotes fatty acid synthesis as a rate-limiting enzyme. The present study demonstrated that increased replacement ratio downregulated expression of PPAR, SREBP-1, FAS, and ACC genes and reduced FAS and ACC activities, indicating weakened fatty acid synthesis capacity and consequently reduced IMF deposition and content. Chen et al. reported that higher replacement ratios decreased PPAR expression in longissimus dorsi muscle, while Peng et al. found that increased replacement lowered dietary energy and downregulated expression of PPAR, SREBP-1, FAS, and ACC genes.

Second, IMF deposition is closely associated with fatty acid degradation capacity, as reduced lipolysis promotes IMF accumulation and increases IMF content. HSL hydrolyzes triglycerides in adipose tissue into glycerol, free fatty acids, and small amounts of diglycerides, while CPT-1 facilitates fatty acid -oxidation to degrade fatty acids. The current results showed that increased replacement ratio enhanced both activities and gene expression levels of HSL and CPT-1 in longissimus dorsi muscle, strengthening fatty acid degradation capacity and thereby reducing IMF deposition and content, consistent with Peng et al.

#### 4 Conclusion

The replacement ratio of distiller's grains with sweet potato residue should not be excessive and should be controlled within 10% of the total diet composition. Elevated replacement ratios reduce IMF deposition in the longissimus dorsi muscle by downregulating expression of fatty acid synthesis-related genes (SREBP-1, FAS, ACC, and PPAR) and upregulating expression of lipolysis-related genes (HSL and CPT-1).

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