Effects of Dietary Leucine and Glutamate Supplementation on Muscle Fatty Acid Composition and Lipid Metabolism-Related Gene Expression in Finishing Pigs: Postprint

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Abstract

This experiment aimed to investigate the effects of dietary leucine and glutamate supplementation on muscle fatty acid composition and lipid metabolism-related gene expression in finishing pigs. Sixty three-way crossbred finishing pigs with an average body weight of approximately 77 kg were selected and randomly divided into 5 groups with 12 pigs per group, half male and half female. The control group was fed a basal diet, and the experimental groups were fed the basal diet supplemented with 2.05% L-alanine (isonitrogenous control group), 1.0% leucine + 1.37% L-alanine (leucine group), 1.0% glutamate + 1.44% L-alanine (glutamate group), and 1.0% leucine + 1.0% glutamate (leucine + glutamate group), respectively. After 60 days of feeding, the pigs were slaughtered and muscle samples were collected to determine fatty acid content and the relative mRNA expression levels of lipid metabolism-related genes. The results showed: Compared with the control group, in the longissimus dorsi muscle, the leucine group had significantly increased contents of C18:2n-6 and C20:1 ($P<0.05$) and significantly decreased C18:0 content ($P<0.05$), while the glutamate group had significantly decreased contents of C14:0 and C16:0 ($P<0.05$) and significantly increased contents of C17:0 and C18:2n-6 ($P<0.05$); in the biceps femoris muscle, the leucine group had significantly decreased C16:0 content ($P<0.05$), and the glutamate group had significantly increased C18:2n-6 content ($P<0.05$). Compared with the control group, the leucine group showed significantly upregulated relative mRNA expression of fatty acid transport protein 1 (FATP-1) in the longissimus dorsi muscle ($P<0.05$), while the leucine + glutamate group showed significantly downregulated relative mRNA expression of fatty acid transport protein 4 (FATP-4) in both the longissimus dorsi and biceps femoris muscles ($P<0.05$). Compared with the isonitrogenous con-

trol group, the glutamate and leucine + glutamate groups showed significantly upregulated relative mRNA expression of lipoprotein lipase (LPL) in the longissimus dorsi muscle (P<0.05). These results suggest that dietary supplementation with 1.00% leucine or 1.00% glutamate can regulate fatty acid composition and lipid metabolism-related gene expression in the muscles of finishing pigs.

Full Text

Effects of Dietary Leucine and Glutamic Acid on Fatty Acid Composition and Expression of Lipid Metabolism-Related Genes in Muscle of Finishing Pigs

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Abstract

This study investigated the effects of dietary leucine (Leu) and glutamic acid (Glu) on fatty acid composition and expression of lipid metabolism-related genes in muscle of finishing pigs. Sixty Duroc × Large White × Landrace pigs with an average body weight of approximately 77 kg were randomly assigned to five groups (n = 12 per group, 1:1 male-to-female ratio). The control group received a basal diet, while experimental groups received the basal diet supplemented with: 2.05% L-alanine (iso-nitrogenous control group), 1.00% Leu + 1.37% L-alanine (Leu group), 1.00% Glu + 1.44% L-alanine (Glu group), or 1.00% Leu + 1.00% Glu (Leu+Glu group). After 60 days of feeding, pigs were slaughtered and muscle samples were collected to determine fatty acid content and relative mRNA expression levels of lipid metabolism-related genes.

The results showed that, compared with the control group, the Leu group exhibited significantly increased C18:2n-6 and C20:1 contents ($P < 0.05$) and decreased C18:0 content ($P < 0.05$) in the longissimus dorsi muscle. The Glu group showed significantly reduced C14:0 and C16:0 contents ($P < 0.05$) and increased C17:0 and C18:2n-6 contents ($P < 0.05$) in the longissimus dorsi muscle. In the biceps femoris muscle, the Leu group had significantly lower C16:0 content ($P < 0.05$), while the Glu group had significantly higher C18:2n-6 content ($P < 0.05$). Compared with the control group, fatty acid transport protein 1 (FATP-1) mRNA expression in the longissimus dorsi muscle was significantly

upregulated in the Leu group ($P < 0.05$), whereas fatty acid transport protein 4 (FATP-4) mRNA expression was significantly downregulated in both the longissimus dorsi and biceps femoris muscles in the Leu+Glu group ($P < 0.05$). Relative to the iso-nitrogenous control group, lipoprotein lipase (LPL) mRNA expression in the longissimus dorsi muscle was significantly upregulated in both the Glu and Leu+Glu groups ($P < 0.05$). These findings indicate that dietary supplementation with 1.00% Leu or 1.00% Glu can regulate fatty acid composition and expression of lipid metabolism-related genes in muscle of finishing pigs.

Keywords: leucine; glutamic acid; finishing pigs; muscle; lipid metabolism

Introduction

As living standards improve, Chinese consumers increasingly demand higher pork quality, with premium pork products being more favored. Fat is a critical factor affecting carcass quality and meat flavor. Excessive abdominal and subcutaneous fat deposition reduces carcass quality, whereas increased intramuscular fat content enhances meat tenderness and flavor. Fatty acid composition is also a key determinant of meat quality, influencing not only tenderness and juiciness but also nutritional value. While breeding strategies to increase lean meat percentage often reduce intramuscular fat content, nutritional regulation through dietary manipulation effectively modulates lipid metabolism, making it an important approach for producing high-quality pork.

Recent studies have demonstrated that leucine regulates mammalian skeletal muscle protein metabolism and accelerates fat oxidation while reducing fat deposition and triglyceride content in adipocytes. Additionally, leucine supplementation significantly increases intramuscular fat content in finishing pigs. Dietary glutamic acid supplementation reduces backfat thickness, increases intramuscular fat content, and improves fatty acid composition in finishing pigs, although some studies have reported that it may increase fat deposition and cause obesity. Our previous research showed that dietary supplementation with 1.00% leucine increased intramuscular fat content in both longissimus dorsi and biceps femoris muscles without affecting growth performance; 1.00% glutamic acid reduced backfat thickness by 34.3%; and the combination of 1.00% leucine + 1.00% glutamic acid increased intramuscular fat content in the biceps femoris muscle without affecting growth performance. These findings indicate that leucine and glutamic acid both regulate lipid metabolism but through different mechanisms, which remain unclear. Fat deposition depends on the dynamic balance between synthesis and degradation, processes regulated by multiple genes. Therefore, this study further investigated the effects of dietary leucine and glutamic acid on muscle fatty acid composition and lipid metabolism-related gene expression to elucidate their molecular mechanisms and provide a basis for using functional amino acids in premium pork production.

Materials and Methods

1.1 Experimental Animals, Grouping and Management Sixty Duroc × Large White × Landrace finishing pigs with an average body weight of approximately 77 kg were randomly allocated to five groups (n = 12 per group, 1:1 male-to-female ratio) and housed in an HHIS-02A automatic feeding system (Henan Heshun Automation Equipment Co., Ltd.). The control group received a basal diet, while the iso-nitrogenous control group received the basal diet supplemented with 2.05% L-alanine (which can be catabolized by multiple tissues to regulate dietary nitrogen balance). Experimental groups received the basal diet supplemented with: 1.00% leucine + 1.37% L-alanine (Leu group), 1.00% glutamic acid + 1.44% L-alanine (Glu group), or 1.00% leucine + 1.00% glutamic acid (Leu+Glu group). A corn-soybean meal basal diet was formulated according to NRC (2012) nutrient recommendations, with composition and nutrient levels shown in Table 1 . The feeding trial was conducted at the Animal Experimental Base of the Institute of Subtropical Agriculture, Chinese Academy of Sciences, located at Yong' an Branch of Hunan Xinwufeng Co., Ltd., for a period of 60 days. Pigs had ad libitum access to feed and water throughout the trial.

1.2 Sample Collection At the end of the feeding trial, pigs were euthanized by exsanguination via carotid artery. Longissimus dorsi and biceps femoris muscles were collected, snap-frozen in liquid nitrogen, and stored at -80 °C for molecular analysis. Additional muscle samples (100 g each) were stored at -20 °C for fatty acid analysis.

1.3 Determination of Fatty Acid Content Freeze-dried muscle samples were extracted for fat using petroleum ether-ethyl ether (1:1, v/v), esterified with 1 mL of potassium hydroxide-methanol solution (0.4 mol/L) for 30 minutes, concentrated in a hot water bath, and layered with water. The upper layer (500 L) was analyzed by gas chromatography-mass spectrometry (Agilent Technologies, USA). Fatty acid composition was calculated by area normalization using computer software, with results expressed as percentages of total fatty acids.

1.4 Determination of Lipid Metabolism-Related Gene Expression Total RNA was extracted from muscle samples using Trizol reagent (TaKaRa, Dalian). RNA quality was assessed by measuring the 260/280 nm absorbance ratio (1.80–2.10) using a NanoDrop® ND-1000 spectrophotometer (Thermo Fisher, USA) and by agarose gel electrophoresis. RNA concentration was adjusted to 1,000 ng/ L, and 1.0 L of total RNA was reverse-transcribed to cDNA according to the TaKaRa reverse transcription kit protocol.

Primers were designed using Primer 5.0 software, with sequences listed in Table 2 . Relative mRNA expression levels of lipid metabolism-related genes were determined by quantitative real-time PCR using cDNA as template. The 10

L reaction mixture contained: 5 L 2×SYBR Green PCR Master Mix, 2 L cDNA, 0.4 L forward primer, 0.4 L reverse primer, and 3.2 L ddH O. Cycling conditions were: 95 °C for 10 s; 40 cycles of 95 °C for 15 s and 60 °C for 30 s. Relative mRNA expression was calculated using the 2^(-ΔΔCt) method based on threshold cycle (Ct) values of target and reference genes.

1.5 Data Processing Data were analyzed using SPSS 17.0 software with Duncan' s multiple comparison test. Results are expressed as "mean ± standard error," with $P < 0.05$ considered statistically significant.

Results

2.1 Effects of Dietary Leucine and Glutamic Acid on Fatty Acid Composition in Longissimus Dorsi Muscle of Finishing Pigs As shown in Table 3 , compared with the control group, the Leu group exhibited significantly decreased C18:0 content and increased C18:2n-6 and C20:1 contents in the longissimus dorsi muscle ($P < 0.05$). The Glu group showed significantly reduced C14:0 and C16:0 contents and increased C17:0, C18:2n-6, and C20:1 contents ($P < 0.05$).

2.2 Effects of Dietary Leucine and Glutamic Acid on Fatty Acid Composition in Biceps Femoris Muscle of Finishing Pigs Table 4 shows that, compared with the control group, the Leu group had significantly lower C16:0 content in the biceps femoris muscle ($P < 0.05$), while the Glu group had significantly higher C18:2n-6 content ($P < 0.05$).

2.3 Effects of Dietary Leucine and Glutamic Acid on Expression of Lipid Metabolism-Related Genes in Longissimus Dorsi Muscle of Finishing Pigs As shown in Table 5 , compared with the control group, the Leu group exhibited significantly upregulated FATP-1 mRNA expression in the longissimus dorsi muscle ($P < 0.05$), while the Leu+Glu group showed significantly downregulated FATP-4 mRNA expression ($P < 0.05$). Relative to the iso-nitrogenous control group, the Glu and Leu+Glu groups had significantly upregulated LPL mRNA expression in the longissimus dorsi muscle ($P < 0.05$).

2.4 Effects of Dietary Leucine and Glutamic Acid on Expression of Lipid Metabolism-Related Genes in Biceps Femoris Muscle of Finishing Pigs Table 6 indicates that, compared with the control group, the Glu and Leu+Glu groups showed significantly downregulated FATP-4 mRNA expression in the biceps femoris muscle ($P < 0.05$).

Discussion

Muscle fat content and fatty acid composition significantly influence pork quality. Understanding the molecular mechanisms by which nutrients regulate muscle lipid metabolism can improve carcass traits and meat quality, providing

consumers with superior products. Fatty acid composition is a crucial factor affecting pork flavor, nutritional value, and oxidative stability. This study found that dietary leucine supplementation significantly reduced C18:0 (a saturated fatty acid, SFA) and increased C18:2n-6 (a polyunsaturated fatty acid, PUFA) in the longissimus dorsi muscle of finishing pigs. Glutamic acid supplementation significantly decreased C14:0 and C16:0 (SFAs) and increased C18:2n-6 (PUFA) in the longissimus dorsi muscle, as well as increased C18:2n-6 in the biceps femoris muscle. The combination of leucine and glutamic acid did not significantly affect muscle fatty acid composition but upregulated LPL gene expression in the longissimus dorsi muscle.

Excessive SFA intake increases consumers' risk of cardiovascular disease and can induce insulin resistance in muscle tissue, whereas PUFA helps prevent cardiovascular disease. C18:2n-6 (linoleic acid) is an essential fatty acid with anti-inflammatory and immunomodulatory functions. These results suggest that dietary leucine or glutamic acid supplementation can improve muscle fatty acid composition by reducing SFA content, thereby enhancing pork nutritional value. However, Kong et al. reported that dietary glutamic acid increased C16:0 content in the longissimus dorsi muscle of growing pigs, possibly due to differences in basal diet composition and growth stage.

Our previous study found that dietary supplementation with 1.00% leucine and 1.00% glutamic acid significantly increased intramuscular fat content in finishing pigs. Therefore, this study further examined lipid metabolism-related gene expression to explore the underlying mechanisms. Leucine supplementation significantly upregulated FATP-1 and FATP-4 expression in the longissimus dorsi muscle. FATP-1 and FATP-4 are fatty acid transport proteins that play important roles in fatty acid uptake. FATP-1 knockout reduces long-chain fatty acid (LCFA) uptake and triglyceride deposition in muscle cells, while FATP-1 overexpression enhances glucose oxidation. FATP-4 expression is associated with obesity. These findings suggest that leucine may promote LCFA uptake by muscle cells, thereby enhancing fat deposition. Interestingly, although our previous study showed that combined leucine and glutamic acid supplementation increased intramuscular fat content, the current study found that this combination downregulated FATP-1 and FATP-4 expression, warranting further investigation.

Acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) are key enzymes in fatty acid synthesis. Increased FAS expression promotes triglyceride deposition and obesity. Peroxisome proliferator-activated receptor (PPAR) is a master regulator of adipocyte differentiation that directly participates in fat differentiation and modulates expression of FAS, ACC, LPL, and hormone-sensitive lipase (HSL). In this study, dietary leucine, glutamic acid, or their combination did not significantly affect ACC, FAS, or PPAR expression, suggesting that increased intramuscular fat content may not result from upregulated fat synthesis genes. This aligns with Madeira et al., who found that leucine supplementation did not affect ACC and FAS expression in longissimus dorsi muscle. However,

Kong et al. reported that glutamic acid increased intramuscular fat content and upregulated ACC expression, possibly because they used a high-fat basal diet.

FAS catalyzes the conversion of acetyl-CoA to C16:0. In this study, glutamic acid significantly reduced C16:0 content in the longissimus dorsi muscle, while leucine decreased C16:0 in the biceps femoris muscle, without significantly affecting FAS mRNA expression. This suggests that leucine and glutamic acid may not influence C16:0 synthesis but could upregulate genes involved in C16:0 degradation. LPL catalyzes hydrolysis of triglycerides in chylomicrons and very low-density lipoproteins, and its activity determines peripheral tissue uptake of triglycerides from plasma. Increased LPL expression significantly enhances fat deposition in subcutaneous tissue and muscle. This study demonstrated that glutamic acid and leucine+glutamic acid supplementation significantly upregulated LPL expression in the longissimus dorsi muscle, indicating they may influence muscle fat deposition by modulating LPL expression.

Conclusion

Dietary leucine supplementation significantly reduced C18:0 content and increased C18:2n-6 content in the longissimus dorsi muscle of finishing pigs. Dietary glutamic acid supplementation significantly decreased C14:0 and C16:0 contents while increasing C18:2n-6 content in the longissimus dorsi muscle. Combined leucine and glutamic acid supplementation did not significantly affect muscle fatty acid composition but significantly upregulated LPL gene expression in the longissimus dorsi muscle.

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