Effects of Eucommia ulmoides Extract on Growth Performance, Serum Biochemical Indices, and Liver Lipid Metabolism in Ningxiang Pigs (Post-print)

Authors: Li Chenyan, Zhang Yang, Xiao Dingfu, Xing Yueteng, Yu Yuanian, Wu Xin, Liu Xingliang, Xie Xianming, Zhang Xu, Zeng Yongbo, Zhang Bin

Date: 2018-12-25T00:00:00+00:00

Abstract

This experiment was conducted to investigate the effects of dietary Eucommia extract supplementation on growth performance, serum biochemical indices, and hepatic lipid metabolism in Ningxiang pigs. Twenty-four healthy castrated female Ningxiang pigs with an average body weight of approximately 43 kg were selected and randomly allocated into 2 groups, with 4 replicates per group and 3 pigs per replicate. The control group was fed a basal diet, while the experimental group was fed the basal diet supplemented with 1,500 mg/kg Eucommia extract. The experimental period lasted 56 days. The results showed that, compared with the control group: 1) Dietary Eucommia extract supplementation slightly increased the average daily gain, decreased average daily feed intake by 46.88 g/d, and reduced feed-to-gain ratio by 2.74% in Ningxiang pigs, but these differences were not significant (P>0.05). 2) Dietary Eucommia extract supplementation increased serum triglyceride (TG) and high-density lipoprotein (HDL) levels by 33.33% (0.05 P<0.10) and 20.29% (P<0.05), respectively, and significantly decreased serum low-density lipoprotein (LDL) level by 13.21% (P<0.05) in Ningxiang pigs. 3) Dietary Eucommia extract supplementation increased palmitoleic acid content in monounsaturated fatty acids (MUFA) by 19.64% (0.05 P<0.10) and decreased linoleic acid content in polyunsaturated fatty acids (PUFA) by 10.71% (0.05 P<0.10) in the liver of Ningxiang pigs. 4) Dietary Eucommia extract supplementation significantly downregulated the mRNA expression levels of adipose triglyceride lipase (ATGL), acetyl-CoA carboxylase (ACC), liver X receptor (LXR), and fatty acid synthase (FAS) in the liver of Ningxiang pigs by 62.00%, 76.00%, 77.00%, and 82.00%, respectively (P<0.05). In conclusion, dietary Eucommia extract supplementation can increase serum HDL level, decrease serum LDL level, and downregulate the

mRNA expression levels of FAS and ACC in the liver, thereby affecting fatty acid metabolism in Ningxiang pigs.

Full Text

Effects of Eucommia ulmoides Oliver Extracts on Growth Performance, Serum Biochemical Indexes and Liver Lipid Metabolism of Ningxiang Pigs

LI Chenyan[1], ZHANG Yang[1], XIAO Dingfu[1], XING Yueteng[1], YU Yuannian[1], WU Xin[2], *LIU Xingliang[3], XIE Xianming[3], ZHANG Xu[3], ZENG Yongbo[3], ZHANG Bin[1]*

[1]Hunan Co-Innovation Center of Safety Animal Production, College of Animal Science and Technology, Hunan Agricultural University, Changsha 410128, China
[2]Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha 410125, China
[3]Ningxiang Animal Husbandry, Veterinary and Aquatic Products Bureau, Ningxiang 410600, China

Abstract

This study investigated the effects of dietary Eucommia ulmoides Oliver extracts (EUOE) on growth performance, serum biochemical indexes, and liver lipid metabolism in Ningxiang pigs. Twenty-four healthy castrated female Ningxiang pigs (approximately 43 kg body weight) were randomly allocated to two groups with four replicates per group and three pigs per replicate. The control group received a basal diet, while the experimental group received the basal diet supplemented with 1,500 mg/kg EUOE. The 56-day trial revealed the following: (1) Compared with the control group, EUOE supplementation slightly increased average daily gain, decreased average daily feed intake by 46.88 g/d, and reduced the feed-to-gain ratio by 2.74%, though these differences were not statistically significant (P>0.05). (2) EUOE significantly increased serum high-density lipoprotein (HDL) content by 20.29% (P<0.05) and tended to increase serum triglyceride (TG) content by 33.33% (0.05 P<0.10), while significantly decreasing serum low-density lipoprotein (LDL) content by 13.21% (P<0.05). (3) EUOE tended to increase hepatic palmitoleic acid (a monounsaturated fatty acid, MUFA) by 19.64% (0.05 P<0.10) and tended to decrease hepatic linoleic acid (a polyunsaturated fatty acid, PUFA) by 10.71% (0.05 P<0.10). (4) EUOE significantly downregulated hepatic mRNA expression of adipose triglyceride lipase (ATGL), acetyl-CoA carboxylase (ACC), liver X receptor (LXR), and fatty acid synthase (FAS) by 62.00%, 76.00%, 77.00%, and 82.00%, respectively (P<0.05). In conclusion, dietary EUOE supplementation increased serum HDL content, decreased serum LDL content, and downregulated hepatic mRNA expression of FAS and ACC, thereby influencing fatty acid metabolism in Ningxiang pigs.

Keywords: Eucommia ulmoides Oliver extracts; growth performance; serum biochemical indexes; fatty acids; Ningxiang pig

Introduction

As national regulations on antibiotic use in animal husbandry become increasingly stringent, identifying safe and beneficial antibiotic alternatives has become fundamental to the advancement of the livestock industry. Eucommia ulmoides Oliver extracts (EUOE) contain various bioactive components including chlorogenic acid, aucubin, and flavonoids [1-2], and exhibit antimicrobial, anti-inflammatory, and antioxidant functions [3-5], making them increasingly popular in animal production. Ningxiang pig is a famous meat-lard type breed in China characterized by strong fat deposition capacity, wide adaptability, and tender meat quality [6]. While numerous studies have investigated the effects of EUOE on growth performance, carcass traits, and meat quality in lean-type pigs [7-8], research on meat-lard type pigs remains limited, with scarce reports on Ningxiang pigs. This study explored the effects of dietary EUOE supplementation on growth performance, serum biochemical indexes, and liver lipid metabolism in Ningxiang pigs to provide a theoretical basis for the scientific application of EUOE in local pig breeds.

Materials and Methods

1.1 Experimental Material The main components of EUOE were chlorogenic acid (3.5%), flavonoids (20%), and aucubin.

1.2 Experimental Animals and Design The experiment was conducted at the Ningxiang Pig Ecological Farming Base of Hunan Liusahe Flower Pig Ecological Husbandry Co., Ltd. Using a single-factor design, 24 healthy castrated female Ningxiang pigs from the same barn and batch (approximately 43 kg body weight) were randomly divided into two groups with four replicates per group and three pigs per replicate. The control group received a basal diet, while the experimental group received the basal diet supplemented with 1,500 mg/kg EUOE. The basal diet was formulated according to the nutrient requirements for meat-lard type pigs in the *Feeding Standard of Swine* (NY/T 65-2004) and the *Ningxiang Pig Feeding Technical Regulations* (2009); its composition and nutrient levels are described in reference [9]. Pigs were fed three times daily (08:00, 12:00, and 18:00), with disinfection and immunization following the company' s standard procedures. The experimental period lasted 56 days.

1.3 Measurements

1.3.1 Growth Performance At the beginning and end of the experiment, body weight was measured for each group, and total feed intake per pen was

recorded to calculate average daily feed intake, average daily gain, and feed-to-gain ratio.

1.3.2 Serum Biochemical Indexes At the end of the experiment, one pig per replicate was selected for jugular vein blood collection (10 mL). After standing at room temperature for 1 hour, serum was collected by centrifugation at 3,000 r/min for 10 minutes at 4°C, aliquoted into 1.5 mL sterile tubes, and stored at -20°C. Serum glucose (GLU), triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) contents were measured using an automatic biochemical analyzer (Beckman, USA).

1.3.3 Hepatic Long-Chain Fatty Acid Content At the end of the experiment, after 24-hour fasting with free access to water, one pig per replicate was slaughtered. Liver middle lobes were wrapped in tin foil, snap-frozen in liquid nitrogen, and stored at -80°C. Freeze-dried liver samples (approximately 0.5 g) were extracted with benzene-petroleum ether, methyl-esterified with potassium hydroxide-methanol, and analyzed for long-chain fatty acid content by gas chromatography with FID detection using the external standard GC-MS method described by Yu et al. [10]. Total fat content was determined according to the *National Food Safety Standard—Determination of Fat in Foods* (GB 5009.6—2016).

1.3.4 Hepatic Lipid Metabolism-Related Gene Expression Total RNA was extracted from liver tissue using Trizol (Invitrogen, USA). Reverse transcription was performed on 1,000 ng RNA using a kit from TaKaRa according to the manufacturer' s instructions. Real-time quantitative PCR was used to measure mRNA expression levels of lipid metabolism-related genes. Primers (sequences shown in) were synthesized by Shanghai Bioengineering Co., Ltd. Using -actin as the internal reference gene, relative quantification was performed for peroxisome proliferator-activated receptor (PPAR), adipose triglyceride lipase (ATGL), acetyl-CoA carboxylase (ACC), liver X receptor (LXR), and fatty acid synthase (FAS).

1.4 Statistical Analysis Data were initially processed using Excel 2016. Independent samples t-tests in SPSS 21.0 were used for inter-group comparisons. Results are expressed as "mean ± standard error (mean±SE)." $P<0.05$ was considered statistically significant, and $0.05 P<0.10$ was considered a tendency toward significance.

Results

2.1 Effects of EUOE on Growth Performance of Ningxiang Pigs As shown in , compared with the control group, the experimental group exhibited slightly increased average daily gain, decreased average daily feed intake by 46.88

g/d, and reduced feed-to-gain ratio by 2.74%, though none of these differences were statistically significant (P>0.05).

2.2 Effects of EUOE on Serum Biochemical Indexes of Ningxiang Pigs As shown in , EUOE supplementation increased serum TG and HDL contents by 33.33% (0.05 P<0.10) and 20.29% (P<0.05), respectively, and significantly decreased serum LDL content by 13.21% (P<0.05). Serum GLU and TC contents were reduced but without significant differences (P>0.05).

2.3 Effects of EUOE on Hepatic Long-Chain Fatty Acid Contents of Ningxiang Pigs As shown in , EUOE supplementation tended to increase total fat content in liver, though the difference was not significant (P>0.05). Palmitoleic acid content in hepatic monounsaturated fatty acids (MUFA) tended to increase by 19.64% (0.05 P<0.10), while linoleic acid content in polyunsaturated fatty acids (PUFA) tended to decrease by 10.71% (0.05 P<0.10).

2.4 Effects of EUOE on Hepatic Lipid Metabolism-Related Gene Expression in Ningxiang Pigs As shown in , EUOE supplementation significantly downregulated hepatic mRNA expression of ATGL, ACC, LXR , and FAS by 62.00%, 76.00%, 77.00%, and 82.00%, respectively (P<0.05), while PPAR mRNA expression showed no significant difference (P>0.05).

Discussion

3.1 Effects of EUOE on Growth Performance of Ningxiang Pigs Growth performance directly determines meat production capacity and economic efficiency in finishing pigs. As a green additive with both pharmacological and physiological functions, EUOE can improve dietary nutrient utilization [11] and plays an important role in intestinal health [12]. Previous studies have shown that dietary EUOE or Eucommia leaf supplementation significantly increased average daily gain and decreased feed-to-gain ratio in finishing pigs [4,7], while other reports indicated that Eucommia leaf powder significantly reduced both average daily feed intake and average daily gain in growing-finishing pigs [13]. In this study, EUOE supplementation reduced feed-to-gain ratio and slightly increased average daily gain, though differences were not significant. This may be attributed to chlorogenic acid and flavonoids in EUOE inhibiting hepatic TNF- mRNA expression, while geniposide promotes bile secretion [14]. Alternatively, since the experimental animals were finishing-stage Ningxiang pigs with relatively mature intestinal development, significant improvements in average daily feed intake may not be evident.

3.2 Effects of EUOE on Serum Biochemical Indexes of Ningxiang Pigs Serum biochemical indexes influence animal metabolism and nutrient deposition, and are affected by growth stage, endocrine status, and dietary nutrition levels

[15]. This study showed that EUOE increased serum TG content in Ningxiang pigs, consistent with results from lactating sows [16]. Research indicates that chlorogenic acid in EUOE inhibits intestinal pancreatic lipase and hepatic HMG-CoA reductase activity, thereby affecting fat absorption and cholesterol synthesis and reducing fat deposition [17]. EUOE supplementation significantly decreased serum LDL content while increasing serum HDL content, likely due to chlorogenic acid effectively regulating lipid metabolism and protecting liver function, which aligns with findings by Choi et al. [18] and Liu [19].

3.3 Effects of EUOE on Hepatic Long-Chain Fatty Acid Contents of Ningxiang Pigs Fatty acids are classified as saturated fatty acids (SFA), MUFA, and PUFA. SFA and MUFA positively correlate with meat quality, improving tenderness, aroma, and flavor, though excessive SFA can be converted to cholesterol in blood, causing arteriosclerosis. High PUFA content may lead to muscle oxidation, rancidity, soft fat, and reduced meat quality [20-21]. This study showed that EUOE supplementation reduced hepatic SFA content by 0.43% and increased MUFA and PUFA contents by 0.43% and 0.49%, respectively, though none were significant, indicating minimal effect on hepatic fatty acid composition. However, palmitoleic acid in MUFA tended to increase, while linoleic acid in PUFA tended to decrease. Palmitoleic acid can reduce serum TC content in humans [22], while linoleic acid can reduce serum TG content [23], consistent with our findings. Additionally, EUOE is rich in antioxidants like chlorogenic acid and Eucommia flavonoids that may delay oxidative loss of unsaturated fatty acids (UFA) in liver, thereby increasing certain UFA contents, though the specific mechanism requires further investigation.

3.4 Effects of EUOE on Hepatic Lipid Metabolism-Related Gene Expression in Ningxiang Pigs Animal fat is synthesized endogenously in the liver through enzymatic catalysis of glucose and amino acid metabolic intermediates. The liver contains diverse lipid metabolism enzymes and serves as a crucial site for fatty acid synthesis, playing a key role in lipid metabolism [24]. Key enzymes in this process include PPAR , ATGL, ACC, FAS, and LXR , where ACC and FAS are involved in fatty acid synthesis while ATGL, PPAR , and LXR participate in lipolysis [9]. Studies have shown that EUOE promotes hepatic fatty acid oxidation and reduces CCl -induced lipid accumulation in rats [25-26]. This study demonstrated that EUOE significantly downregulated hepatic mRNA expression of ATGL, ACC, LXR , and FAS. The main chemical components of EUOE are chlorogenic acid, flavonoids, and aucubin. Chlorogenic acid protects liver function [3]. ACC catalyzes acetyl-CoA to malonyl-CoA, providing two-carbon units for fatty acid synthesis and thus is considered the rate-limiting enzyme in fatty acid synthesis [27]. FAS catalyzes synthesis of fatty acids from acetyl-CoA and malonyl-CoA, and EUOE supplementation inhibits FAS mRNA expression, thereby reducing body fat deposition [28]. EUOE decreased hepatic mRNA expression of FAS and ACC, leading to reduced hepatic fatty acid synthesis and body fat deposition [17,27]. ATGL is a rate-limiting

enzyme in lipid metabolism that hydrolyzes TG to diglycerides and free fatty acids [29]; downregulation of hepatic ATGL mRNA expression may contribute to increased serum TG content. LXR , a nuclear receptor superfamily member involved in lipid homeostasis, is significantly inhibited by chlorogenic acid [30]. EUOE may suppress TG content by regulating LXR mRNA expression. These results demonstrate that EUOE influences fatty acid metabolism by modulating hepatic expression of lipid metabolism-related genes.

Conclusion

Dietary EUOE supplementation had no significant effect on growth performance in Ningxiang pigs but increased serum HDL content, decreased serum LDL content, and inhibited hepatic mRNA expression of lipid metabolism-related genes including FAS and ACC, thereby influencing palmitoleic acid and linoleic acid metabolism.

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