Effects of Eucommia Leaf on Lipid Metabolism in Sheep and Its Mechanism: Postprint

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Abstract

To investigate the effects of Eucommia leaf on fat metabolism and meat quality in sheep, this study randomly selected 30 Hu sheep aged 70–80 days with a body weight of 25–30 kg, which were equally divided into three groups: a control group (CTL group, diet without Eucommia leaf), a low-level Eucommia leaf group (EUL1 group, diet containing 10% Eucommia leaf), and a high-level Eucommia leaf group (EUL2 group, diet containing 20% Eucommia leaf), with 10 sheep per group. The pre-trial period was 15 days, and the experimental period was 90 days. Blood was collected to determine plasma lipid metabolism indices, longissimus dorsi muscle was collected to determine nutrient contents, and liver tissue was collected to determine the mRNA expression levels and protein levels of fat metabolism-related enzymes and nuclear transcription factors. The results showed that: 1) Dry matter intake in each group followed the order EUL1 group > EUL2 group > CTL group, with significant differences among groups ($P<0.05$). 2) Compared with the CTL group, plasma very low-density lipoprotein (VLDL), high-density lipoprotein (HDL), and non-esterified fatty acid (NEFA) contents were significantly increased in the EUL2 group ($P<0.05$), while plasma low-density lipoprotein (LDL) ($0.05\leq P<0.10$) and total cholesterol (TC) ($P<0.05$) contents were decreased in both the EUL1 and EUL2 groups. 3) Compared with the CTL group, muscle crude protein content in the EUL1 group and muscle crude fat content in the EUL2 group were significantly increased ($P<0.05$), while muscle shear force in the EUL1 group was significantly decreased ($P<0.05$). 4) Compared with the CTL group, muscle saturated fatty acid (SFA) content was slightly decreased in both the EUL1 and EUL2 groups ($P>0.05$), while muscle unsaturated fatty acid (USFA) content was significantly increased ($P<0.05$). Specifically, muscle MUFA and PUFA contents and the USFA/SFA ratio were significantly increased in the EUL2 group ($P<0.05$). 5) Compared with the CTL group, Eucommia leaf significantly downregulated the mRNA expression levels of the liver fat synthesis-related enzyme stearoyl-

CoA desaturase 1 (SCD1) and the nuclear transcription factors sterol regulatory element-binding protein 1c (SREBP-1c) and peroxisome proliferator-activated receptor γ (PPARγ) (P<0.05), and significantly upregulated the mRNA expression levels of the liver fatty acid oxidation-related enzymes carnitine palmitoyltransferase 1A (CPT1A) and lipoprotein lipase (LPL) (P<0.05). Compared with the CTL group, liver SCD1 protein level was significantly decreased in the EUL2 group (P<0.05). These results suggest that Eucommia leaf significantly affects fat metabolism and muscle fatty acid composition in sheep by regulating the expression of genes related to fat synthesis and breakdown.

Full Text

Effects of Eucommia ulmoides Leaves on Sheep Lipid Metabolism and the Mechanism

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Abstract

To investigate the effects of Eucommia ulmoides leaves (EUL) on lipid metabolism and meat quality in sheep, thirty healthy Hu sheep aged 70–80 days with body weights of 25–30 kg were randomly divided into three groups (n=10): a control group (CTL) fed diets without EUL, a low-level EUL group (EUL1) fed diets containing 10% EUL, and a high-level EUL group (EUL2) fed diets containing 20% EUL. The pre-experimental period lasted 15 days, followed by a 90-day formal experimental period. Blood samples were collected to determine plasma lipid metabolism indices. Longissimus dorsi muscle was harvested to analyze nutrient contents, and liver tissue was collected to measure mRNA and protein expression levels of lipid metabolism-related enzymes and nuclear transcription factors. The results demonstrated: (1) Dry matter intake followed the pattern EUL1 > EUL2 > CTL, with significant differences among groups ($P<0.05$). (2) Compared with the CTL group, plasma contents of total cholesterol (TC) ($P<0.05$) and low-density lipoprotein (LDL) ($0.05\leq P<0.10$) were reduced, while plasma contents of very low-density lipoprotein (VLDL) ($P<0.05$), high-density lipoprotein (HDL), and non-esterified fatty acid (NEFA) in the EUL2 group were significantly increased ($P<0.05$). (3) Compared with the CTL group, crude protein content in muscle was significantly elevated in the EUL1 group ($P<0.05$), ether extract content in muscle was significantly increased in the EUL2 group ($P<0.05$), and muscle shear force was significantly decreased in the EUL1 group ($P<0.05$). (4) Compared with the CTL group, saturated fatty acid (SFA) content in muscle decreased slightly in both EUL1 and EUL2 groups ($P>0.05$), while unsaturated fatty acid (USFA) content was significantly increased ($P<0.05$). Specifically, monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) contents, and the USFA/SFA ratio were significantly elevated in the EUL2 group ($P<0.05$). (5) Compared with the CTL group, EUL supplementation significantly down-regulated hepatic mRNA expression levels of the lipogenic enzyme stearoyl-CoA desaturase 1 (SCD1) and nuclear transcription factors sterol regulatory element-binding protein 1c (SREBP-1c) and peroxisome proliferator-activated receptor γ (PPARγ) ($P<0.05$), while significantly up-regulating mRNA expression levels of lipid catabolism-related enzymes carnitine palmitoyltransferase 1A (CPT1A) and lipoprotein lipase (LPL) ($P<0.05$). Additionally, hepatic SCD1 protein level was significantly decreased in the EUL2 group ($P<0.05$). These findings indicate that EUL significantly influences lipid metabolism and muscle fatty acid composition in sheep by regulating the expression of genes related to lipid synthesis and catabolism.

Keywords: Eucommia ulmoides leaves; lipid metabolism; plasma biochemical indices; fatty acid composition; liver; gene expression; sheep

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Introduction

Eucommia ulmoides Oliver is a valuable traditional Chinese medicinal herb whose bark has been used medicinally to tonify the liver and kidneys, strengthen bones and muscles, replenish qi, and calm the fetus. Modern research has revealed that Eucommia possesses hypolipidemic, antihypertensive, antioxidant, and immune-enhancing properties. Eucommia leaves are abundant and readily obtainable, with chemical compositions and pharmacological activities similar to Eucommia bark. The leaves contain numerous active components that can be classified by chemical structure into lignans and glycosides (including 28 compounds such as syringin, pinoresinol diglucoside, and medioresinol), iridoids (including 15 compounds such as geniposidic acid, geniposide, and aucubin), phenylpropanoids (including 11 compounds such as chlorogenic acid, vanillic acid, and caffeic acid), flavonoids (including 10 compounds such as flavonols, rutin, and quercetin), gutta-percha, amino acids (16 types), vitamins (4 types), trace elements (13 types), and other constituents. The main active component contents (on a dry matter basis) are: syringin 0.003%, geniposidic acid 0.07%, geniposide 0.08%, aucubin 2.44%, chlorogenic acid 2.84%-5.28%, and flavonoids 2.21%. The crude fat content in dried leaves is 7.25%, with a fatty acid composition comprising myristic acid 0.3%, palmitic acid 10.70%, stearic acid 1.72%, hexadecatrienoic acid 0.75%, linoleic acid 1.59%, linolenic acid 45.85%, arachidic acid 5.01%, behenic acid 16.36%, tricosanoic acid 9.95%, tetracosanoic acid 7.10%, and other volatile fatty acids. Notably, the three unsaturated fatty acids (USFA)—hexadecatrienoic acid, linoleic acid, and linolenic acid—account for 48.19% of total fatty acids. China possesses abundant Eucommia leaf resources, which besides being used as tea, can be applied as a special feed additive in animal production. As a feed ingredient, Eucommia leaves not only improve animal performance but also enhance product quality, making research on their effects on livestock product quality highly significant.

Numerous studies have demonstrated that dietary supplementation with Eucommia leaves or their extracts can enhance immune function, improve growth performance, and ameliorate meat quality in animals. The bioactive functional components in Eucommia leaves (chlorogenic acid, geniposidic acid, aucubin, flavonoids, etc.) exhibit antibacterial, anti-inflammatory, antiviral, antioxidant properties, and can reduce hepatic fat deposition and alleviate hepatocyte damage. Eucommia leaves and their extracts are increasingly being utilized as feed additives in animal production, showing considerable development value and market prospects. Although some reports exist on the application of Eucommia leaves as feed or additives in animal production, most have focused on monogastric animals such as pigs and poultry, with research content largely limited to production effects, product quality improvement, and blood biochemistry, lacking in-depth mechanistic studies. Research in ruminants is particularly scarce, and the few mechanistic studies available have been conducted in rats, mice, or cell cultures, lacking comprehensiveness and systematicity. Our previous research investigated the effects of Eucommia leaves on nutrient utilization, growth performance, and slaughter characteristics in sheep, but did not explore the mechanistic aspects of lipid metabolism. This study aims to investigate the mechanisms by which Eucommia leaves regulate lipid metabolism and affect meat quality in sheep at the blood, tissue, and molecular levels, providing a theoretical basis for the development and utilization of Eucommia leaf feed resources.

Materials and Methods

1.1 Source, Nutritional Composition of Eucommia Leaves, and Diet Formulation The Eucommia leaves used in this study were collected from Eucommia trees planted in Ruyang County, Henan Province, harvested in late autumn, sun-dried, and stored in bales. Analysis revealed that the conventional nutrient contents of Eucommia leaves (air-dry basis) were: dry matter (DM) 89.46%, gross energy (GE) 19.47 MJ/kg, crude protein (CP) 12.38%, calcium

(Ca) 2.06%, and phosphorus (P) 0.05%. Diets were formulated according to NRC (2007) nutrient requirements for sheep and mixed as total mixed rations (TMR) using a TMR mixer (9JSG-5).

1.2 Experimental Design and Animal Management The experiment was conducted from April 20 to August 22, 2015, at the breeding base of Luoyang Longxupo Agriculture and Livestock Co., Ltd. (Xiaodian Town, Ruyang County, Henan Province). Thirty Hu sheep aged 70–80 days with body weights of 25–30 kg were selected. A single-factor completely randomized block design was employed with diet as the experimental factor. Based on similar body weight principles, sheep were randomly divided into three groups: a control group (CTL) fed diets without Eucommia leaves, a low-level Eucommia leaf group (EUL1) fed diets containing 10% Eucommia leaves, and a high-level Eucommia leaf group (EUL2) fed diets containing 20% Eucommia leaves, with 10 sheep per group. The dietary composition and nutrient levels are presented in Table 1 . Prior to the experiment, all sheep were dewormed uniformly. The pre-experimental period lasted 15 days, followed by a 90-day formal experimental period. Sheep were fed twice daily (morning and evening). Experimental sheep were housed in group pens with 4-5 more feeding slots than the number of sheep in each pen to ensure all animals could access feed. Feed was evenly distributed in troughs with leftover feed controlled at approximately 5%. Sheep had free access to water and exercise. Water troughs were cleaned and pens were swept daily.

1.3 Dietary Nutrient Analysis Dietary CP content was determined by the Kjeldahl method using an automatic Kjeldahl nitrogen analyzer (SKD-2000, Shanghai Peiou Analytical Instrument Co., Ltd.). Ether extract (EE) content was measured by Soxhlet extraction using a Soxhlet extractor (BSXT-06, Shanghai Bilang Instrument Co., Ltd.). Calcium content was determined by atomic absorption spectrophotometry using an atomic absorption spectrometer (Z-2000, Hitachi). Total phosphorus content was measured by molybdenum yellow spectrophotometry using a UV-visible spectrophotometer (T6, Beijing Puxi General Analytical Instrument Co., Ltd.). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined according to national standards.

1.4 Feed Intake Measurement During the formal experimental period, feed intake was recorded daily, and nutrient intake was calculated based on dietary nutrient contents.

1.5 Blood Sample Collection and Processing One week before the end of the formal experimental period, blood samples (5 mL) were collected from the jugular vein of each sheep before morning feeding (07:00) using vacuum anticoagulant tubes. Tubes were gently inverted twice to ensure adequate mixing of anticoagulant and blood, then placed in an incubator at approximately 4

°C. After collection, samples were centrifuged at 1,500×g for 15 minutes (centrifuge model: TDZS-WS, Xiangyi Centrifuge Factory). Plasma was separated, transferred to EP tubes, and stored at -20 °C until analysis.

1.6 Plasma Lipid Metabolism Index Determination Plasma triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) contents were measured using an automatic biochemical analyzer (AU640, Olympus, Japan). Very low-density lipoprotein (VLDL) and non-esterified fatty acid (NEFA) contents were determined by enzyme-linked immunosorbent assay (ELISA) using kits purchased from Shanghai Lanji Biotechnology Co., Ltd., following the manufacturer' s instructions.

1.7 Meat Sample Collection After the feeding trial, sheep were weighed. Five sheep were randomly selected from each group, fasted for 24 h, and deprived of water for 12 h, then re-weighed and slaughtered according to methods described in reference [18]. Carcasses were held at room temperature for 24 h of aging, after which approximately 200 g of thoracolumbar longissimus dorsi muscle was collected, wrapped in aluminum foil, placed in plastic self-sealing bags, stored in an ice-box (2 °C), transported to the laboratory, and stored at -20 °C until analysis.

1.8 Muscle Nutrient Content Determination Appropriate meat samples were collected, visible fascia and tendons were removed, and the samples were minced for moisture content determination using a thermostatic blast drying oven (DHG-9123A, Shanghai Yunan Instrument Co., Ltd.). Additional samples were freeze-dried, ground, and passed through a 60-mesh sieve for determination of: crude protein content by the Kjeldahl method using an automatic Kjeldahl nitrogen analyzer (SKD-2000, Shanghai Peiou Analytical Instrument Co., Ltd.); ether extract content by Soxhlet extraction using a Soxhlet extractor (BSXT-06, Shanghai Bilang Instrument Co., Ltd.); crude ash content by gravimetric method using a muffle furnace (SX2-8-10, Jiangsu Taizhou Best Electric Appliance Co., Ltd.); fatty acid composition by gas chromatography (Thermo Trace 1300, Thermo Fisher, USA); and cholesterol content by high-performance liquid chromatography (Waters 2695 HPLC, Waters, USA).

1.9 Determination of mRNA Expression Levels of Lipid Metabolism-Related Enzymes and Nuclear Transcription Factors Real-time fluorescent quantitative PCR was used to determine mRNA expression levels of lipid metabolism-related enzymes and nuclear transcription factors in liver tissue. Lipid metabolism-related enzymes included stearoyl-CoA desaturase 1 (SCD1), acetyl-CoA carboxylase-α (ACC-α), fatty acid synthase (FASN), carnitine palmitoyltransferase 1A (CPT1A), lipoprotein lipase (LPL), and adipose triglyceride lipase (ATGL). Nuclear transcription factors included sterol regulatory element-binding protein 1c (SREBP-1c), peroxisome proliferator-activated receptor γ (PPARγ), peroxisome proliferator-activated receptor α (PPARα), and CCAAT/enhancer-binding protein α (C/EBPα). Primers for these genes were designed based on corresponding sheep gene sequences searched from GenBank using online primer design software (http://primer5.ut.ee/). Primer sequences were synthesized by Sangon Biotech (Shanghai) Co., Ltd. and validated for specificity. Detailed primer information is provided in Table 2 .

The assay procedure was as follows: liver tissue was quickly removed from liquid nitrogen, approximately 0.5 g was cut, total RNA was extracted using RNAiso Plus, and reverse-transcribed to cDNA using a reverse transcription kit (PrimeScript™ RT reagent Kit with gDNA Eraser, TaKaRa, Dalian). Real-time fluorescent quantitative PCR was performed using a real-time PCR instrument (Mastercycler nexus, Eppendorf, Germany) with the SYBR Green method. Reaction parameters were set as: pre-denaturation at 95 °C for 2 min, followed by 40 cycles of denaturation at 95 °C for 15 s and annealing at 60 °C for 20 s.

1.10 Determination of Protein Levels of Lipid Metabolism-Related Enzymes and Nuclear Transcription Factors For genes showing significant differences ($P<0.05$) in mRNA expression, protein levels were determined by Western blot. The method was as follows: approximately 0.1 g of frozen sheep liver tissue was lysed with 1 mL of RIPA lysis buffer containing 1% protease inhibitor phenylmethylsulfonyl fluoride (PMSF). The tissue was ground in liquid nitrogen and homogenized for 30 s using a tissue homogenizer, left on ice for 30 min, then centrifuged at 12,000×g for 20 min. The middle aqueous phase was collected, excluding the fat layer on the surface and the precipitate at the bottom. The sample was centrifuged again at 12,000×g for 20 min, and the middle aqueous phase was collected. Protein concentration was determined by bicinchoninic acid (BCA) assay, and proteins were denatured by boiling at 99 °C for 10 min. SDS-PAGE gels were prepared, and protein samples (50 µg) were loaded. Electrophoresis was performed at 100 V for 30 min, and after protein bands appeared in the separating gel, voltage was increased to 120–140 V until bromophenol blue migrated to approximately 0.5–1.0 cm from the gel bottom. Proteins were wet-transferred to polyvinylidene fluoride (PVDF) membranes that had been activated by soaking in methanol for 15 min. Transfer was performed at 105 V for 70 min. After transfer, membranes were blocked with 5% skim milk blocking solution for 1 h, then incubated with primary antibody (diluted 1:1,000 with 5% skim milk) overnight at 4 °C. Membranes were washed three times with TBST for 5 min each, then incubated with secondary antibody [horseradish peroxidase (HRP)-labeled goat anti-rabbit immunoglobulin G (IgG) diluted 1:3,000 with 5% skim milk] at room temperature for 2 h, followed by three additional TBST washes (5 min each). Bands were visualized using the ECL method, and grayscale optical density analysis of target bands was performed using Image J for quantification. β-actin was used as an internal reference for each sample.

1.11 Statistical Analysis Data were initially processed using Excel 2007, and inter-group differences were analyzed by one-way ANOVA using GraphPad

Prism 5.0 statistical software. When ANOVA showed significant differences, Turkey' s multiple comparison test was performed. $P<0.05$ was considered statistically significant, $P<0.01$ as highly significant, and $0.05 \leq P<0.10$ as a trend toward difference. Data are expressed as mean±SD.

Results

2.1 Effects of Eucommia ulmoides Leaves on Feed Intake in Sheep The effects of Eucommia ulmoides leaves on dry matter intake (DMI) and main nutrient intakes in sheep are shown in Table 3 . The results indicate that DMI in the EUL1 and EUL2 groups was significantly increased compared with the CTL group ($P<0.05$), with the EUL1 group being significantly higher than the EUL2 group ($P<0.05$). Metabolic energy intake and nitrogen intake in the EUL1 and EUL2 groups were significantly higher than in the CTL group ($P<0.05$), with no significant difference between the EUL1 and EUL2 groups ($P>0.05$). Calcium and phosphorus intakes did not differ significantly among groups ($P>0.05$).

2.2 Effects of Eucommia ulmoides Leaves on Plasma Lipid Metabolism Indices in Sheep The effects of Eucommia ulmoides leaves on plasma lipid metabolism indices are presented in Table 4 . Compared with the CTL group, plasma HDL, VLDL, and NEFA contents in the EUL2 group were significantly increased ($P<0.05$), while no significant differences were observed between the EUL1 and CTL groups ($P>0.05$). Plasma TC content was significantly reduced in both EUL1 and EUL2 groups compared with the CTL group ($P<0.05$), with no significant difference between the EUL1 and EUL2 groups ($P>0.05$). Plasma LDL content in the EUL1 and EUL2 groups showed a decreasing trend compared with the CTL group ($0.05 \leq P<0.10$).

2.3.1 Effects of Eucommia ulmoides Leaves on Muscle Nutrient Contents in Sheep The effects of Eucommia ulmoides leaves on muscle nutrient contents are shown in Table 5 . No significant differences were observed among groups in muscle moisture, cholesterol, or crude ash contents ($P>0.05$). Compared with the CTL group, muscle crude protein content was increased in both EUL1 and EUL2 groups, with a significant elevation in the EUL1 group ($P<0.05$) but no significant difference in the EUL2 group ($P>0.05$). Muscle ether extract content followed the pattern EUL2 > EUL1 > CTL, with the EUL2 group being significantly higher than the CTL group ($P<0.05$) but no significant difference between the EUL1 group and the other two groups ($P>0.05$). Muscle shear force was decreased in both EUL1 and EUL2 groups compared with the CTL group, with a significant reduction in the EUL1 group ($P<0.05$) but no significant difference in the EUL2 group ($P>0.05$).

2.3.2 Effects of Eucommia ulmoides Leaves on Muscle Fatty Acid Composition in Sheep The effects of Eucommia ulmoides leaves on muscle fatty acid composition are presented in Table 6 . Fourteen fatty acids were detected in muscle, categorized as saturated fatty acids (SFA) and unsaturated fatty acids (USFA), with USFA further divided into monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). Analysis revealed that muscle SFA content gradually decreased with increasing EUL supplementation levels, though differences among groups were not significant (P>0.05). In contrast, muscle USFA content was significantly increased (P<0.05), with significant inter-group differences (P<0.05). Specifically, compared with the CTL group, muscle MUFA and PUFA contents in the EUL2 group were significantly increased (P<0.05), while no significant changes were observed in the EUL1 group (P>0.05). Total fatty acid content also gradually increased with higher EUL supplementation levels. The USFA/SFA ratio increased from 1.49 in the CTL group to 1.85 in the EUL1 group and 2.22 in the EUL2 group, with the EUL2 group being significantly higher than the CTL group (P<0.05). Further analysis showed no significant differences among groups in individual SFA contents (P>0.05), while various USFA contents increased to different extents. Pentadecenoic acid, palmitoleic acid, linoleic acid, and arachidonic acid contents in muscle were significantly higher in both EUL1 and EUL2 groups compared with the CTL group (P<0.05). α-linolenic acid content showed a trend toward being higher than the CTL group (0.05≤P<0.10), and oleic acid content in the EUL2 group was significantly higher than in the CTL group (P<0.05).

2.4.1 Effects of Eucommia ulmoides Leaves on Hepatic mRNA Expression Levels of Lipid Metabolism-Related Enzymes and Nuclear Transcription Factors in Sheep The effects of Eucommia ulmoides leaves on hepatic mRNA expression levels of lipid metabolism-related enzymes and nuclear transcription factors are shown in Table 7 . For lipogenic enzymes, compared with the CTL group, hepatic SCD1 mRNA expression levels were decreased in both EUL1 and EUL2 groups, with a significant reduction in the EUL2 group (P<0.05), while ACC-α and FASN mRNA expression levels showed no significant changes (P>0.05). For lipolytic enzymes, compared with the CTL group, hepatic CPT1A mRNA expression levels were increased in both EUL1 and EUL2 groups, with a significant elevation in the EUL1 group (P<0.05). Hepatic LPL mRNA expression level was significantly increased in the EUL1 group (P<0.05) but showed no significant change in the EUL2 group (P>0.05). Hepatic ATGL mRNA expression levels did not differ significantly among groups (P>0.05). For lipogenesis-related nuclear transcription factors, compared with the CTL group, hepatic SREBP-1c mRNA expression levels were significantly decreased in both EUL1 and EUL2 groups (P<0.05), with no significant difference between the EUL1 and EUL2 groups (P>0.05). Hepatic PPARγ mRNA expression level was significantly decreased in the EUL2 group (P<0.05) but showed no significant change in the EUL1 group (P>0.05). For lipolysis-related nuclear transcription factors, hepatic PPARα mRNA expression levels did not differ significantly among groups (P>0.05), and C/EBPα mRNA expression levels also showed no significant differences among groups (P>0.05).

2.4.2 Effects of Eucommia ulmoides Leaves on Hepatic Protein Levels of Lipid Metabolism-Related Enzymes and Nuclear Transcription Factors To further investigate the relationship between nuclear transcription factors and lipid metabolism enzymes, genes showing significant differences in mRNA expression levels (SCD1, LPL) and nuclear transcription factors (SREBP-1c, PPARγ) were selected for protein-level analysis by Western blot. The results are shown in Figure 2 [Figure 2: see original paper]. Changes in protein levels of these lipid metabolism enzymes and nuclear transcription factors were generally consistent with their mRNA expression patterns. Compared with the CTL group, hepatic PPARγ and SCD1 protein levels in both EUL1 and EUL2 groups remained decreased, with a greater reduction observed in the EUL2 group. Specifically, hepatic SCD1 protein level was significantly decreased in the EUL2 group ($P<0.05$), confirming that PPARγ and SCD1 are important regulators of lipid metabolism. However, hepatic SREBP-1c and LPL protein levels did not differ significantly among groups ($P>0.05$).

Discussion

3.1 Effects of Eucommia ulmoides Leaves on Lipid Metabolism Numerous studies have demonstrated that Eucommia ulmoides leaves significantly affect blood physiological indices. Ma et al. found that dietary supplementation with Eucommia leaves reduced blood TG and TC contents in pigs. Other studies have shown that adding Eucommia leaves to chicken diets decreased serum TG, TC, and LDL contents while increasing HDL content, enhanced catalase (CAT) and peroxidase (POD) activities, and significantly reduced malondialdehyde (MDA) content, liver fat content, and liver index. Plasma TG, TC, VLDL, LDL, and HDL contents are important indicators reflecting lipid metabolism status. Blood VLDL is the form in which the liver transports TG to peripheral tissues, showing an inverse relationship with TG. In this study, the increased plasma VLDL in the EUL1 and EUL2 groups indicated enhanced TG export from the liver, which corresponded with reduced plasma TG content. LDL is derived from VLDL degradation and transports cholesterol synthesized in the liver to extrahepatic tissues for metabolism. The significantly reduced LDL and TC contents in the EUL1 and EUL2 groups indicated decreased cholesterol synthesis in the liver. HDL is primarily synthesized in the intestine and can also be converted from VLDL, functioning to transport cholesterol from peripheral tissues to the liver for metabolism into bile acids that are excreted. The significantly increased plasma HDL content in the EUL1 and EUL2 groups was associated with reduced TC content and increased VLDL content, as VLDL degradation produces HDL. The TG generated from VLDL degradation is further broken down into NEFA, leading to significantly increased NEFA content. These changes are related to the ability of bioactive components in Eucommia leaves, such as syringin, geniposidic acid, and aucubin, to inhibit lipid absorption and synthesis while promoting lipid catabolism.

3.2 Effects of Eucommia ulmoides Leaves on Muscle Fat Composition and Meat Quality in Sheep Fat is a crucial indicator of meat quality, with intramuscular fat content directly affecting meat flavor and quality. Increased intramuscular fat content reduces muscle shear force and improves tenderness. Cao et al. reported that dietary supplementation with a Chinese herbal feed additive primarily composed of Eucommia leaf extract increased average daily gain by 9.43%, decreased feed-to-gain ratio by 10.23%, reduced backfat thickness by 20.35%, increased lean meat percentage by 15.1%, increased loin eye area by 6.7%, and decreased fresh meat drip loss by 12.02% in finishing pigs. Wang et al. demonstrated that dietary Eucommia extract supplementation significantly increased intramuscular fat, total amino acid, and umami amino acid contents in pigs, while reducing drip loss and improving meat color score, loin eye area, and marbling score. These studies indicate that Eucommia leaves can alter lipid metabolism and distribution in the body, reduce subcutaneous fat deposition, increase intramuscular fat content, and improve meat quality.

Further analysis revealed that while muscle SFA contents did not differ significantly between the EUL1 and EUL2 groups, the significant increase in USFA content was an important factor contributing to improved meat quality. It is widely recognized that dietary fatty acid composition substantially influences muscle fat content and fatty acid composition. The increased USFA content in the EUL1 and EUL2 groups may be attributed to several factors: (1) Eucommia leaves are rich in USFA such as hexadecatrienoic acid, linoleic acid, α-linolenic acid, and arachidic acid, which increase precursor availability for muscle USFA synthesis; (2) Lignans, iridoids, flavonoids, and other compounds in Eucommia leaves can affect lipid metabolism and inhibit SFA synthesis; and (3) Eucommia leaves influence lipid distribution in the body, with active components reducing blood lipid levels while increasing intramuscular fat content. Our previous research indicated that Eucommia leaves can increase feed intake and daily gain to some extent, though effects on pre-slaughter weight were not significant, and impacts on slaughter characteristics varied, with increased bone-to-meat ratio but decreased dressing percentage and carcass lean meat percentage. In this study, the increased crude protein and ether extract contents in muscle suggest that active components in Eucommia leaves alter nutrient partitioning in animals. However, the overall effects on body fat distribution require further investigation.

Different fatty acids exert varying effects on meat quality. Fat in ruminant muscle is primarily composed of long-chain saturated and monounsaturated fatty acids. Degradation of fatty acids produces different short-chain fatty acids such as hexanoic and octanoic acids, which are important compounds contributing to meat flavor. Palmitoleic acid, oleic acid, and linoleic acid significantly influence meat flavor, whereas stearic acid, α-linolenic acid, capric acid, and butyric acid are associated with off-flavors. Therefore, the content and composition of intramuscular fatty acids are primary determinants of meat flavor. Research has shown that most SFAs, such as lauric acid, myristic acid, and palmitic acid, are considered risk factors for cardiovascular disease. Keys et al. and Schroeder et al. found that SFAs (except stearic acid) can elevate blood cholesterol, whereas USFA can reduce blood glucose, lipid, and cholesterol contents. Oleic acid, a MUFA comprising 34%–43% of muscle fatty acids, can lower cholesterol and is considered a beneficial fatty acid. PUFA (n-3 and n-6 series) exert multiple biological effects including hypolipidemic, hypotensive, and anti-atherosclerotic activities. The PUFA/SFA ratio is commonly used as a meat quality indicator, and an increased ratio within a certain range is beneficial for reducing blood TG, VLDL, and TC contents. In this study, the decreased SFA content and increased USFA content and PUFA/SFA ratio in the EUL1 and EUL2 groups contributed to improved mutton quality. Nevertheless, due to the unique rumen digestion characteristics of ruminants, the extent to which active components in Eucommia leaves affect fatty acid composition in mutton requires further investigation.

3.3 Effects of Eucommia ulmoides Leaves on Hepatic Lipid Metabolism Gene Expression The liver is the central organ of lipid metabolism and can reflect the overall lipid metabolic status of the body. Active components in Eucommia leaves, such as syringin, aucubin, geniposidic acid, chlorogenic acid, and flavonoids, can affect lipid synthesis and catabolism through intracellular signaling pathways. Pan et al. reported that Eucommia leaves could inhibit hepatic FASN synthesis, reduce lipid synthesis, increase hormone-sensitive lipase (HSL) protein and gene expression levels, promote lipid catabolism, and enhance mRNA expression levels of acyl-CoA oxidase (ACO) and carnitine acylcarnitine translocase (CACT), thereby promoting lipid oxidation and reducing body fat deposition. Li et al. demonstrated that chlorogenic acid extract from Eucommia leaves could reduce dietary fat, cholesterol, and bile acid absorption, decrease pancreatic lipase and hydroxymethylglutaryl-CoA (HMG-CoA) reductase activities, and inhibit cholesterol micelle formation. In addition to these specific medicinal components, the abundant PUFA in Eucommia leaves also significantly affect hepatic lipogenic enzyme gene expression. Studies have shown that n-3 and n-6 PUFA can inhibit expression of hepatic lipogenic enzymes such as FASN, acetyl-CoA carboxylase (ACC), and stearoyl-CoA desaturase (SCD), while promoting expression of genes related to fatty acid β-oxidation, thereby reducing body fat content and risk of cardiovascular disease.

PPARα is a ligand-activated nuclear transcription factor belonging to the peroxisome proliferator-activated receptor (PPARs) family, which is involved in glucose and lipid metabolism. Long-chain fatty acids (LCFA) are the primary endogenous ligands for PPARs. The PPAR family mainly includes three isoforms: PPARα, PPARβ/δ, and PPARγ. PPARα primarily regulates fatty acid transport, esterification, and oxidation, inhibits inflammatory responses, and is highly expressed in hepatocytes and cardiomyocytes. Genes regulated by PPARα include CPT1A, electron transfer flavoprotein dehydrogenase (ETFDH), mitochondrial trifunctional protein (HADHA), hydroxymethylglutaryl-CoA synthase 2 (HMGCS2), and LPL. The first three genes are involved in mitochondrial

β-oxidation of LCFA, while CPT1A is a key regulatory enzyme that transports LCFA into mitochondria. In this study, the slightly increased hepatic PPARα mRNA expression level in the EUL1 and EUL2 groups indicated enhanced hepatic fatty acid oxidation.

PPARγ is primarily expressed in differentiated adipocytes and controls transcription of genes involved in lipid synthesis and energy storage, such as adipocyte fatty acid-binding protein (AFABP), acyl-CoA synthetase, fatty acid synthase, stearoyl-CoA desaturase, LPL, and fatty acid transport protein 1 (FATP1), regulating lipid synthesis, transport, storage, and adipocyte differentiation. When TG content is insufficient in adipocytes, exogenous LCFA activate PPARγ and induce expression of genes related to TG storage. In this study, the significantly decreased hepatic PPARγ mRNA expression level in the EUL1 and EUL2 groups indicated reduced adipocyte numbers in the liver and confirmed the inhibitory effect of active components in Eucommia leaves on PPARγ gene expression.

SREBP-1c is an important member of the sterol regulatory element-binding protein (SREBPs) family of nuclear transcription factors, primarily regulating expression of genes related to fatty acid synthesis and glucose metabolism, including ACC, FASN, SCD1, and low-density lipoprotein receptor (LDLR), thereby promoting hepatic lipid synthesis. In this study, the decreased hepatic SREBP-1c mRNA and protein levels in the EUL1 and EUL2 groups predicted reduced transcription of fatty acid synthesis-related enzymes. Although hepatic FASN and ACC-α mRNA expression levels did not change significantly, hepatic SCD1 mRNA and protein levels were significantly decreased, indicating an overall trend of reduced fatty acid synthesis. SCD1 is a key enzyme in fatty acid synthesis, catalyzing the desaturation of palmitoyl-CoA and stearoyl-CoA to produce palmitoleoyl-CoA and oleoyl-CoA. The significantly decreased hepatic SCD1 mRNA and protein levels in this study indicated inhibition of fatty acid synthesis. Additionally, PPARα regulates transcription of fatty acid catabolism-related enzymes. The slightly increased hepatic PPARα mRNA expression level in this study predicted elevated transcription of fatty acid catabolism enzymes. CPT1A, ATGL, and LPL are enzymes involved in lipid catabolism, and their mRNA expression levels were increased to varying degrees in the EUL1 and EUL2 groups (except for LPL in the EUL2 group), confirming the PPARα expression results and indicating enhanced hepatic lipid catabolism. This corresponded with reduced plasma TG, VLDL, TC, and LDL contents in the EUL1 and EUL2 groups and suggested that the increased muscle USFA content in these groups did not originate from hepatic synthesis. The mechanisms by which active components in Eucommia leaves affect intramuscular lipid metabolism remain unclear and warrant further investigation.

Conclusion

This study demonstrates that Eucommia ulmoides leaves significantly affect lipid metabolism in sheep by: (1) influencing lipid metabolism throughout the body; (2) modulating muscle fatty acid composition by increasing the proportion of unsaturated fatty acids and improving mutton quality; and (3) regulating hepatic lipid metabolism through effects on expression of lipid metabolism-related enzymes and nuclear transcription factor genes.

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