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Effects of perilla frutescens seed supplemented to diet on fatty acid composition and lipogenic gene expression in muscle and liver of Hu lambs

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ABSTRACT

The objective of this study was to determine the effect of perilla (*perilla frutescens* L.) seed (PFS) supplementation on serum lipids metabolism, intramuscular adipocyte size, fatty acid composition and lipogenic genes expression in muscle and liver of Hu lambs. Sixty male Hu lambs (23.02 ± 1.36 kg body weight and approximately 3 months of age) were randomly assigned to four dietary treatments receiving diets containing 0%, 5%, 10% or 15% perilla seed (CD, 5%PFSD, 10%PFSD and 15%PFSD, respectively). During the 84 days experimental period, these groups were fed the assigned diets *ad libitum*. Compared with CD group, LDL-cholesterol, total cholesterol, and triglyceride levels in the serum significantly ($P < 0.05$) increased with the supplementation of PFS to lamb diets. The supplementation of PFS promote intramuscular adipocyte hypertrophy ($P < 0.05$). Adding PFS rich in α-linolenic acid (ALA) increased the contents of ALA, vaccenic acid (VA), eicosapentaenoic acid (EPA) and docosapentaenoic acid (DPA), in *longissimus* muscle and liver ($P < 0.05$). However, the contents of C18:1*cis*9, linoleic acid (LA), and total saturated fatty acids (SFA) and n-6 to n-3 ratio decreased in muscle and liver in PFS groups compared with CD group ($P < 0.05$). The supplementation of PFS significantly increased *PPARα* and *PPARγ* expression and significantly decreased *LPL*, *ACC*, *FASN*, *SCD*, *FADS*1, and *FADS2* expression ($P < 0.05$). These results suggest that PFS supplementation in lambs' diets can influence the metabolism of lipids and promotes intramuscular adipocyte hypertrophy and deposition of n-3 polyunsaturated fatty acids (n-3 PUFA) in muscle and live tissues.

1. Introduction

It is widely recognized that human nutrition and health were affected by the content and composition of dietary fat. High consumption of saturated fatty acids (SFA) has been associated with numerous cancers, atherosclerosis and cardiovascular disease (Siri-Tarino et al., 2010; Laviano et al., 2013), while replacing SFA with polyunsaturated fatty acids (PUFA) can reduces the risk of coronary heart disease (Endo and Arita, 2016). Generally, in ruminant meat, the proportion of SFA are often higher (Bas and Morand-Fehr, 2000), the PUFA to SFA ratio lower and the n-6 to n-3 ratio higher, because biohydrogenation in ruminants leads to extensive loss of dietary unsaturated fat. Feeding whole oilseeds is one of the strategies to reduce rumen biohydrogenation and to manipulate the fatty acid composition of ruminant, as the seed coat provides protection for unsaturated fatty acids from rumen microorganisms (Aldrich et al., 1997).

Perilla frutescens, a plant native to Asian countries, is one of the richest botanical sources of α-linoleic acid (ALA) (Sargi et al., 2013). Perilla seed (PFS) as a good feed ingredients are not only rich in ALA but also crude protein. Previous research showed that PFS might be used in the diet of rabbits to increase PUFA concentration in muscles (Peiretti et al., 2011). In addition, our previous found that dietary supplementation with PFS improves meat quality and has no adverse effect on growth performance and carcass characteristics (Deng et al., 2017a, b). Therefore, perilla seed may be a promising feed supplement that modify fatty acid composition of animal-derived food product. However, little is known about the effect of dietary whole PFS on the fatty acid composition of ruminants.

Several lipogenic genes are regulated by genetic and dietary fatty acid (Hocquette et al., 2007; Corazzin et al., 2013), however, little information about the effect of dietary lipids supplementation (especially PFS) on the expression of genes involved in lipid metabolism in lambs,

Abbreviations: ALA, α-linolenic acid; CLA, Conjugated linoleic acid; DPA, Docosapentaenoic acid; FADS1, Fatty acid desaturase-1; FADS2, Fatty acid desaturase-2; EPA, Eicosapentaenoic acid; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; LPL, Lipoprotein lipase; LA, Linoleic acid; PFS, Perilla. Seed; PPARα, Peroxisome proliferator-activated receptor alpha; PPARγ, Peroxisome proliferator-activated receptor gamma; PUFA, Polyunsaturated fatty acids; SCD, Stearoyl CoA desaturase; SFA, Saturated fatty acid; VA, Vaccenic acid

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especially for long-chain PUFA synthesis-related genes (such as *FADS1*, *FADS2*). It was hypothesized that PFS can manipulate the fatty acid composition of lambs by providing increased quantities of n-3 PUFA and influencing the expression of specific genes. Except for fatty acid composition, intramuscular adipose tissue amount also plays important role in meat quality. Moreover, polyunsaturated fatty acids influence adipogenesis through regulating related to it transcription factors (such as *PPARγ* and *CEBPA*). Therefore, this study aims to evaluate the effect of whole PFS as a dietary source of n-3 PUFA on serum lipid metabolites concentration, intramuscular adipocyte size, fatty acid composition, and the expression of some lipogenic genes in muscle and liver of Hu lambs.

2. Materials and methods

2.1. Experimental design, animals, and diets

All trials were approved by the Nanjing Agricultural University China Animal Care and Use Committee, and the experiment was carried out at the Jiangsu Taizhou Helen Sheep Industry Co. LTD.

Sixty male Hu lambs with an average initial body weight of 23.02 ± 1.36 kg and approximately 3 months of age were randomly assigned into four diet treatments, and each treatment was replicated five times. Every three lambs were housed in pen with slatted floor (4.0 × 4.0 m, animal density of 5.3 m^2 per lamb). During the entire experimental period of 84 days, these groups were fed an isocaloric and isonitrogenous diet *ad libitum*, containing different levels of PFS (0%, 5%, 10%, and 15%, respectively). The PFS was purchased from Zheng Ning Jinniu Industrial Co. Ltd. The ingredient list and chemical composition of the experimental diets are shown in Table 1 and Table 2. The four experimental treatments were fed the assigned diets as total mixed ration *ad libitum*. Clean water was available *ad libitum* throughout the experiment.

2.2. Slaughtering procedure and sampling

In the morning of day 80 before feed were offered, blood samples (10 ml) were collected from all lambs by jugular vein puncture using vacutainers. To separate out the serum, blood samples were placed for 45 min, followed by centrifugation at 1000 × *g* for 15 min. Then the serum was immediately stored at − 20 °C for later analysis. At the end of experiment, animals were fasted for 16 h and transported to slaughter house. At 30 min post-slaughter, *longissimus* muscle samples were obtained from the 11th to 12th thoracic ribs, and liver samples were sampled from the left side of the carcass. Samples were dived into two aliquots: one aliquot was fixed in 4% formalin for 24 h, then these tissues were embedded into paraffin, blocked and cut for haematoxylin and eosin (H&E) staining; the other were immediately frozen in liquid nitrogen, and stored at − 80 °C until analysis.

Table 1
Ingredients (%) and chemical composition (on dry matter basis) of experimental diets.

Item	Treatments			
	CD	5%PFSD	10%PFSD	15%PFSD
Ingredients, % of DM				
Soybean straw	35.64	39.14	41.64	45.14
Corn	30.00	25.50	20.60	14.50
Soybean meal	21.00	17.00	14.40	12.00
Perilla seed	0	5.00	10.00	15.00
Corn bran	3.00	3.00	3.00	3.00
Cassava residue	6.00	6.00	6.00	6.00
Salt	0.36	0.36	0.36	0.36
Vitamin and mineral Premix[a]	4.00	4.00	4.00	4.00
Chemical composition, % of Dry matter				
Organic matter	90.51	91.72	91.21	90.28
Crude protein	15.81	15.19	15.59	14.60
Ether extract	1.86	3.89	6.01	8.09
Ash	9.49	8.29	8.79	9.72
Neutral detergent fiber	33.68	36.71	39.21	42.33
Acid detergent fiber	23.64	26.13	28.20	30.80
Metabolizable energy[b], MJ/kg	9.38	9.36	9.38	9.36

[a] Provided 56 mg Fe, 15 mg Cu, 30 mg Mn, 40 mg Zn, 1.5 mg I, 0.2 mg Se, 0.25 mg Co, 3.2 g S, 2150 IU Vitamin A, 170 IU Vitamin D, 13 IU Vitamin E, 2 1.6 g 2% monensin, 10.1 g Sodium sulfate per kg of diet.

[b] Metabolizable energy values were calculated based on the feed composition (DM basis), whereas the others were measured values.

Table 2
Fatty acid composition (g/100 g of total fatty acids) of PFS and diets.

Item	Flaxseed[a]	PFS[b]	Treatments			
			CD	5%PFSD	10%PFSD	15%PFSD
Fatty acid composition, g/100 g fatty acids						
C14:0	–	0.04	0.33	0.29	0.26	0.24
C16:0	4.13	9.30	24.84	21.18	18.50	16.39
C16:1	–	0.08	0.27	0.25	0.23	0.23
C17:0	–	0.05	0.24	0.21	0.19	0.17
C18:0	2.67	2.10	12.11	10.82	9.84	9.01
C18:1*cis*9	15.4	12.73	4.53	4.52	4.60	4.85
cis-9, *trans*-11 CLA	0.02	0.23	0.37	0.38	0.35	0.36
C18:2n-6 (LA)	14.00	13.78	47.57	37.84	30.79	25.06
C20:0	0.17	0.16	0.66	0.55	0.46	0.39
C18:3n-3 (ALA)	48.35	57.57	6.01	18.25	27.12	34.44
Other fatty acids	–	1.43	2.11	2.77	1.47	1.30
n-6: n-3	0.25	0.19	7.92	2.07	1.35	0.73

[a] The data of fatty acid composition of flaxseed from the study of Sargi et al. (2013).

[b] PFS: perilla seed.

2.3. H&E staining

The muscle and liver sections were deparaffinized, rehydrated and the nuclei stained with haematoxylin for 15 min. These sections were rinsed in running tap water and stained with eosin for 1 min, dehydrated and mounted. Whole-slide digital images were collected at × 20 magnification with a microscope (Nikon, Japan). Scan images of H&E staining were analyzed by Image J to determine the mean cell area per section. The mean cell area was recorded in μm^2.

2.4. Chemical analyses

The standard method of AOAC ((1990) was followed to determine the proximate chemical composition of diets and PFS. Dry matter was determined by oven-drying at 105 °C for 24 h. The nitrogen content of diets and PFS was determined by Kjeldahl method using Kjeltec Auto Analyzer (Tecator, Hoganas, Sweden), and then converted to crude protein (CP = N × 6.25). Lipids were extracted by the soxhlet method using ether as the solvent and ash by using a muffle furnace at 550 °C for 5 h. The neutral detergent fiber and acid detergent fiber were determined according to Vansoest et al. (1991).

2.5. Biochemical analyses

Serum was used for assessment of free fatty acid, high density lipoprotein-cholesterol, low-density lipoprotein cholesterol, total cholesterol and triglyceride concentrations. The total cholesterol, HDL-cholesterol, LDL-cholesterol and triglyceride was determined using automatic analyzer (AU 5800, Olympus, Japan). The free fatty acid and