The mechanism through which dietary supplementation with heated linseed grain increases n-3 long-chain polyunsaturated fatty acid concentration in subcutaneous adipose tissue of cashmere kids¹

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ABSTRACT: The aim of this study was to investigate the effects of dietary supplementation with heated linseed on the fatty acid (FA) composition of the plasma, liver, and subcutaneous adipose tissue (SADT) of Albas white cashmere kids, particularly the effect on n-3 long-chain polyunsaturated FA profiles and the mRNA expression of genes related to lipid metabolism in SADT. Sixty 4-month-old castrated male kids (average BW 18.6 ± 0.1 kg) were selected and randomly allocated into three groups in a randomized block design. Three dietary treatments were used: (1) basal diet without supplementation (Control), (2) basal diet supplemented with linseed oil (LSO), and (3) basal diet supplemented with heated linseed grain (HLS). The diets were fed for 104 d, consisting of 14 d for adaptation followed

by 90 d of measurement. Different FA profiles were found in SADT between LSO and HLS. Kids fed HLS had more C18:3n3 ($P < 0.0001$), C22:6n3 ($P = 0.007$), and n-3 PUFA ($P < 0.0001$) and a less ($P < 0.0001$) n-6/n-3 ratio than LSO kids. These FA differences between LSO and HLS kids were due to the increased expression of elongation of very long chain FA protein 5 ($P < 0.0001$), delta-6 desaturase ($P < 0.0001$), and peroxisome proliferator-activated receptor α ($P = 0.003$) in SADT of HLS kids and was also associated with liver fat metabolism. Together, these results suggest that the consumption of HLS leads to more C22:6n3 than LSO in SADT by increasing liver C22:6n3 content and by increasing SADT mRNA expression of ELOVL5 and FADS2 through promoting PPAR α expression.

Key words: fatty acids, gene expression, heated linseed, linseed oil, liver, subcutaneous adipose tissue

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INTRODUCTION

There is an imbalance in the proportion of fatty acids (FAs) in the typical western diet, with

a high ratio of n-6 unsaturated FA to n-3 polyunsaturated FA (n-6/n-3 PUFA; Williams, 2000). Additional dietary n-3 PUFA has benefits because more of the substrate C18:3n3 is converted to the functional n-3 long-chain PUFAs (n-3 LCPUFA, C20:5n3 and C22:6n3). An important source of n-3 PUFA is meat from ruminants. In a previous study with Albas white cashmere goats, it was found that the concentrations of n-3 PUFA and n-3 LCPUFA in muscle and adipose tissue were less in animals fed a total mixed ration than in animals fed pasture, but the n-6/n-3 ratio was greater in total mixed ration-fed animals than in pasture-fed animals. These differences were associated with a greater

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intake of C18:3n3 by the pasture-fed goats (Wang et al., 2018).

The n-3 PUFA content in meat can be increased by including linseed (oil) in the diet of the animals. However, linseed oil (LSO) has only a limited use in ruminant diets because it is hydrogenated in the rumen (Doreau and Ferlay, 1994), so oil supplements have to be protected to increase postruminal C18:3n3 flow. A simpler alternative was proposed by Shuang et al. (2014), who found that supplementing sheep with linseed grain that had been heated to approximately 130 °C for 20 min was more effective in increasing n-3 LCPUFA content in adipose tissue than supplementing with untreated linseed grain. However, the mechanism that explains this response is not understood, and it is not known if there is a similar effect in cashmere kids.

With respect to the mechanism, feeding strategies can modify the FA profile in sheep meat by altering the expression of genes for enzymes related to fat metabolism (Dervishi et al., 2011). Dietary FAs are converted by the liver into LCPUFA and secreted into the blood, where they are taken up by adipose and muscle tissues (Cherfaoui et al., 2012). Therefore, we tested the hypothesis that inclusion of heated linseed grain (HLS) in the diet of cashmere kids would increase n-3 LCPUFA content in subcutaneous adipose tissue (SADT) more than LSO, by increasing liver and plasma n-3 LCPUFA content and by upregulating the expression of genes for enzymes involved in n-3 LCPUFA synthesis in SADT.

MATERIALS AND METHODS

Animals, Diets, and Feeding Management

This study was conducted at the Inner Mongolia White Cashmere Goat Breeding Farm, Wulan Town, Etuoque Banner, Ordos City, Inner Mongolia Autonomous Region, China (39°12' N, 107°97' E), in accordance with the Animal Care and Use Committee of Inner Mongolia Agriculture University (Hohhot, Inner Mongolia, China) for the care and use of animals for experimental and other scientific purposes. Sixty 4-month-old castrated male kids (average BW 18.6 ± 0.1 kg) were selected and randomly allocated into three groups in a randomized block design, with each group composed of four units of five kids. Three dietary treatments were used (Table 1): (1) basal diet without supplementation (Control), (2) basal diet supplemented with LSO, and (3) basal diet supplemented with HLS. The oil-supplemented diets were prepared by manually blending the oil thoroughly into the ground concentrate to ensure homogenous

distribution of the oil in the ration. Supplemented linseed grain was heated using a hot air roaster for 10 min at 120 °C. The temperature was dictated by the specific roaster used and was measured between seeds with a needle thermometer immediately after the treatment. After heat treatment, the linseed grain was spread on the ground for cooling and stored at 4 °C until utilizing. No antioxidant was added. The diets were prepared fresh twice a day and were offered as total mixed ration (concentrate:forage ratio of 5:5) in two equal meals at 0830 and 1630 hours. The kids were given free access to drinking water. The diets were fed for 104 d, consisting of 14 d for adaptation followed by 90 d of measurement. The treatment period was divided into early (1 to 30 d), middle (31 to 60 d), and late periods (61 to 90 d) so levels of nutrition could be increased to meet the needs of growing cashmere kids (Table 1). Feed intake was recorded daily for the groups of five kids based on the amount of feed offered and refusals. The amount of feed offered was adjusted daily in the morning to ensure a 10% refusal (on a fresh basis). The major FAs of LSO, flaxseed cake, and HLS are C16:0, C18:0, C18:1n9c, C18:2n6c, and C18:3n3. The values for LSO are 4.50, 3.17, 15.07, 13.71, and 52.81, respectively (percent identified total FA); for flaxseed cake are 6.00, 2.65, 17.06, 13.88, and 53.56, respectively; and for HLS are 5.07, 3.02, 18.27, 15.89, and 55.87, respectively. The consumption of FA was calculated for each 30-d period as a proportion of metabolic weight.

Sampling and Slaughtering Procedures

Residuals were collected and weighed 30 min before each feeding at 0830 hours daily to estimate intake. Samples of total mixed ration were collected in separate plastic bags at the beginning of each period and stored at -20 °C for chemical analysis. At the end of the experiment, two kids from each unit were randomly selected and slaughtered by exsanguination. Before slaughter, the kids were prevented from consuming food for 24 h and from drinking for 2 h. Jugular blood (20 mL) was sampled into EDTA-containing Vacutainer tubes after the kids were prevented from consuming feed for 12 h. Blood was centrifuged at 3,000 × g for 15 min and plasma was harvested. Immediately after death, liver and SADT samples were collected, snap frozen in liquid N₂ and stored at -80 °C until analysis.

Chemical Analysis

Analysis of feed. Samples of dietary ingredients were analyzed for DM (method 930.15), CP (N

Table 1. Composition and analysis of experimental diets

Item	Diets ¹								
	1–30 d			31–60 d			61–90 d		
	Control	LSO	HLS	Control	LSO	HLS	Control	LSO	HLS
Ingredient, percent air dry basis									
Alfalfa hay particles	25	25	25	15	15	15	12.5	12.5	12.5
Maize straw particles	5	5	5	20	20	20	25	25	25
Tall oat grass particles	20	20	20	15	15	15	12.5	12.5	12.5
Corn	28.41	23.37	23.17	30.8	30.4	29.9	31.3	29.9	29.4
Soybean meal	11.7	10.5	11.5	9.5	11.4	11.9	8	10.4	10.9
Distillers dried grains with solubles	3	7.24	7.74	4	0.5	0.5	4	0.5	0.5
Flax cake	4.8	4.8	0	3.5	3.5	0	4.5	4.5	0
Heated linseed	0	0	5.5	0	0	5.5	0	0	7
Linseed oil LSO	0	2	0	0	2	0	0	2.5	0
Premix ²	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Calcium carbonate	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
CaHPO ₄	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Sodium chloride	0.54	0.54	0.54	0.5	0.5	0.5	0.5	0.5	0.5
Sodium bicarbonate	0.35	0.35	0.35	0.8	0.8	0.8	0.8	0.8	0.8
Magnesia	0.3	0.3	0.3	0	0	0	0	0	0
Chemical composition									
Digestible energy, MJ/kg DM ³	12.83	13.09	13.06	12.87	13.00	12.96	12.74	13.09	13.05
Crude protein, g/kg DM	188.73	188.13	188.2	162.84	158.71	159.69	153.52	151.34	151.85
Ether extract, g/kg DM	29.12	53.97	53.97	28.99	45.84	46.14	26.85	48.99	49.92
Neutral detergent fiber, g/kg DM	425.31	431.18	441.60	448.6	427.41	439.12	457.42	436.06	450.68
Acid detergent fiber, g/kg DM	232.2	231.71	243.3	242.59	235.23	248.4	247.69	240.32	256.96
Calcium, g/kg DM	11.25	11.11	11.00	10.48	10.89	10.78	10.26	10.67	10.56
Phosphorus, g/kg DM	4.65	4.67	4.78	4.50	4.44	4.56	4.31	4.22	4.33

¹LSO = linseed oil diet; HLS = heated linseed grain diet.

²Provided per kg of premix: iron (Fe) 4 g, copper (Cu) 0.8 g, zinc (Zn) 5 g, manganese (Mn) 3 g, iodine (I) 30 mg, selenium (Se) 30 mg, cobalt (Co) 25 mg, vitamin A (VA) 600,000 IU, vitamin D (VD3) 250 000 IU, vitamin E (VE) 1,250 IU, vitamin K (VK3) 180 mg, vitamin B1 (VB1) 35 mg, vitamin B2 (VB2) 850 mg, vitamin B6 (VB6) 90 mg, nicotinic acid 2,200 mg, K-pantothenic acid 1,700 mg, vitamin B12 (VB12) 3 mg, biotin 14 mg, folic acid 150 mg.

× 6.25; method 984.13), ether extract (method 920.39), and calcium and phosphorous (method 935.13) according to AOAC (2000). NDF and ADF were determined according to the methods described by Van Soest et al. (1991) with an Ankom 220 Fiber Analyzer (Ankom Co., USA) and were expressed inclusive of residual ash. Heat stable amylase was not used in the NDF determination.

Measurement of FA. Fatty acid methyl esters (FAMES) were produced from samples of 0.5 g of feed, 0.02 g of SADT, 0.5 g of liver, and 1 mL of plasma according to the method of O'Fallon et al. (2007). Briefly, samples were placed in a screw-cap Pyrex culture tube to which 0.7 mL of 10 N KOH in water and 5.3 mL of methanol were added. The tube was incubated in a 55 °C water bath for 1.5 h with vigorous 5 s hand shaking every 20 min

to properly permeate, dissolve, and hydrolyze the sample. After cooling below room temperature in a cold tap water bath, 0.58 mL of 24 N H₂SO₄ in water was added. The tube was mixed by inversion, and with precipitated H₂SO₄ present, was incubated again in a 55 °C water bath for 1.5 h with 5 s hand shaking every 20 min. After FAMES synthesis, the tube was cooled in a cold tap water bath. Hexane was added and the tube was vortex mixed for 5 min. The tube was centrifuged at 1,500 × g for 5 min and the hexane layer containing the FAMES was placed into a gas chromatography (GC) vial.

The FAMES was analyzed using a GC-2014 gas chromatograph (Shimadzu International Trading Co., Ltd) equipped with a flame-ionization detector, an automatic injector AOC-20I (Shimadzu), and a capillary column (SP-2560 for FAME; 100 m × 0.25 mm i.d., 0.20-µm film thickness, Supelco).

The temperature program was as follows: 120 °C for 5 min, increased at 3.0 °C/min to 230 °C and held for 3 min, and finally, at 1.5 °C/min to 240 °C and held for 20 min. Nitrogen (1 mL/min flow rate) was used as the carrier gas with a pressure of 233.6 kPa. The split ratio was 1:9. The injector temperature was set at 260 °C and the detector temperature was set at 280 °C. The FAMES peaks were routinely identified by comparing their retention times with those of known external standard mixes of 37 FAMES (Sigma Aldrich, China). After determining individual FA, the sums of saturated fatty acid (SFA), MUFA, PUFA, n-6 PUFA, and n-3 PUFA were calculated, and the ratios of n-6/n-3, PUFA/SFA (P/S) and unsaturated FA/saturated FA (U/S) were determined.

RNA extraction and real-time PCR. Total RNA was extracted from 0.05 g of frozen SADT using the Trizol reagent (TaKaRa, Dalian, China) according to the manufacturer's recommendations. The concentration, purity, and integrity of the RNA were assessed using 2% agarose gel electrophoresis and a microplate reader (Synergy H4, BioTek, USA) at 260/280 nm (OD260/OD280 = 1.8 to 2.0). First-strand cDNA was synthesized in a volume of 20 µL using 1 µg of total RNA and the PrimeScript RT reagent (TaKaRa, Dalian, China). Real-time PCR reactions were carried out in a final volume of 20 µL containing 10 µL of 1×SYBR Premix Ex TaqTM (TaKaRa, Dalian, China), 2 µL of cDNA, 0.4 µL each of 0.2 µM forward and reverse primers, and 7.2 µL of RNase-free water. Real-time PCR was performed on a Light Cyber Roche 480 PCR real-time PCR system with an initial denaturing step of 95 °C for 30 s followed by 40 cycles of 95 °C for 30 s (denaturation), various annealing temperatures (shown in Table 2), 72 °C for 20 s (extension) and then 51 cycles of 70 °C for 0.06 s (drawing melting curve). The specificity of the PCR amplification was confirmed by melting curve analysis and 2% agarose gel electrophoresis of the PCR products. The RT-qPCR analyses of each studied gene were performed using cDNA from eight biological replicates, with three technical replicates per biological replicate. In particular, β -2-microglobulin, tyrosine 3-monooxygenase and β -actin were treated as reference genes. The primers used are presented in Table 2. The $2^{-\Delta\Delta C_t}$ method was used to analyze the RT-qPCR data (Livak and Schmittgen, 2001). For the normalization of the RT-qPCR data, the geometric mean C_t of three reference genes was used (Vandesompele et al., 2002). The data are presented in a fold-change ratio with the Control group

as a reference, and primer efficiency was calculated and verified using the standard curve obtained by serial dilution of the pooled cDNA (Pfaffl, 2001).

Statistical Analysis

A one-way ANOVA was used to test the effects of dietary treatments on gene expression and FA composition using GLM (SAS Institute, 2002). Differences among means were tested using Duncan multiple range tests (Duncan, 1955). The results are presented as the mean values and SEM. The data means were considered significantly different at $P < 0.05$, and tendencies were considered at $0.05 < P < 0.10$.

RESULTS

FA Composition

The major ingested FAs were C18:3n3, C18:2n6c, C18:1n9c, and C16:0 (Table 3). Comparing to Control kids, LSO and HLS kids ingested more C18:1n9c ($P = 0.040$), C18:3n3 ($P = 0.009$), PUFA ($P = 0.030$), n-3 PUFA ($P = 0.010$), U/S ($P = 0.002$), and P/S ($P = 0.002$), but with a lesser n-6/n-3 ratio ($P = 0.0001$). The feed content of those FA did not differ ($P > 0.05$) between HLS and LSO kids. Compared to Control and HLS, LSO kids ingested greater amounts of C10:0 ($P = 0.035$), C14:0 ($P = 0.048$), C15:0 ($P = 0.041$), C16:1 ($P = 0.061$), C17:0 ($P = 0.049$), C17:1 ($P = 0.046$), C18:1n9t ($P = 0.009$), C18:3n6 ($P = 0.001$), and C20:4n6 ($P = 0.005$); for those FA, there was no difference ($P > 0.05$) between Control and HLS kids. Ingested MUFA and C18:0 contents were markedly greater ($P = 0.038$ and $P = 0.029$, respectively) in LSO kids than in Control kids, but in HLS kids, it was not different ($P > 0.05$) from LSO or Control.

Plasma FA composition is presented in Table 4. Dietary supplementation with LSO or HLS decreased the plasma concentration of C20:4n6 ($P = 0.0001$) and the n-6/n-3 ratio ($P = 0.0003$) but increased the concentrations of C18:3n3 ($P < 0.0001$), C20:5n3 ($P < 0.0001$) and n-3PUFA ($P = 0.0002$) compared to Control. The plasma concentration of those FA did not differ ($P > 0.05$) between HLS and LSO kids with two exceptions: C20:4n6 values were greater ($P < 0.05$) in LSO kids than in HLS kids, while C18:3n3 values were less ($P < 0.05$) in LSO than HLS. In addition, compared with Control and LSO, there were increases in plasma concentrations of C18:1n9t ($P = 0.006$), C18:2n6t ($P = 0.051$), and C22:6n3 ($P = 0.028$) in HLS; those FAs did not differ ($P > 0.05$) between

Table 2. Primer pairs sequences for quantitative real-time PCR

Gene ¹	Primer pairs (5' to 3')	Accessions number	Annealing temperature, °C	Length, bp
FADS1	F:ATGCAACGTCCACAAGTCAG R:GGGAGCCACTTTGTGGTAAT	XM_004019593	56	115
FADS2	F:GTTCAGTGGGCACCTCAACT R:TACTCAATGCCGTGCTTGG	XM_004019592	57	106
ELOVL5	F:GGAAGGCCGGTACAACCTCT R:TGTCCATGAACTCGATGAGC	XM_004018905	56	118
CPT1 β	F:TGTTCAACACCACTCGCATC R:CTCGTAGAGCCACAGCTTGA	AJ272435	58	116
SLC27A2	F:AAGGCCCGCTTTCTAAG R:TCGGTGTTTAAAAGTTCCAGTG	XM_615837	56	61
LPL	F:TCAGGCGTCTTTGCGGGTAT R:GCCTGTTTCATTGCGTTTG	X68308	60	194
PPAR α	F: TGCCAAGATCTGAAAAAGCA R: CCTCTTGGCCAGAGACTTGA	FJ200440.1	57	101
ACOX1	F:GAGTGAGCTGCCTGAGCTTC R:TTGTCCAGGACGTGAAAGC	NM_001035289	59	62
SCD	F:CCCAGCTGTCAGAGAAAAGG R:GATGAAGCACACAGCAGGA	AJ001048	60	115
SREBP1c	F:GCCTTCTATATTACATCCACAACCTTG R:AGATTATCCAGCATCCGCATGAG	AB355703	60	221
PPAR γ	F:GACCACTCCCATGCCTTTGATA R:TCTTGAGCTTCAGGTCATACTTAT	AY137204	58	140
ACC	F:ACCCAACCCAGAAAGGTCAGT R:TCCCACGGGTATTCTCTCTA	NM_001009256	60	125
Pref1	F:CGACCGCAGCCCTCCTG R:TGTCATCGTCGCAGAATCCATT	AB009278	60	114
FAS	F:GCAACCAGGGGAGACCGT R:CTGAGGGCAATGGCGATGG	AB011671	60	300
DGAT1	F:ACGTCCCGGTAGGAAAACAG R:CGTGGCCTTCCTCCTAGAGT	EU178818	60	100
β -actin	F:ACTGGGACGACATGGAGAAGA R:GCGTACAGGGACAGCACAG	U39357	60	199
B2M	F:GGTGCTGCTTAGAGGTCTCG R:ACGCTGAGTTCACTCCCAAC	NM_001009284	59	109
YWHAZ	F:TGTAGGAGCCCGTAGGTCATCT R:TTCTCTCTGTATTCTCGAGCCATCT	AY970970	59	102

¹PPAR γ = peroxisome proliferator-activated receptor gamma, PPAR α = peroxisome proliferator-activated receptor alpha, SREBP1c = sterol regulatory element binding transcription factor 1c, FAS = fatty acid synthetase, ACC = acetyl-CoA carboxylase, SCD = stearoyl-CoA desaturase, FADS1 = delta-5 desaturase, FADS2 = delta-6 desaturase, ELOVL5 = elongation of very long chain fatty acids protein 5, ACOX1 = acyl-coenzyme A oxidase 1, SLC27A2 = solute carrier family 27 member, CPT1 β = carnitine palmitoyltransferase I, DGAT1 = acyl-CoA diacylglycerol acyltransferase 1, Pref1 = pre-adipocyte factor-1, LPL = lipoprotein lipase, B2M = β -2-microglobulin, YWHAZ = tyrosine 3-monooxygenase, β -actin = beta-actin.

Control and LSO. Meanwhile, plasma PUFA tended ($P = 0.070$) to be reduced in Control kids compared to HLS kids, but LSO kids did not differ ($P > 0.05$) from either Control or HLS kids.

Liver FA composition is shown in Table 5. Dietary supplements with LSO or HLS decreased liver concentrations of C20:4n6 ($P < 0.0001$) and n-6 PUFA ($P < 0.0001$), as well as the n-6/n-3 ratio ($P < 0.0001$), but increased the concentrations of C12:0 ($P = 0.030$), C18:3n3 ($P < 0.0001$), C20:5n3 ($P = 0.012$), and n-3 PUFA ($P = 0.0002$) compared to Control kids. Liver concentrations of those FA

did not differ ($P > 0.05$) between HLS and LSO kids with the exceptions of C18:3n3 and the n-6/n-3 ratio. The concentration of C18:3n3 was greater ($P < 0.05$) in HLS than LSO, while the n-6/n-3 ratio was less ($P < 0.05$). Compared to Control and LSO, HLS kids had increased concentrations of C18:1n9t ($P = 0.051$) and C22:6n3 ($P = 0.030$) in liver, and these FA did not differ ($P > 0.05$) between Control and LSO kids.

The FA concentrations in SADT are reported in Table 6. Dietary supplementation with LSO or HLS decreased the concentration of C20:4n6 ($P = 0.005$)

Table 3. Average intake of dietary fatty acid during the whole period (gram per kilogram metabolic weight, DM basic)

Fatty acid ¹	Diets ²			SEM	P-value
	Control	LSO	HLS		
C10:0	0.011 ^b	0.018 ^a	0.001 ^b	0.002	0.035
C12:0	0.013	0.013	0.012	0.001	0.841
C14:0	0.016 ^b	0.024 ^a	0.015 ^b	0.002	0.048
C15:0	0.008 ^b	0.013 ^a	0.008 ^b	0.001	0.041
C16:0	0.222	0.286	0.259	0.025	0.272
C16:1	0.009 ^b	0.015 ^a	0.009 ^b	0.001	0.061
C17:0	0.011 ^b	0.017 ^a	0.011 ^b	0.002	0.049
C17:1	0.008 ^b	0.014 ^a	0.008 ^b	0.001	0.046
C18:0	0.042 ^b	0.095 ^a	0.078 ^{ab}	0.01	0.029
C18:1n9t	0.006 ^b	0.012 ^a	0.006 ^b	0.001	0.009
C18:1n9c	0.206 ^b	0.445 ^a	0.416 ^a	0.054	0.040
C18:2n6t	0.007	0.007	0.006	0.0005	0.681
C18:2n6c	0.46	0.685	0.62	0.091	0.274
C18:3n6	0.005 ^b	0.014 ^a	0.005 ^b	0.001	0.001
C18:3n3	0.197 ^b	1.097 ^a	0.912 ^a	0.141	0.009
C20:4n6	0.007 ^b	0.013 ^a	0.006 ^b	0.001	0.005
C20:5n3	0.01	0.01	0.01	0.001	0.866
C22:6n3	0.012	0.012	0.011	0.002	0.867
SFA	0.386	0.563	0.455	0.050	0.116
MUFA	0.261 ^b	0.572 ^a	0.469 ^{ab}	0.065	0.038
PUFA	0.730 ^b	1.903 ^a	1.602 ^a	0.236	0.030
n-3 PUFA	0.234 ^b	1.143 ^a	0.948 ^a	0.145	0.010
n-6 PUFA	0.496	0.76	0.655	0.095	0.223
n-6/n-3	2.126 ^a	0.667 ^b	0.698 ^b	0.108	0.0001
U/S	2.562 ^b	4.360 ^a	4.526 ^a	0.230	0.002
P/S	1.891 ^b	3.350 ^a	3.500 ^a	0.196	0.002
Dry matter intake, kg/d	0.91	0.9	0.87	0.080	0.925

^{a-c}Means within the same row followed by the same superscript letters are not significantly different at $P < 0.05$. The number of observations for each mean value was four ($n = 4$).

¹SFA = saturated fatty acids (10:0 + 11:0 + 12:0 + 13:0 + 14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0 + 21:0 + 22:0 + 23:0 + 24:0), MUFA = mono-unsaturated fatty acids (14:1 + 15:1 + 16:1 + 17:1 + 18:1n9t+18:1n9c+20:1 + 22:1 + 24:1), n-6 PUFA = n-6 polyunsaturated fatty acids (18:2n6t+18:2n6c+18:3n6 + 20:2n6 + 20:3n6 + 20:4n6 + 22:2n6), n-3PUFA = n-3 polyunsaturated fatty acids (18:3n3 + 20:3n3 + 20:5n3 + 22:6n3), PUFA n-6 PUFA+ n-3 PUFA, n-6/n-3 n-6 polyunsaturated fatty acids/n-3 polyunsaturated fatty acids, U/S = unsaturated fatty acids/saturated fatty acids, P/S = polyunsaturated fatty acids/saturated fatty acids.

²LSO = linseed oil diet, HLS = heated linseed grain diet.

and the n-6/n-3 ratio ($P < 0.0001$) but increased the concentrations of C18:3n3 ($P < 0.0001$), C20:5n3 ($P = 0.0002$), PUFA ($P = 0.006$), and n-3PUFA ($P < 0.0001$) compared to Control. The concentrations of C20:5n3 and PUFA did not differ ($P > 0.05$) between HLS and LSO kids, while the concentrations of C18:3n3 and n-3PUFA were greater ($P < 0.05$) in HLS kids than in LSO kids. In addition, compared with Control and LSO, HLS kids had increased concentrations of C17:1 ($P = 0.010$) and C22:6n3 ($P = 0.007$) in SADT, yet concentrations of C17:1 and C22:6n3 did not differ ($P > 0.05$) between Control and LSO kids. Meanwhile, the concentration of C18:1n9c tended ($P = 0.063$) to be greater in Control kids than in HLS kids, but values for LSO kids did not differ ($P > 0.05$) from those for either Control or HLS kids.

Gene Expression

The relative expression of genes in SADT is presented in Table 7. In comparison to Control, mRNA expression of sterol regulatory element-binding transcription factor 1c (**SREBP1c**, $P < 0.0001$), peroxisome proliferator-activated receptor-gamma (**PPAR γ** , $P = 0.001$), acyl-CoA diacylglycerol acyltransferase 1 (**DGAT1**, $P < 0.0001$), FA synthetase (**FAS**, $P = 0.0002$), acetyl-CoA carboxylase (**ACC**, $P < 0.0001$), and lipoprotein lipase (**LPL**, $P = 0.0004$) was less in both LSO and HLS. In contrast, expression of carnitine palmitoyltransferase I (**CPT1 β** , $P < 0.0001$) and pre-adipocyte factor-1 (**Pref1**, $P = 0.0002$) was greater in SADT of LSO and HLS kids than in Control kids. The expression of those genes did not differ ($P > 0.05$) between

Table 4. Fatty acids profile in plasma of kids (percentage of total identified fatty acids)

Fatty acid ¹	Diets ²			SEM	P-value
	Control	LSO	HLS		
C10:0	1.68	1.82	1.49	0.11	0.48
C12:0	1.57	1.68	1.53	0.09	0.621
C14:0	2.23	2.26	2.15	0.09	0.823
C15:0	1.23	1.21	1.21	0.04	0.941
C16:0	14.22	13.35	13.00	0.41	0.173
C16:1	1.48	1.45	1.36	0.05	0.392
C17:0	2.02	1.83	1.88	0.06	0.158
C17:1	1.26	1.28	1.11	0.06	0.301
C18:0	18.46	19.37	19.77	0.72	0.558
C18:1n9t	1.43 ^b	1.60 ^b	2.22 ^a	0.09	0.006
C18:1n9c	18.984	17.91	17.643	0.95	0.517
C18:2n6t	1.04 ^b	1.06 ^b	1.35 ^a	0.06	0.051
C18:2n6c	11.5	10.99	10.58	0.56	0.670
C18:3n6	1.08	1.1	0.99	0.04	0.321
C18:3n3	2.40 ^c	3.80 ^b	4.63 ^a	0.14	<0.0001
C22:0	1.66	1.80	1.46	0.09	0.333
C20:1	0.92	1.01	0.86	0.05	0.375
C24:1	0.8	0.88	0.81	0.05	0.760
C20:4n6	2.34 ^a	1.99 ^b	1.78 ^c	0.04	0.0001
C20:5n3	1.25 ^b	2.13 ^a	2.16 ^a	0.06	<0.0001
C22:6n3	1.33 ^b	1.28 ^b	2.07 ^a	0.15	0.028
SFA	48.09	46.98	46.68	0.69	0.387
MUFA	27.73	26.97	27.44	0.47	0.629
PUFA	24.34 ^b	26.38 ^{ab}	26.68 ^a	0.52	0.070
n-3 PUFA	5.70 ^c	8.04 ^a	9.54 ^a	0.38	0.0002
n-6 PUFA	18.62	18.34	17.07	0.53	0.274
n-6/n-3	3.26 ^a	2.28 ^b	1.79 ^b	0.21	0.0003

^{a-c}Means within the same row followed by the same superscript letters are not significantly different at $P < 0.05$. The number of observations for each mean value was eight ($n = 8$).

¹SFA = saturated fatty acids (10:0 + 11:0 + 12:0 + 13:0 + 14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0 + 21:0 + 22:0), MUFA = monounsaturated fatty acids (14:1 + 15:1 + 16:1 + 17:1 + 18:1n9t+18:1n9c+20:1 + 22:1 + 24:1), n-6 PUFA = n-6 polyunsaturated fatty acids (18:2n6t+18:2n6c+18:3n6 + 20:2n6 + 20:3n6 + 20:4n6 + 22:2n6), n-3 PUFA = n-3 polyunsaturated fatty acids (18:3n3 + 20:3n3 + 20:5n3 + 22:6n3), PUFA n-6 PUFA + n-3 PUFA, n-6/n-3 n-6 polyunsaturated fatty acids/n-3 polyunsaturated fatty acids.

²LSO = linseed oil diet, HLS = heated linseed grain diet.

HLS and LSO kids with one exception—the values for CPT1 β were greater ($P < 0.05$) in LSO kids than in HLS kids. In addition, compared to Control and LSO, HLS had increased ($P < 0.05$) expression of delta-6 desaturase (**FADS2**), elongation of very long chain FA protein 5 (**ELOVL5**), and peroxisome proliferator-activated receptor- α (**PPAR α**). The mRNA expression of those genes did not differ ($P > 0.05$) between Control and LSO. Compared to Control, mRNA expression of stearoyl-CoA desaturase (**SCD1**) was decreased ($P = 0.031$) in HLS, while expression of solute carrier family 27 member (**SLC27A2**) was increased ($P = 0.003$). Kids fed LSO did not differ ($P > 0.05$) in mRNA expression of SCD1 and SLC27A2 in either Control or HLS kids.

The relative expression of genes in the liver is presented in Table 8. Compared to Control,

mRNA expression of FADS1 ($P < 0.001$), ELOVL5 ($P = 0.024$), and SLC27A2 ($P < 0.0001$) was significantly increased in LSO and HLS. The expression of those genes did not differ ($P > 0.05$) between HLS and LSO with the exception of SLC27A2, the value was greater ($P < 0.05$) in HLS than in LSO. Compared to Control and LSO, HLS showed an increased expression of FADS2 ($P = 0.006$); mRNA expression of FADS2 did not differ ($P > 0.05$) between Control and LSO. In addition, mRNA expression of PPAR α was significantly greater ($P = 0.003$) in HLS kids than Control kids, but LSO kids did not differ ($P > 0.05$) from Control or HLS kids.

DISCUSSION

In the present study of cashmere kids, both LSO and HLS provided the same amount of

Table 5. Fatty acids profile in liver of kids (percentage of total identified fatty acids)

Fatty acid ¹	Diets ²			SEM	P-value
	Control	LSO	HLS		
C10:0	1.21	1.36	1.25	0.08	0.55
C12:0	0.73 ^b	1.32 ^a	1.28 ^a	0.09	0.03
C14:0	1.09	1.25	1.25	0.11	0.677
C15:0	0.7	0.7	0.82	0.04	0.276
C16:0	15.27	14.26	14.99	0.58	0.641
C16:1	1.22	1.02	0.92	0.07	0.088
C17:0	2.17	2.2	1.97	0.07	0.214
C17:1	0.81	0.8	0.66	0.05	0.168
C18:0	19.58	20.28	20.04	0.43	0.777
C18:1n9t	1.03 ^b	1.02 ^b	1.32 ^a	0.06	0.051
C18:1n9c	15.17	14.95	15.84	0.36	0.489
C18:2n6t	0.65	0.6	0.84	0.06	0.208
C18:2n6c	10.72	9.98	9.21	0.46	0.208
C18:3n6	0.68	0.78	0.62	0.06	0.573
C18:3n3	1.56 ^c	3.18 ^b	3.60 ^a	0.09	<0.0001
C22:0	0.45	0.43	0.47	0.04	0.902
C20:1	0.63	0.76	0.77	0.05	0.394
C24:1	0.4	0.66	0.61	0.06	0.272
C20:4n6	14.39 ^a	8.99 ^b	7.15 ^b	0.69	<0.0001
C20:5n3	3.16 ^b	5.71 ^a	5.75 ^a	0.35	0.012
C22:6n3	2.26 ^b	2.25 ^b	3.45 ^a	0.21	0.03
SFA	43.57	45.7	45.79	0.81	0.346
MUFA	20.64	20.69	21.52	0.65	0.758
PUFA	35.74	33.71	32.55	1.13	0.317
n-3 PUFA	7.67 ^b	11.79 ^a	13.44 ^a	0.62	0.0002
n-6 PUFA	28.08 ^a	21.92 ^b	19.46 ^b	0.73	<0.0001
n-6/n-3	3.67 ^a	1.86 ^b	1.46 ^c	0.07	<0.0001

^{a-c}Means within the same row followed by the same superscript letters are not significantly different at $P < 0.05$. The number of observations for each mean value was eight ($n = 8$).

¹SFA = saturated fatty acids (10:0 + 12:0 + 13:0 + 14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0 + 21:0 + 22:0 + 23:0 + 24:0), MUFA = monounsaturated fatty acids (14:1 + 15:1 + 16:1 + 17:1 + 18:1n9t+18:1n9c+20:1 + 22:1 + 24:1), n-6 PUFA = n-6 polyunsaturated fatty acids (18:2n6t+18:2n6c+18:3n6 + 20:2n6 + 20:3n6 + 20:4n6 + 22:2n6), n-3 PUFA = n-3 polyunsaturated fatty acids (18:3n3 + 20:3n3 + 20:5n3 + 22:6n3), PUFA n-6 PUFA + n-3 PUFA, n-6/n-3 n-6 polyunsaturated fatty acids/n-3 polyunsaturated fatty acids.

²LSO = linseed oil diet, HLS = heated linseed grain diet.

C18:3n3 to the animals, both diets increased the content of C18:3n3, C20:5n3, and n-3 PUFA in SADT, and both diets decreased the n-6/n-3 ratio. However, only the HLS increased the C22:6n3 content in SADT, in association with increased liver and plasma C22:6n3 contents, and with upregulated expression of genes for enzymes involved in n-3 LCPUFA synthesis in SADT. Moreover, HLS was more effective at decreasing the n-6/n-3 ratio, and more effective at increasing C18:3n3 and n-3 PUFA.

These observations add to evidence that diet rich in C18:3n3 increase the C18:3n3 and n-3 PUFA content of meat from sheep and goats (Ebrahimi et al., 2014; Kronberg et al., 2015). Ebrahimi et al. (2013) also used dietary LSO to increase the proportion of C18:3n3 in SADT. They observed a dose-response and concluded that a greater dose of

linseed oil results in more C18:3n3 escaping ruminal biohydrogenation. A similar outcome was achieved by using HLS and therefore proposes that C18:3n3 from this source also escaped ruminal biohydrogenation, thus increased postruminal C18:3n3 flow. Importantly, in the present study, the amount of C22:6n3 ingested did not differ between the LSO and HLS treatments and only the HLS increased the C22:6n3 content in SADT. This observation can be explained in two ways, as illustrated in Figure 1: (1) in HLS-supplemented kids, the high postruminal flow of C18:3n3 into the blood provides more substrate to the liver where it is used to synthesize more C22:6n3, and then the extra C22:6n3 is transported to and taken up by SADT, as suggested by Cherfaoui et al. (2012); (2) SADT directly takes up extra dietary C18:3n3 from the blood and uses it to synthesize more C22:6n3, a process aided by the

Table 6. Fatty acids profile in subcutaneous adipose tissue of kids (percentage of total identified fatty acids)

Fatty acid ¹	Diets ²			SEM	P-value
	Control	LSO	HLS		
C10:0	0.4	0.4	0.4	0.02	0.984
C12:0	0.32	0.38	0.38	0.02	0.075
C14:0	4.02	4.1	3.97	0.26	0.948
C15:0	0.86	0.87	0.88	0.05	0.974
C16:0	24.54	24.68	25.16	1.13	0.974
C16:1	1.77	2.23	2.22	0.19	0.313
C17:0	2.83	2.73	2.79	0.17	0.934
C17:1	1.27 ^b	1.23 ^b	1.94 ^a	0.10	0.01
C18:0	16.67	16.31	15.57	1.70	0.939
C18:1n9t	0.19	0.19	0.16	0.01	0.463
C18:1n9c	37.54 ^a	35.66 ^{ab}	35.00 ^b	0.70	0.063
C18:2n6t	0.16	0.18	0.16	0.01	0.29
C18:2n6c	3.65	3.47	3.41	0.11	0.576
C18:3n6	0.2	0.17	0.17	0.01	0.099
C18:3n3	1.14 ^c	1.88 ^b	2.13 ^a	0.04	<0.0001
C22:0	0.27	0.3	0.29	0.01	0.375
C20:1	0.25	0.27	0.26	0.01	0.578
C24:1	0.15	0.15	0.15	0.01	0.877
C20:4n6	0.40 ^a	0.27 ^b	0.31 ^b	0.02	0.005
C20:5n3	0.16 ^b	0.23 ^a	0.23 ^a	0.01	0.0002
C22:6n3	0.18 ^b	0.25 ^b	0.39 ^a	0.03	0.007
SFA	51.95	52.53	52.35	1.59	0.974
MUFA	41.51	40.2	40.1	0.99	0.551
PUFA	6.51 ^b	7.25 ^a	7.53 ^a	0.14	0.006
n-3 PUFA	1.63 ^c	2.51 ^b	2.90 ^a	0.05	<0.0001
n-6 PUFA	4.89	4.74	4.63	0.24	0.826
n-6/n-3	3.00 ^a	1.88 ^b	1.58 ^c	0.06	<0.0001

^{a-c}Means within the same row followed by the same superscript letters are not significantly different at $P < 0.05$. The number of observations for each mean value was eight ($n = 8$).

¹SFA = saturated fatty acids (8:0 + 10:0 + 12:0 + 13:0 + 14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0 + 21:0 + 22:0 + 23:0), MUFA = monounsaturated fatty acids (14:1 + 15:1 + 16:1 + 17:1 + 18:1n9t+18:1n9c+20:1 + 22:1 + 24:1), n-6PUFA = n-6 polyunsaturated fatty acids (18:2n6t+18:2n6c+18:3n6 + 20:2n6 + 20:3n6 + 20:4n6 + 22:2n6), n-3PUFA = n-3 polyunsaturated fatty acids (18:3n3 + 20:3n3 + 20:5n3 + 22:6n3), PUFA n-6 PUFA+ n-3 PUFA, n-6/n-3 n-6 polyunsaturated fatty acids/n-3 polyunsaturated fatty acids.

²LSO = linseed oil diet, HLS = heated linseed grain diet.

upregulation of enzymes involved in n-3 LCPUFA synthesis (Sprecher et al., 1999, 2000). In mammals, C22:6n3 is synthesized from C18:3n3 by a series of steps catalyzed by FADS1, FADS2, ELOVL5, and ELOVL2, and then β -oxidation by acyl-coenzyme A oxidase 1 (ACOX1) and SLC27A2 (Sprecher et al., 1999, 2000). In goats fed HLS increased mRNA expression for FADS2, ELOVL5, and SLC27A2 (Figure 1) in SADT, compared to the control diet (no LSO or grain), and expression of those three genes did not differ between goats fed the Control or the LSO diet.

In both SADT and liver cells, the regulation of FADS2, ELOVL5, and SLC27A2 probably involves the transcription factor PPAR α (Figure 1), as shown in several studies in a variety of species (Reddy and Hashimoto, 2001; Matsuzaka et al., 2002; Wang et al., 2005; Dong et al., 2017). Moreover, in sheep

meat, Ebrahimi et al. (2014) reported a dose-response in the effect of dietary C18:3n3 on the upregulation of PPAR α . It therefore seems likely that upregulation of mRNA expression for FADS2, ELOVL5, and SLC27A2 in SADT and liver by C18:3n3 involves PPAR α , a concept supported by our observation of increased PPAR α expression in goats fed HLS. However, some responses were not consistent with this concept. For example, in the liver, expression of ELOVL5 was increased in LSO compared to Control, but the expression of PPAR α was not. A possible explanation is that C18:3n3 directly increases the mRNA expression of genes for enzymes involved in n-3 LCPUFA synthesis in liver. On the other hand, activation of PPAR α could increase the expression of FA translocase/CD36, a key regulator of the uptake of FA, such as n-3 LCPUFA, across the plasma membrane of

Table 7. Relative expression of genes related to lipid metabolism in subcutaneous adipose tissue of kids

Gene ¹	Diets ²			SEM	P-value
	Control	LSO	HLS		
Fatty acid desaturation					
FADS1	1.00	0.94	1.03	0.08	0.83
FADS2	1.00 ^b	0.90 ^b	2.54 ^a	0.19	<0.0001
SCD1	1.00 ^a	0.84 ^{ab}	0.73 ^b	0.06	0.031
Fatty acid elongation					
ELOVL5	1.00 ^b	1.05 ^b	1.55 ^a	0.06	<0.0001
Peroxisomal oxidation					
ACOX1	1.00	1.01	1.11	0.07	0.619
SLC27A2	1.00 ^b	1.36 ^{ab}	1.80 ^a	0.12	0.003
CPT1β	1.00 ^c	1.32 ^b	1.64 ^a	0.06	<0.0001
Transcription factors					
PPARα	1.00 ^b	0.98 ^b	1.27 ^a	0.04	0.003
PPARγ	1.00 ^a	0.76 ^b	0.65 ^b	0.06	0.001
Pref1	1.00 ^b	1.86 ^a	2.44 ^a	0.2	0.0002
SREBP1c	1.00 ^a	0.49 ^b	0.59 ^b	0.04	<0.0001
Fatty acid synthesis <i>de novo</i>					
FAS	1.00 ^a	0.61 ^b	0.61 ^b	0.05	0.0002
ACC	1.00 ^a	0.67 ^b	0.66 ^b	0.04	<0.0001
Lipogenesis					
LPL	1.00 ^a	0.71 ^b	0.78 ^b	0.04	0.0004
DGAT1	1.00 ^a	0.69 ^b	0.56 ^b	0.04	<0.0001

^{a-c}Means within the same row followed by the same superscript letters are not significantly different at $P < 0.05$. The number of observations for each mean value was eight ($n = 8$).

¹PPAR γ = peroxisome proliferator-activated receptor gamma, PPAR α = peroxisome proliferator-activated receptor alpha, SREBP1c = sterol regulatory element binding transcription factor 1c, FAS = fatty acid synthetase, ACC = acetyl-CoA carboxylase, SCD = stearoyl-CoA desaturase, FADS1 = delta-5 desaturase, FADS2 = delta-6 desaturase, ELOVL5 = elongation of very long chain fatty acids protein 5, ACOX1 = acyl-coenzyme A oxidase 1, SLC27A2 = solute carrier family 27 member, CPT1 β = carnitine palmitoyltransferase I, DGAT1 = acyl-CoA diacylglycerol acyltransferase 1, Pref1 = pre-adipocyte factor-1, LPL = lipoprotein lipase.

²LSO = linseed oil diet, HLS = heated linseed grain diet.

Table 8. Relative expression of genes related to lipid metabolism in liver of kids

Gene ¹	Diets ²			SEM	P-value
	Control	LSO	HLS		
Fatty acid desaturation					
FADS1	1.00 ^b	2.33 ^a	2.88 ^a	0.17	<0.0001
FADS2	1.00 ^b	1.29 ^b	2.19 ^a	0.17	0.006
Fatty acid elongation					
ELOVL5	1.00 ^b	1.50 ^a	1.58 ^a	0.15	0.024
Peroxisomal oxidation					
ACOX1	1.00	1.16	1.4	0.15	0.183
SLC27A2	1.00 ^c	1.32 ^b	1.59 ^a	0.06	<0.0001
Transcription factors					
PPAR α	1.00 ^b	1.33 ^{ab}	1.64 ^a	0.11	0.003

^{a-c}Means within the same row followed by the same superscript letters are not significantly different at $P < 0.05$. The number of observations for each mean value was eight ($n = 8$).

¹FADS1 = delta-5 desaturase, FADS2 = delta-6 desaturase, ELOVL5 = elongation of very long chain fatty acids protein 5, ACOX1 = acyl-coenzyme A oxidase 1, SLC27A2 = solute carrier family 27 member, PPAR α = peroxisome proliferator-activated receptor alpha.

²LSO = linseed oil diet, HLS = heated linseed grain diet.

adipocytes (Figure 1; Bonen et al., 2004). Therefore, the increased expression of PPAR α in SADT was observed in HLS-fed kids would be expected to increase the amount of CD36, thus increasing the

uptake of n-3 LCPUFA from blood by adipocytes (Figure 1).

The present observations are consistent with others made in cattle and sheep fed linseed. For

Figure 1. Schema illustrating our current understanding of the ways in which the addition of heated linseed grain (HLS) affects the flows and synthesis of n-3 long-chain polyunsaturated fatty acids (FAs) in cashmere goats. Black solid arrows are used where the effect of HLS was greater than the effect of LSO (both greater than control values). Black broken arrows and grey solid arrows are used where the effects of HLS and LSO did not differ significantly (but both greater than control values). Grey broken arrows indicate hypothetical changes (not measured). PPAR α = peroxisome proliferator-activated receptor alpha; FADS1 = delta-5 desaturase; FADS2 = delta-6 desaturase; ELOVL5 = elongation of very long chain FAs protein 5; ACOX1 = acyl-coenzyme A oxidase 1; SLC27A2 = solute carrier family 27 member; CD36 = FA translocase/CD36. For the sake of clarity, only the pathways that show clear responses to linseed treatment are shown: for example, FADS1, ACOX1 are not affected in SADT or liver by either LSO or HLS. Other components of FA pathways are also omitted because they were not tested in the present study: elongation of very long chain FAs protein 2; Enoyl-Coenzyme A, hydratase/3-hydroxyacyl Co A deshydrogenase; Sterol-carrier protein 2; 2,4-dienoyl coA reductase 2 and 3-oxoacyl-Coenzyme A thiolase.

example, with non-HLS fed to cattle, the C18:3n3 concentration in meat was increased but not the C22:6n3 concentration (Vatansever et al., 2016). This observation agrees with Ebrahimi et al. (2013), who pointed out that incremental amounts of LSO in the diet resulted in dose-dependent increases in C22:6n3 in SADT of goats. However, in the present study, the content of C20:5n3 did not differ between the linseed treatments, probably because C20:5n3 is more readily β -oxidized than C22:6n3 (Gavino and Gavino, 1991; Chen et al., 2009).

Ebrahimi et al. (2013) found that supplementing with LSO decreased the n-6/n-3 ratio in diet and reduced C20:4n6 content in SADT, suggesting that decreasing the dietary n-6/n-3 ratio reduces the synthesis of C20:4n6 from C18:2n6c. These outcomes are explained by competition between C18:2n6c and C18:3n3 for the same desaturation and elongation enzymes that convert them to LCPUFA derivatives. Bioconversion of n-3 FA into higher homologs depends on the ratio of ingested n-6/n-3 PUFA, a ratio of 1/1 being proposed to lead to the highest formation of n-3 LCPUFA (Harnack et al., 2009). In the present study, the n-6/n-3 ratio in the diet, plasma, liver, and SADT was closer to 1/1 for the two

linseed supplementation groups than for the control group. Therefore, the C20:4n6 content was reduced by both linseed treatments.

It was also found that, independently of supplementation, the predominant FAs in SADT were C16:0 and C18:0 as SFAs, C18:1n9c as MUFAs, and C18:2n6c as PUFAs, as also seen by Ebrahimi et al. (2013) in goats. Among these predominant FAs, the C18:1n9c content in SADT was less with the HLS diet than with the Control diet, perhaps because in SADT from HLS-fed goats, there is a reduction in the expression of SCD1, a key enzyme for FA desaturation (Dervishi et al., 2015). Again, these observations agree with those of Ebrahimi et al. (2014), who reported that expression of SCD1 in sheep meat could be downregulated by LSO (linseed) or n-3PUFA. Additionally, inhibition of SCD1 catalytic activity increased the expression of genes associated with FA oxidation, such as CPT1 β (Kadegowda et al., 2013), as we observed in goat tissues.

Adipose tissue is the principal site possessing the ability to synthesize FA (Favarger, 1965) and, in this tissue, gene expression for enzymes that control fat metabolism, and thus the FA profile, can be modified by the feeding strategy. Therefore, the

identification of these genes is a good starting point for identification and selection of reference genes that can influence *de novo* FA synthesis. For this purpose, the effect of linseed supplementation on mRNA expression of genes implicated in FA synthesis *de novo* (ACC, FAS) and triglyceride synthesis (DGAT1, LPL) as well as some transcriptional factors (SREBP1, PPAR γ , and Pref1) were studied in SADT. Pref-1 belongs to the Notch/Delta/Serrate family of epidermal growth factor-like repeat-containing proteins. It inhibits adipocyte differentiation by suppressing PPAR γ expression (Sul et al., 2009), so it was expected that the mRNA expression of Pref1 and PPAR γ would be negatively correlated. Target genes of PPAR γ are SREBP1, FAS, ACC, DGAT1 (Grauagnard et al., 2009; Corazzin et al., 2013), and LPL (Hosseini and Loor, 2004). The present results indicated that linseed supplementation decreases adipogenesis in SADT. The total weight of SADT was not measured because it is too difficult to collect the complete tissue. However, reductions in adipogenesis are accompanied by downregulated mRNA expression for SREBP1, FAS, PPAR γ , DGAT1, and LPL (Vlassara et al., 1986; Lin et al., 2002; Chen and Farese, 2005; Yang et al., 2007), and up-regulated mRNA expression for Pref1 and phosphorylated ACC (Ejaz et al., 2009; Moon et al., 2002). The current observations agree with others showing that supplementation with a linseed source decreases mRNA expression for SREBP1c, PPAR γ , LPL, FAS, and DGAT1 (Chechi et al., 2010; Corazzin et al., 2013; Kim et al., 2014; Zhu et al., 2014).

In conclusion, compared to LSO, a supplement of HLS was more effective at increasing SADT content of C18:3n3 and n-3 PUFA and decreasing the n-6/n-3 ratio. Feeding HLS leads to the deposition of more C18:3n3 in SADT and liver, in turn increasing the expression of FADS2, ELOVL5, and SLC27A2 and subsequently increasing the C22:6n3 content. Therefore, C18:3n3 seems to be acting as a regulatory factor for transcriptional and translational control of these genes, either directly or through a mechanism that involves an increase in PPAR α mRNA expression.

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