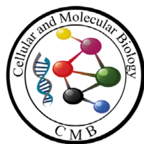

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Original Research

Metabolic and molecular responses to calcium soap of fish oil fed to ewes during peripartal period

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Abstract: It has been shown that n-3 long chain fatty acids (n-3 LCFA) are involved in energy/lipid metabolisms, reproductive parameters, and molecular regulations leading to maintained homeostasis. We hypothesized that supplementation of peripartal diets with fish oil (FO), as a source of n-3 LCFA, could improve energy balance and modulate metabolic pressure in a sheep model. Prepartum ewes (n = 24) were fed control (CON) or calcium soap of fish oil (FO) supplemented-diet from four weeks before until three weeks after parturation. Feed intake, body weight (BW) change, plasma metabolites, colostrums/milk composition, and fatty acids profile of milk along with the expression of core microRNAs in glucose and lipid metabolism were evaluated. Prepartal feed intake decreased in FO group (1674 ± 33.26 *vs.* 1812 ± 35.56) though post-partal intake was similar. Differences in BW were not also significant (55.47 ± 2.07 in CON *vs.* 53.69 ± 1.94 in FO). No differences were observed in plasma metabolites except for cholesterol that was lower in FO group (56.25 ± 0.71 *vs.* 53.09 ± 0.61). Milk fat percentage was reduced (8.82 ± 0.49 *vs.* 7.03 ± 0.45) while the percentage of milk total n-3 LCFA increased in FO group. In accordance, the relative transcript abundance of miR-101 (0.215 ± 0.08) and miR-103 (0.37 ± 0.15) decreased by FO supplementation. Results showed that FO supplementation during peripartal period decreased milk fat, feed intake, plasma cholesterol, milk n-6:n-3 ratio and the expression of miR-101. Although the trend indicated that FO could alter lipid metabolism during transition period, further studies are needed to fully address its effect on energy balance and homeorhetic processes.

Key words: Long chain fatty acids; Metabolite; MicroRNA; Peripartum period.

Introduction

Sheep experience some degree of negative energy balance especially in the case of twin pregnancy during late gestation to early lactation (1, 2). Adipose tissue mobilization in order to meet extra energy demands in ruminants transitioning from a pregnant non-lactating to non-pregnant lactating state is commonly associated with excessive triglyceride (TG) accumulation in the liver (3, 4). These physiologic conditions are associated with increased risk of metabolic- and production-related diseases (5). Therefore, managing pre- and postpartum diets by increasing the nutrient intake (6) or net energy density (7) suggested to improve transition success. In a review (8), dietary fat was hypothesized to contribute in lowering fatty acid mobilization and sparing glucose by decreasing the synthesis of NADPH that is required for milk fat synthesis. Generally, fats have received considerable attention for meeting the elevated energy demands of pregnant ewes carrying multiple fetuses during the last stage of pregnancy and early phase of lactation (9). However, specific polyunsaturated fatty acids (PUFA) have been shown to have different effects on adipose tissue metabolism that is beyond their energetic role. For example, n-3 long chain fatty acids (LCFA) could modify milk fat composition and maintain immune system function (10, 11). While postpartal supplementation of the diet with fatty acids has been the subject of many studies, data on prepartal PUFA supplementation is limited. A study reported that feeding a source of n-3 LCFA in transition period decreased TG accumulation and prevented fatty liver (12). Prepartal fish oil (FO) supplementation, a rich source of n-3 LCFA, was shown to decrease plasma concentration of β-hydroxybutyrate (BHB) and NEFA during the postpartum period and was highly correlated with energy status (13). Moreover, n-3 LCFAs has recently been shown to modulate metabolic pathways related to the energy and lipid metabolism thorough a group of non-coding RNAs called microRNAs (14, 15). MicroRNAs directly affect post-transcriptional regulation of gene expression through binding to the 3′ untranslated regions (UTRs) of messenger RNAs (mRNA) and inhibit their translation (16). Recently, it has been shown that microRNAs including miR-101, 103 and 27b and their downstream genes are involved in cholesterol and lipid metabolism that could be used as a marker of postpartal energy status in ruminants (14, 17). Therefore, the objectives of the present study were to determine effects of feeding FO in peripartal ewes on feed intake, plasma metabolites concentrations, colostrum and milk composition, and relative expression of microRNAs related to adipogenesis.

Materials and Methods

Animals and Treatments

The study was conducted at the experimental research

Table 1. Ingredients and chemical composition of the experimental diets fed to peripartal ewes.

Diet ingredient (% dry matter)	Prepartum	Postpartum
Alfalfa hay	30	35
Barley straw	33	-
Corn silage	-	7
Whole grain barley	27	20
Whole grain corn	-	16.5
Wheat bran	-	11
Soybean meal, solvent extracted	-	8
Canola meal	9.7	-
Vitamin and mineral premix	0.3	0.7
Sodium chloride	-	0.2
Sodium bentonite	-	1
Magnesium oxide	-	0.3
Calcium carbonate	-	0.3
Chemical analysis		
Fat (%)	3.15	6.05
Crude Protein (%)	12.50	14.20
Ash (%)	8.28	7.82
Calcium (%)	0.52	0.85
Phosphorus (%)	0.35	0.45
NDF (%)	34.4	35.5
ME (Mcal/kg dry matter)1	2.13	2.66

[1]Calculated from NRC (2007).

facility of the University of Tehran (College of Aburaihan, Pakdasht, Iran). Twenty-four healthy Zandi ewes were estrus-synchronized with CIDR and introduced to rams for natural breeding. Rams were with the ewes for 15 days after which the ewes were separated and kept in group pens before they were moved to individual 1.5 × 2.5 m pens approximately four weeks before anticipated lambing date. Time of movement to individual pens was chosen based on the median day of the breeding season (i.e. day 7 after rams introduced to ewes). This ensured that all ewes in the experimental groups received the corresponding diets for at least three weeks. Following moving to individual pens, ewes were randomly assigned to either control (CON) or FO (as calcium soap of FO at 2% of diet dry matter)supplemented group until three weeks after parturition. Ingredients and chemical composition of the diets are shown in Table 1. The fatty acids profile of basal pre and postpartal diet are presented in Table 2. Also, chemical composition of FO, as provided by the manufacturer (Virtus Nutrition LLC., CA, USA), is shown in Table 3.

Data recordings and samplings

Feed intake was recorded daily and body weight weekly. Also, colostrum and milk samples were taken before lamb's first suckling and twice on d 7, respectively.

Colostrum and milk Composition

Colostrum and milk samples were analyzed for fat, protein, lactose, and SNF by infrared spectroscopy (AOAC International, 2002; method 972.16; MilkoScan COMBIFOSS 5000, HillerØd, Denmark). Milk fatty acid profile was determined as follows: fatty acids in

Table 2. Fatty acid profiles of the basal diet.

Fatty acid (gr/100 gr of total fatty acids)	Before lambing	After lambing
C4:0	3.62	5.21
C6:0	0.60	0.86
C8:0	0.05	0.03
C10:0	0.03	0.03
C14:0	0.08	0.21
C14:1	ND[1]	0.02
C16:0	8.08	7.62
C16:1	1.82	1.67
C17:0	0.70	0.61
C18:0	1.84	1.52
Trans C18:1	0.54	0.44
C18:1	20.9	ND
C18:2	15.25	11.75
C18:3	2.11	1.74

[1]ND = not detected.

diet and plasma samples were converted into fatty acid methyl ester (FAME) and then profiled using gas chromatography as described previously (18). Briefly, 1 ml of milk sample was mixed with 1ml of C13:0 internal standard (0.5 mg of C13:0/ml of MeOH), 0.7 ml of 10 M KOH in dH_2O, and 5.3 ml of MeOH. The tube was incubated in a 55 °C water bath for 1.5 h with vigorous hand-shaking every 20 min. After cooling below room temperature, 0.58 ml of 12 M of H_2SO_4 in dH_2O was added. The tube content was mixed by inversion, and with precipitated K_2SO4, incubated again in a 55 °C water bath for 1.5 h with hand-shaking every 20 min. After FAME formed, the tube was cooled and 3 ml of hexane was added; the tube was vortex-mixed for 5 min and centrifuged in a tabletop centrifuge again for 5 min and then the hexane layer containing FAME was transferred into a gas chromatography vial.

The fatty acid profile of the FAME was analyzed by gas chromatography on a SP-2560, 100 m × 0.25 mm × 0.20 mm Supelco capillary column installed on a Hewlett Packard 5890 gas chromatograph equipped with a Hewlett Packard 3396 Series II integrator and 7673 controller, a flame ionization detector, and split

Table 3. Chemical compositions of calcium soap of fish oil used in the experiment[1].

Fat (%)	80
Calcium (min, %)	9.3
Calcium (max, %)	11.1
Moisture (max, %)	5
Lipid (max unbound with calcium salt, %)	5
Fatty acids profile (%)	
C16:0	33
C18:0	15
C18:1	25
C18:2	5
C18:3	2
C22:6 n-3 (DHA) and C20:5 n-3 (EPA)	16
Net energy (MCal /kg)	6.36

[1]As provided by the manufacturer (Virtus nutrition LLC., CA, USA).

injection (Agilent Technologies Inc., Santa Clara, CA). After injection of sample to gas chromatography the initial oven temperature was 140 °C, held for 5 min subsequently increased to 240 °C at a rate of 4 °C/min, and then held for 20 min. Helium was supplied as the carrier gas at a flow rate of 0.5 ml/min, and the column head pressure was 280 kPa. Both the injector and the detector were set at 260 °C. The split ratio was 30:1. Fatty acids were identified by comparing their retention times with the FAME standards.

Blood sampling and metabolites analyses

Blood samples were withdrawn immediately before the a.m. feeding from the jugular vein into EDTA-evacuated tubes (10.5 mg, Monoject, Sherwood Medical, St. Louis, MO). Samples were shacked and maintained on an ice pack until plasma was separated by centrifugation at 1500 × g for 15 min at room temperature. Plasma was harvested and stored at −70 °C for further analysis. Plasma concentrations of glucose, albumin, total protein, triglycerides, cholesterol and urea nitrogen (PUN), were measured using enzymatic procedures and commercial kits (Pars Azmon Co., Tehran, Iran) using a spectrophotometer (Shimadzu 2100, Kyoto, Japan). VLDL-cholesterol was estimated as one fifth of the concentration of triglycerides (19).

RNA extraction, reverse transcription, and real-time PCR

Total RNA was extracted from plasma samples using TRI-reagent. Synthesis of cDNA was carried out with M-MuLV reverse transcriptase specific microRNA primers, according to the manufacturer's instructions (Fermentas; St. Leon-Rot, Germany). PCR amplification was performed using a standard procedure with Taq DNA polymerase with denaturation at 94 °C for 15 s, annealing at 55–60 °C for 30 s according to the melting temperature of each primer, and extending at 72 °C for 45 s in 40 cycles.

Real-time PCR reactions of 25 µl were conducted in a Rotor Gene 6000 (Corbett Life Science, Sydney, Australia), by adding 12.5 µl 2× SYBR Premix Ex Taq, 0.4 µM of final concentration for each primer, 2 µl template, and distilled water to reach the volume of 25 µl. Real-time PCR was performed in two steps with the following thermal setting: 3 min at 95 °C for initial enzyme activation, followed by 40 amplification cycles (each 5 s at 95 °C, and 20 s at 60 °C with fluorescence detection) and a final step of melting curve analysis. All samples were analyzed in duplicate, and the average value of the duplicate was used for quantification. Data were normalized to β2m and $2^{-\Delta\Delta Ct}$ methodology was used for relative quantification. Expression of miR-181 was used as an internal control.

Experimental design

Experiment 1: Effect of FO on feed intake and body weight change

In order to measure the effect of FO on feed intake, amount of food offered and of refusal were measured daily. For determining the effect of FO on body weight ewes were weighed before starting the trial, 2 d before expected date of lambing, 24 h after lambing, and 3 weeks after lambing.

Experiment 2: Effect of FO on plasma metabolites

Blood samples were taken from each ewe 1 week before and 1 week after lambing. Plasma was then separated and stored at -70 °C. Plasma metabolites including glucose, protein, urea, triglyceride, VLDL and albumin were measured using commercial kits as mentioned above.

Experiment 3: Effect of FO on colostrum and milk composition and milk fatty acid profile

In order to investigate the effect of FO on colostrum and milk composition, samples were taken on d 0 and 7 of lambing. Samples compositions (lactose, fat, protein, and SNF) were analyzed using MILKOSCAN (COMBIFOSS 5000, HillerØd, Denmark). Milk samples collected on d 7 were used to determine fatty acids profile using gas chromatography.

Experiment 4: Effect of FO on microRNA expression

As mentioned, microRNAs are a class of small molecules regulating some metabolic pathways. To investigate the effect of FO on lipid related microRNAs containing miR-101 (5'-CGA CCG TAC AGT ACT GTG ATA-3', global R: GAG CAG GGT CCG AGG T), miR-103 (F: 5'-CAA GAG CAG CAT GGT ACA G-3', global R: GAG CAG GGT CCG AGG T), and miR-27b (5'-CCG TTC ACA GTG GCT AAG-3', global R: GAG CAG GGT CCG AGG T), plasma samples (n = 3) of each groups on d 7 after lambing were used.

Statistical Analysis

Data for which recordings and analyses were repeated in time were subjected to analysis of variance using REPEATED statement in MIXED procedure of SAS (version 9.1, SAS Institute Inc., Cary, NC) in a completely randomized design with two treatments. Means of other variables with single time measurements were compared using students T-test and the results were expressed as mean ± standard error. For microRNA expression the data were normalized to β2m; then the $2^{-\Delta\Delta Ct}$ methodology was used for relative quantification. $P<0.05$ was considered as significant and $0.05 < P \leq 0.10$ was declared as a trend for significant effect.

Results

Experiment 1: Effect of FO on feed intake and body weight

Average prepartum feed intake was significantly decreased from 1812 ± 35.56 g/d in CON to 1674 ± 33.26 g/d in FO; however, postpartum feed intake was not significantly different (2626 ± 95 *vs.* 2488 ± 89 g /d in CON and FO groups, respectively, Table 4). FO supplementation also did not significantly affect BW, either before (59.6 ± 1.71 kg in CON *vs.* 59.4 ± 1.59 kg in FO) or after (55.5 ± 2.07 kg in CON *vs.* 53.7 ± 1.94 kg in FO) lambing.

Experiment 2: Effect of FO on plasma metabolites

Among plasma metabolites, only the concentration of cholesterol was significantly affected by treatments (56.2 ± 0.71 mg /dl *vs.* 53.1 ± 0.61 mg /dl in CON and FO groups, respectively). No significant difference was

Table 4. Feed intake and body weight of the ewes fed the experimental diets.

Item	Diet		P value		
	Control	Fish oil[1]	Diet	Time	Diet*time
Feed intake					
Prepartum dry matter intake (kg/d)[2]	1812 ± 35	1674 ± 33	0.01	0.00	0.51
Postpartum dry matter intake (kg/d)[3]	2626 ± 95	2488 ± 89	0.31	0.00	0.83
Body weight (kg)					
145 d of pregnancy	59.6 ± 1.71	59.4 ± 1.59	0.91	0.00	0.11
21 d post-lambing	55.5 ± 2.07	53.7 ± 1.94	0.54	0.00	0.30

[1]Calcium soap of fish oil supplemented at 2% of diet dry matter, [2]From 28 d prior to anticipated lambing date until lambing, [3]From d 1 to 21 after parturition.

Table 5. Postpartal plasma metabolites of ewes fed the experimental diets.

Trait	Diet		P value
	Control	Fish oil[1]	
Total Protein (mg/dl)	5.3 ± 0.16	5.1 ± 0.13	0.19
Cholesterol (mg/dl)	56.2 ± 0.66	53.9 ± 0.69	0.02
Albumin (g/dl)	3.7 ± 0.04	3.8 ± 0.13	0.46
Triglyceride (mg/dl)	53.6 ± 1.37	54.3 ± 1.18	0.69
Urea (mg/dl)	32.6 ± 0.73	32.9 ± 0.69	0.84
Glucose (mg/dl)	61.2 ± 1.11	61.5 ± 0.96	0.83
VLDL (mg/dl)	10.7 ± 0.27	10.8 ± 0.29	0.72

[1] Calcium soap of fish oil supplemented at 2% of the diet.

detected in total protein, albumin, triglyceride, urea, glucose and VLDL between treatments (Table 5, $P>0.05$).

Experiment 3: Effect of FO on colostrum and milk composition and milk fatty acid profile

The FO treatment significantly reduced the percentage of fat in colostrum (10.3 ± 0.50 *vs.* 8.5 ± 0.47, Table 6). However, concentrations of protein (CON: 14.0 ± 1.03 *vs.* FO: 12.3 ± 0.97), lactose (CON: 7.5 ± 0.48 *vs.* FO: 7.1 ± 0.45) and SNF (CON: 22.1 ± 1.01 *vs.* FO: 20.4 ± 0.95) were unaffected by FO treatments. The same results were observed for milk components where FO significantly reduced milk fat percentage, while milk protein, lactose and SNF concentrations remained unaffected (Table 6, $P>0.05$).

Supplemental FO had substantial effects on milk fatty acids profile where concentrations (g/ 100 g total fatty acids) of EPA (0.17 *vs.* 1.83), DHA (0.07 *vs.* 0.14), C22:5n-3 (0.16 *vs.* 0.24), C20:3 n-3 (0.35 *vs.* 0.98) and CLA c9t11 (0.34 *vs.* 1.99) significantly increased by FO (Table 6).

Experiment 4: Effect of FO on microRNA expression

Relative transcript abundance of adipogenic related microRNAs showed that miR-101 significantly decreased (0.215 ± 0.08) in FO group. The relative expression of miR-27b did not show any difference (0.98 ± 0.3 in FO group) between groups, while the relative transcript abundance of miR-103 tended to show a significant decrease [0.37 ± 0.15 in FO group, (P< 0.1)]. The relative transcript abundances of miR-101, miR-103, and miR-27b are presented in Figure 1.

Discussion

The hypothesis tested here was that FO supplementation during ewes' transition from pregnant non-lactating to non-pregnant lactating state could affect milk and plasma metabolites and modulate energy-related pathways. Results indicated that following FO supplementation, prepartum feed intake significantly decreased ($p<0.05$) while postpartum feed intake and pre and postpartum BW remained unchanged. In this regard, some studies using FO or fish meal in diets of ewes (20) or lactating cattle (21-24) reported feed intake and milk yield depressions, whereas others reported that fish meal supplementation increased milk yield and feed intake with no changes in milk composition of early lactation cows (25). In a study, FO supplementation (140 g /cow /day) during transition period had no effect on feed intake, milk yield, protein yield and percentage while postpartum milk fat percentage and yield were decreased (26). Although the use of protected PUFA is expected to increase passage of fatty acids to abomasum and reduce the risk of ruminal toxicity, there are studies that reported substantial biohydrogenation of calcium salt of fatty acids in the rumen indicative of incomplete ruminal protection (27). Therefore, the negative effect of FO on prepartal feed intake observed in our study could partially be justified by its toxic effect on rumen microflora (28), which likely reduced fiber digestion and passage (29) ultimately reducing feed intake. Moreover, it has been demonstrated that Ca-salt of fish oil is particularly unpalatable (29) potentially decreasing intake. We postulate that ewes were adapted to and the extent of intake depression was narrowed with time making the postpartum difference between treatments non-significant.

In the present study, FO significantly decreased colostrum and milk fat percentage, while concentrations

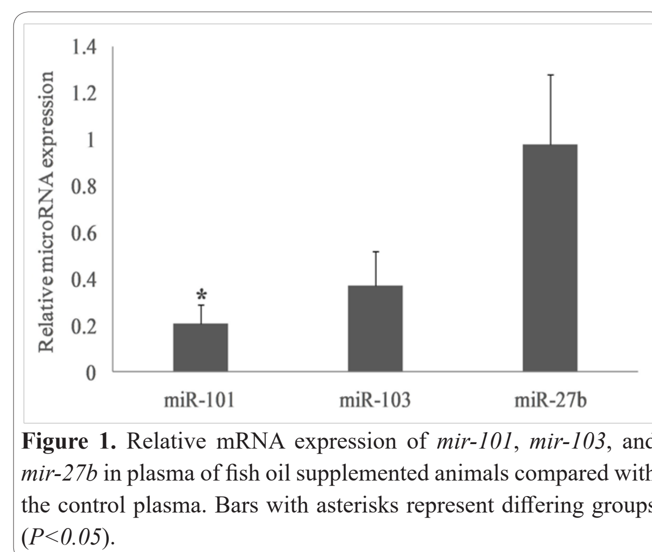

Figure 1. Relative mRNA expression of *mir-101*, *mir-103*, and *mir-27b* in plasma of fish oil supplemented animals compared with the control plasma. Bars with asterisks represent differing groups ($P<0.05$).

Table 6. Colostrum and milk compositions and milk fatty acids profile in control and fish oil supplemented ewes.

Item	Diet		
	Control	Fish oil[1]	P value
Colostrum composition			
Fat (%)	10.3 ± 0.50	8.5 ± 0.47	0.02
Protein (%)	14.1 ± 1.03	12.3 ± 0.97	0.2
Lactose (%)	7.5 ± 0.48	7.1 ± 0.45	0.5
SNF (%)[2]	22.1 ± 1.01	20.4 ± 0.95	0.25
Milk composition			
Fat (%)	8.8 ± 0.49	7.0 ± 0.45	0.02
Protein (%)	4.4 ± 0.27	4.3 ± 0.23	0.78
Lactose (%)	5.1 ± 0.18	5.1 ± 0.16	0.95
SNF (%)	10.4 ± 0.36	10.9 ± 0.3	0.56
Milk fatty acids (g/100g)			
C4:0	0.76 ± 0.2	0.45 ± 0.09	0.23
C6:0	0.62 ± 0.14	0.33 ± 0.09	0.15
C8:0	0.62 ± 0.16	0.37 ± 0.1	0.28
C10:0	1.73 ± 0.43	1.46 ± 0.36	0.65
C16:0	31.15 ± 6.11	28.35 ± 5.02	0.73
C16:1	3.04 ± 0.43	3.44 ± 0.81	0.68
C18:0	6.19 ± 1.18	4.0 ± 1.2	0.2
trans-10 C18:1	1.13 ± 0.2	2.19 ± 0.55	0.14
cis-9 trans-12 C18:2	0.05 ± 0.01	0.06 ± 0.01	0.51
C18:3n3	0.30 ± 0.06	0.23 ± 0.06	0.455
C22:1	0.08 ± 0.01	0.08 ± 0.03	1
C20:3n3	0.35 ± 0.08	0.98 ± 0.18	0.03
C20:4n6	0.09 ± 0.02	0.06 ± 0.01	0.25
C22:2	0.03 ± 0.006	0.47 ± 0.4	<0.001
C24:0	0.31 ± 0.08	0.19 ± 0.03	0.232
C20:5n3 (EPA)	0.17 ± 0.04	0.83 ± 0.21	0.001
C24:1	1.06 ± 0.28	0.05 ± 0.1	0.022
C22:5n3	0.16 ± 0.03	0.24 ± 0.07	0.044
C22:6n3 (DHA)	0.07 ± 0.01	0.14 ± 0.04	0.014
cis-9 trans 11 CLA	0.34 ± 0.01	1.99 ± 0.13	<0.001

[1] Calcium soap of fish oil supplemented at 2% of the diet, [2]SNF: solid non-fat.

of protein, lactose and SNF were not affected by FO supplementation. The same results were reported when lactating ruminants fed FO supplemented diets (23, 30-33). In contrast, Badiei *et al.,* (2014) reported that supplementation of prepartum cows' diet with n-3 LCFA had no effect on milk composition (34). Discrepancies between these studies could be due in part to the differences in the amount, form, and palatability of fat supplements and the duration of the experiments. Feeding FO has been shown to change the bacterial metabolism of lipids and subsequently increase the duodenal flow of PUFA and trans-11 C18:1 and decrease C18:0 (35). It has been fully discussed that specific fatty acids produced as intermediates during ruminal biohydrogenation, including trans-10, cis-12 C18:2 and trans-9, cis-11 CLA, possess inhibitory effects on mammary lipogenesis (33, 36), thereby reducing milk fat concentration. In this regard Carreño *et al.,* (2016) observed that milk fat depression in ewes supplemented with FO was correlated with increases in C18:2 t10c12 and t9c11, and C18:1 c11 (37). This might explain milk fat depression in response to FO supplementation in this study where the amount of c-9,t-11 CLA was significantly increased in milk. Furthermore, FO has a great impact on regulation of lipogenic genes involved in the synthesis of milk fat. Recently, it has been shown that lipogenic genes containing acetyl-CoA carboxylase alpha (ACACA), acyl–CoA synthetase short-chain family member-1 (ACSS1), and 1-acylglycerol-3-phosphate O-acyltransferase 6 (AGPAT6), and the transcription factor sterol regulatory element-binding factor 1 (SREBF1) were down-regulated in the mammary tissue of ewes fed FO (37). Milk fatty acid profile also indicated that the n6:n3 ratio decreased as a result of significant increase in the amount of n-3 LCFA especially EPA and DHA. The results are in agreement with a previous study that reported a significant increase in the proportion of EPA and DHA in ewes' milk fed FO (37).

Except for the cholesterol that significantly reduced in FO group, other plasma metabolites were not affected by feeding FO. Recent studies showed that prepartum supplementation of ewes' diet with FO had no effect on albumin and total protein but significantly increased urea concentration (20, 38). In contrast, Janhani-mo-

ghadam *et al.,* (2015) reported that postpartum linseed supplementation increased the concentration plasma glucose (39). A reason for decreased cholesterol might be related to increase in concentration of other lipoproteins like HDL and increased reverse transport of cholesterol in hepato-peripheral lipid circulation. This might increase supply of cholesterol from the tissues to the liver, thereby increasing cholesterol metabolization in the liver and decreasing its circulation.

As mentioned, it has been shown that diet could modulate adipogenesis and lipid metabolism through regulatory microRNAs. To the best of our knowledge, this is the first study using plasma MicroRNAs as a predictor marker of metabolic alteration following FO supplementation. In this aspect, our results indicated that the transcript abundance of miR-101 was affected by FO supplementation. The relative expression of miR-103 also showed a downward trend, although it was not significant. A previous study in this area reported that a high fat diet decrease the expression of miR-101, but not miR-103, in bovine subcutaneous and visceral fat (14). In another study, ewes fed algae (a rich source of DHA) did not show significant difference in expression of miR-101 in subcutaneous and visceral fat depots (15). As mentioned miR-101 controls lipid metabolism and adipogenesis (14). Hence, it could be inferred that FO changed lipid metabolism in favor of adipogenesis in this study, which might consequently prevent adipose lipolysis and triglyceride accumulation in the liver, a process that might help the liver to supply the mammary gland and the gravid uterus with substantial nutrient demand while avoiding fatty liver.

Feeding of moderate amounts of Ca-soup of FO during peripartal period in comparison to a control diet resulted in prepartal feed intake reduction, milk fat depression, and increases in milk trans fatty acids and n-3 LCFA and altered patterns of microRNA expression. However, postpartal feed intake, plasma metabolites and milk fat and protein remained unaffected. Future research should investigate whether these changes in molecular patterns affect lambs future performance and metabolism.

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Declaration of interests

The authors declare no potential conflicts of interest with respect to the authorship and publication of the article.

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