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Studying the Effect of Source and Feeding Duration of Protected Omega-3 and Omega-9 Fatty Acids on Production Performance and Expression of Genes Involved in Fat Metabolism in Fattening Lambs

Research Article

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ABSTRACT

This experiment was conducted to investigate the effects of calcium salts of unsaturated and saturated fatty acids (omega-3 and omega-9) on fattening performance and genes expression related to fat metabolism in Lori Bakhtiari × Romanov crossbreed male lambs. Forty-nine male lambs, averaging 29.97 ± 0.88 kg were randomly assigned to seven groups. The lambs were fed experimental diets as follows: 1) Basal ration without fat (C), 2 and 3) Basal ration with calcium salt of fish oil (2% DM of diet) for 90 and 45 days (FO-90 and FO-45), respectively, 4 and 5) Basal ration with calcium salt of olive oil (2% DM of diet) for 90 and 45 days (OO-90 and OO-45), respectively, 6 and 7) Basal ration with saturated fat powder (2% DM of diet) for 90 and 45 days (SF-90 and SF-45), respectively. Overall, feeding calcium salts of fish oil and olive oil significantly increased daily weight gain and decreased the feed conversion ratio compared to the control group (P=0.04). Additionally, these diets reduced abdominal and kidney fat (P=0.03). The supplementation of calcium salts of unsaturated and saturated fats did not significantly affect the expression of the delta-6 desaturase (FADS2) and delta-5 desaturase (FADS1) genes in the liver tissue compared to the control group. However, the calcium salts of fish and olive oils administered for 90 and 45 days significantly increased liver mRNA expression of the carnitine palmitoyl transferase-1 (CPT1) (P=0.001) and acyl coenzyme A oxidase-1 (ACOX1) (P=0.002) genes compared to the saturated fat and controls treatments. This study indicated that supplementing diets with calcium salts of unsaturated fatty acids from fish and olive oils improved fattening performance and increased the expression of genes involved in fat metabolism in lamb liver tissue, regardless of feeding duration.

KEY WORDS fattening lambs, gene expression, performance, unsaturated fatty acids.

INTRODUCTION

Monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) have garnered much attention over the last decade due to their evident health benefits (Vassiliou *et al*. 2009; Poorghasemi *et al*. 2017). Vegetable and animal oils, as high-energy sources, are now commonly added to animal rations to increase the energy concentration of diets. Supplementing the rations of fattening lambs with fats, in amounts that do not adversely affect rumen ecology, can increase daily weight gain and reduce feed intake (Huffm *et al*. 1992; Coleman *et al*. 2019). Among unsaturated fatty acids, oleic acid is the primary unsaturated fatty acid found in ruminant feed. Studies have reported that adding olive oil to the diet increase the presence of this fatty acid in sheep's milk and lamb's meat (Manso *et al*. 2011).

The effects of oils in ruminant diets on livestock performance vary and may depend on the livestock type, the composition of the basal diet, and the type and amount of forage and oil used (Jenkins *et al*. 2008; Fekri and GanjKhanlu, 2021). Some reports suggest that the consumption of protected unsaturated fats reduces dry matter intake, and this effect becomes more pronounced with increasing fat unsaturation (Relling and Reynolds, 2007). Gallardo *et al*. (2014) studied the effects of calcium salts of palm, olive, and fish oils (3% of the diet) in the rations of ewes on their suckling lambs and found that the type of fatty acids added to the diet did not significantly affect the growth rate, slaughter weight, or carcass percentage of lambs. Ghoorchi *et al*. (2006) also showed that adding long-chain fatty acids up to 7.5% of ration's dry matter had no effect on lamb carcass weight.

In addition to meeting organisms' energy and protein requirements, many food items contain compounds that affect cellular functions and intracellular signaling pathways which can alter cell function either temporarily or permanently (Abrahams, 2017; Malau-Aduli *et al*. 2019; Poorghasemi *et al*. 2024). Fatty acids influence nuclear processes by binding to nuclear receptors and regulating the activity of nuclear transcription factors. The interaction between dietary nutrients and gene expression involved in lipid metabolism plays a significant role in how fatty acids are stored in different tissues (Ladeira *et al*. 2018). The unsaturation of PUFA in mammals is encoded by fatty acid desaturase genes (FADS) (Matsumoto *et al*. 2014). EPA and DHA, key PUFA components, act as bioactive molecules that can stimulate peroxisome proliferator-activated receptor alpha (PPARα). This stimulation decreases the transcription of lipogenic genes while enhancing the transcription of lipolytic genes (Clarke, 2001). As transcription factors, PPARα and PPARγ regulate fatty acid metabolism and transport by controlling mitochondrial β-oxidation, increasing peroxisomal β-oxidation, and inducing the expression of target genes such as ACOX (acyl coenzyme-A oxidase) and CPT1 (carnitine palmitoyl transferase-1) (Belal *et al*. 2018). Oleic acid may also serve as a signal to create a negative feedback loop, helping to regulate excess fatty acids, maintain lipid homeostasis, and promote fatty acid oxidation through signaling and transcriptional mechanisms (Lim *et al*. 2013). This study aimed to investigate the effects of calcium salts of olive oil, fish oil, and saturated fat on growth performance, carcass traits, and the expression of genes related to liver fat metabolism in Lori Bakhtiari × Romanov crossbred male lambs during both short-term (45 days) and long-term (90 days) feeding periods.

MATERIALS AND METHODS

This research was conducted at the educational research station of the Department of Animal Sciences, Faculty of Agriculture and Natural Resources, University of Tehran. Forty-nine Lori Bakhtiari × Romanov crossbred male lambs, aged 4-5 months with an average initial weight of 29.97 ± 0.88 kg, were randomly housed in individual pens with free access to water and feed. The experiment consisted of seven treatments (7 lambs per treatment), each receiving a basal diet as follows:

Treatment 1: Basal diet without fat powder (Control=C).
Treatments 2 and 3: Basal diet with fish oil calcium salt (as a source of Omega-3) at 2% of the diet's dry matter for 90 and 45 days (FO-90 and FO-45), respectively.
Treatments 4 and 5: Basal diet with olive oil calcium salt (as a source of Omega-9) at 2% of the diet's dry matter for 90 and 45 days (OO-90 and OO-45), respectively.
Treatments 6 and 7: Basal diet with saturated fat powder at 2% of the diet's dry matter for 90 and 45 days (SF-90 and SF-45), respectively.

The rations were formulated according to the recommendations of the NRC for Sheep and Goat (NRC, 2007) and using the fifth version of the Cornell Net Carbohydrate and Protein System (CNCPS) software to ensure equal energy and protein levels across all diets. The components and compositions of the experimental diets are shown in Table 1, and the fatty acid composition of the diets are listed in Table 2. The calcium salts of fish and olive oil contained 85% fat and 9% calcium.

Measurement of dry matter intake and body weight changes

During the experiment, the lambs were fed individually, and the amount of dry matter consumed was recorded daily. Each morning before feeding, the leftover feed from the previous day was collected and weighed. Dry matter intake was calculated by determining the difference between the amount of dry matter offered and the amount remaining in the manger.

To track body weight changes, the lambs were weighed at the start of the experiment and every three weeks thereafter. Weighing was done after 14 to 16 hours of fasting and prior to the morning meal. Body weight changes were recorded, and the feed conversion ratio was calculated based on the dry matter intake and the weight gain of the lambs during the experimental periods.

Table 1 Feed components and chemical composition of the experimental diets (based on 100% DM of the ration)

Feed components	Control	Fish oil	Olive oil	Saturated fat
Alfalfa hay	18.33	18.33	18.33	18.33
Corn silage	8.33	8.33	8.33	8.33
Wheat straw	3.33	3.33	3.33	3.33
Barley	30	28.33	28.33	28.33
Corn	19.33	16.67	16.67	16.67
Soybean meal	7.92	7.92	7.92	7.92
Rice bran	5	5.83	5.83	5.83
Wheat bran	5.83	7.5	7.5	7.5
Calcium carbonate	0.5	0.33	0.33	0.5
MgO	0.17	0.17	0.17	0.17
Mineral and vitamin mix	0.5	0.5	0.5	0.5
NaCl	0.25	0.25	0.25	0.25
$NaHCO_3$	0.5	0.5	0.5	0.5
Fat powder	-	2	2	1.83
Chemical composition[1]				
ME (Mcal/kg DM)	2.64	2.73	2.73	2.73
CP (%)	13.5	13.5	13.5	13.5
NDF (%)	27	27	27	27
NFC (%)	52	51	51	51
Ash (%)	6.6	6.6	6.6	6.8
EE (%)	3.4	5.1	5.1	5.1
Concentrate (%)	70	70	70	70

ME: metabolizable energy; CP: crude protein; NDF: neutral detergent fiber; NFC: non fibrotic carbohydrate and EE: ether extract.

Table 2 Fatty acid profile of dietary treatments (g/100 g total fatty acid)

Fatty acid[1]	Control	Fish oil diet	Olive oil diet	Saturated fat diet
C14:0	1.4	2.09	1.95	2.45
C16:0	17.31	16.04	14.76	21.09
C16:1	1.42	1.73	2.99	1.4
C18:0	4.9	4.42	4.55	26.25
C18:1 trans	1.65	1.72	3.9	1.68
C18:1 cis-9	26.22	24.66	33.49	19.41
C18:2 n-6	32.23	28.44	24.28	16.02
C18:3 n-3	3.96	4.21	3.48	2.14
C20:4 n-6	0.35	0.36	0.4	0.25
C20:5 n-3	0.3	4.38	0.6	0.3
C22:6 n-3	0.25	5.05	0.6	0.2
SFA	26.36	25.5	24.36	54.49
MUFA	30.19	29.06	41.38	23.25
PUFA	37.09	42.44	29.36	18.91
ω3 FA	4.51	13.64	4.68	2.64
ω6 FA	32.58	28.8	24.68	16.27
ω9 FA	26.22	24.66	33.49	19.41
ω6/ω3	7.22	2.11	5.27	6.16

SFA: saturated fatty acids; MUFA: mono unsaturated fatty acids; PUFA: poly unsaturated fatty acids; ω3 FA: omega-3 fatty acids; ω6 FA: omega-6 fatty acids and ω9 FA: omega-9 fatty acids.

Measurement of carcass characteristics

At the end of the experiment, 28 lambs were weighed and slaughtered after 14 to 16 hours of feed and water deprivation. Thirty minutes post-slaughter, a 2 g sample was taken from the right lobe of the liver and immediately frozen in liquid nitrogen. Liver tissue samples were stored at -80 °C until gene expression analyses were conducted. After slaughter, the weights of various carcass parts were measured. A cross-section of the longissimus muscle between the 12^{th} and 13^{th} ribs was outlined on chalk paper and measured using a digital planimeter. Body fat was determined by measuring the thickness of adipose tissue and

muscle at the $11^{th}/12^{th}$ ribs with calipers.

Measurement of liver (mRNA) gene expression

Total RNA was extracted from liver tissue samples using an RNA extraction kit. RNA concentration was measured using a nanodrop device at 260 nm wavelength, and its purity was calculated by calculating the absorption ratios at 260/280 nm. cDNA was synthesized from 100 ng of total RNA using a cDNA synthesis kit. To design primers, the nucleotide sequence of the candidate genes and reference gene were obtained from the public database GenBank (National Center for Biotechnology Information). The sequences of the designed primers are presented in Table 3. To control for potential errors in gene expression measurements, the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the reference gene in liver tissue. The target genes studied in liver tissue included delta-5 desaturase (FADS1), delta-6 desaturase (FADS2), carnitine palmitoyl transferase-1 (CPT1A) and acyl coenzyme A oxidase-1 (ACOX1).

Real-time PCR was performed using a SYBR GREEN mixture on iQ5 system (BioRad, USA). RT-PCR data analysis was conducted following the Livak and Schemittgen method (Livak and Schmittgen, 2001). The geometric mean of the reference genes was used to calculate the initial Ct, and ΔCt was determined using the following formula:

$$\Delta Ct = Ct\ (Target\ gene) - Ct\ (Reference\ gene)$$

After calculating ΔCt for all samples, the expression level of the target genes was measured relative to the mean of the reference genes using the following formula:

$$RE = 2^{-\Delta\Delta Ct} = 2^{-(\Delta Ct\ (target\ gene) - \Delta Ct\ (Reference\ gene)}$$

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) with the SAS statistical package (SAS, 2013). The analysis was performed using a completely randomized design with one replication, and mean comparison were made using Duncan's multiple range test. Feed consumption and weight gain data were analyzed as repeated measurements, and comparison of means were also done using Duncan's test.

RESULTS AND DISCUSSION

Feeding lambs with calcium salts of fish oil, olive oil, and saturated fat, both throughout the 90-day fattening period and the last 45 days, had no significant effect on dry matter intake compared to the control group (Table 4). Some studies have shown that calcium salts of fatty acids can increase the energy concentration of the diet by bypassing the rumen without affecting fiber digestion. These calcium salts are broken down in the low pH of abomasum and become available for absorption in the small intestine (Alba *et al*. 2021). In a study conducted by Ferreira *et al*. (2014) and Parvar *et al*. (2017) on sheep, when lambs were fed various levels (2.5% to 7.5%) of polyunsaturated fatty acids (fish oil), no difference was observed in dry matter intake, daily weight gain, final body weight, or feed efficiency. However, Hernández-García *et al*. (2017) reported a quadratic response in weight gain when increasing the concentration of fish oil in the diet. As shown in Table 4, feeding calcium salts of unsaturated fish and olive oils for both 45 and 90-day periods significantly increased final weight, total weight gain, and daily weight gain in lambs compared to the control group (P=0.04). Given the same feed intake but higher weight gain, lambs in the unsaturated fat groups exhibited significantly lower feed conversion ratios compared to the saturated fat and control groups.

In line with these findings, Ponnampalam *et al*. (2015) investigated the effects of supplementing fattening lamb diets with alpha-linolenic acid (linseed) and docosahexaenoic acid (algae) over 56 days. They found that the lambs fed linseed had greater body weight, body fat, and carcass yield than the control group. In their study, DHA from algae reduced feed intake, but despite this reduction, carcass performance was higher in lambs consuming DHA and the linseed × DHA mixture. This suggests that supplementing lamb diets with DHA led to less feed consumption but improved energy conversion into carcass tissue. This improvement may be due to reduced methane production, thereby transferring more dietary energy to body tissues (fat and muscle) (Ponnampalam *et al*. 2015). Similarly, Arana *et al*. (2006) evaluated the effect of calcium soaps of olive fatty acids (5% of the diet) in male Rasa Aragonesa lambs and found that daily weight gain, dry matter intake and feed conversion ratios were similar to those of the control lambs.

The results of the effects of calcium salts of unsaturated and saturated fatty acids on carcass characteristics are presented in Table 5. The use of calcium salts from unsaturated fatty acids (fish and olive oils) and saturated fat had no significant effect on carcass yield, carcass length, the weight of head and legs, full and empty digestive tracts, tail, backfat thickness, or the cross-section of longissimus muscle in the lambs. However, lambs consuming fat powder, compared to the control group, had higher carcass weight and yield. The improvement in carcass yield with the addition of fish oil may be attributed to the positive effects of fish oil on metabolic energy production (Lough *et al*. 1993; Mirshamsollahi *et al*. 2022). Consistent with the findings of this study, adding 4% saturated palm fat and sunflower oil to barley-based diets in fattening lambs showed no significant effect on hot and cold carcass weights (Manso *et al*. 2009).

Table 3 Primers used to measure the expression of genes involved in the liver metabolism of fatty acids

Gene[1]	Forward and reverse sequence
ACOX1	(fwd) TGGAGAGCCCTCAGCTATGG (rev) CGTTTCACCGCCTCGTAAG
CPT1A	(fwd) CTGTATCGTCGCACATTAGACCGT (rev) CAGACCTTGAAGTACCGCCCTCT
FADS1	(fwd) AGGAACAGTGTGACCCCTTG (rev) AAAAACAGCTTCTCCCAGCA
FADS2	(fwd) ATCTGCCCTACAACCACCAG (rev) TGTGACCCACACAAACCAGT

Table 4 Effect of omega-3, omega-9, and saturated fatty acids on fattening performance

Trait	Rations							SEM	P-value
	C	FO-45	FO-90	OO-45	OO-90	SF-45	SF-90		
Initial body weight (kg)	29.3	30.4	30.8	29.8	29.9	29.8	29.4	0.88	1
Final body weight (kg)	47.1^{b}	49.6^{a}	51.7^{a}	49.5^{a}	49.8^{a}	48.4^{ab}	48.3^{ab}	0.87	0.04
Total weight gain (kg)	17.7^{b}	19.2^{a}	20.9^{a}	19.7^{a}	19.9^{a}	18.5^{ab}	18.9^{ab}	0.519	0.04
Mean daily gain (g)	201^{b}	219^{a}	238^{a}	224^{a}	226^{a}	210^{ab}	214^{ab}	0.005	0.04
Daily DMI (g)	1.42	1.40	1.58	1.46	1.44	1.46	1.49	0.019	0.30
FCR	7.17^{a}	6.55^{b}	6.78^{b}	6.70^{b}	6.54^{b}	7.21^{a}	7.27^{a}	0.18	0.03

DMI: dry matter intake and FCR: feed conversion ratio.
C: control; FO-45: fish oil for 45 days; FO-90: fish oil for 90 days; OO-45: olive oil for 45 days; OO-90: olive oil for 90 days; SF-45: saturated fatty acid for 45 days and SF-90: saturated fatty acid for 90 days.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Effect of omega-3, omega-9, and saturated fatty acids on carcass traits

Trait	Rations							SEM	P-value
	C	FO-45	FO-90	OO-45	OO-90	SF-45	SF-90		
Live weight (kg)	44.1	46.8	48.8	46.6	46.8	42.7	44.3	1.48	0.95
Warm carcass weight (kg)	19.2	21.4	21.9	21.2	21.3	18.8	20.1	0.71	0.92
Carcass yield (%)	43.15	45.84	44.84	48.39	45.47	44.03	45.23	0.31	0.27
Head and legs (%)	7.35	7.59	7.07	7.93	7.07	7.63	7.62	0.10	0.31
Carcass length (cm)	70.33	72	77.33	72.66	73.33	70.33	73	1.06	0.71
Full digestive tract (%)	11.7	9.9	10.6	10.2	10.6	12.1	12	0.35	0.55
Empty digestive tract (%)	3.9	3.1	3.5	3.3	3.2	3.9	3.5	0.098	0.22
Fat around the kidney (%)	1.11^{a}	0.72^{b}	0.86^{b}	0.83^{b}	0.50^{b}	1.02	1.18^{a}	0.06	0.03
Visceral fat (%)	2.75^{a}	1.53^{b}	1.75^{b}	1.26^{b}	1.26^{b}	2.33	2.36^{a}	0.14	0.03
Tail (%)	1.18	1.34	1.48	0.99	1.11	0.97	1.49	0.084	0.21
Backfat thickness (mm)	4.9	5.8	5.1	4	4.9	4.3	5.4	0.25	0.63
Cross-section of the longissimus muscle (cm^2)	21.3	20.3	24	22	19.7	21.3	25	0.98	0.83

C: control; FO-45: fish oil for 45 days; FO-90: fish oil for 90 days; OO-45: olive oil for 45 days; OO-90: olive oil for 90 days; SF-45: saturated fatty acid for 45 days and SF-90: saturated fatty acid for 90 days.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 The effect of omega-3, omega-9, and saturated fatty acids on the expression of studied genes in liver tissue

The studied genes	Rations							SEM	P-value
	C	FO-45	FO-90	OO-45	OO-90	SF-45	SF-90		
FADS1	1.48	1.55	1.64	1.63	1.22	1.32	1.43	0.18	0.67
FADS2	1.09	1.15	1.03	1.32	1.26	1.21	1.25	0.099	0.69
CPT1	0.8^{b}	1.79^{a}	1.84^{a}	1.85^{a}	1.87^{a}	0.73^{b}	0.84^{b}	0.091	0.01
ACOX1	0.83^{c}	1.66^{a}	1.32^{ab}	1.18^{ab}	1.25^{ab}	0.95^{c}	0.85^{c}	0.063	0.02

FADS1: fatty acid desaturase-1; FADS2: fatty acid desaturase-2; CPT1: carnitine palmitoyl transferase-1 and ACOX1: acyl coenzyme-A oxidase-1.
C: control; FO-45: fish oil for 45 days; FO-90: fish oil for 90 days; OO-45: olive oil for 45 days; OO-90: olive oil for 90 days; SF-45: saturated fatty acid for 45 days and SF-90: saturated fatty acid for 90 days.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

However, feeding with calcium salts of fish and olive oil significantly decreased abdominal fat and fat around the kidney (P=0.03) (Table 5). The highest amount of abdominal fat was observed in the control group, while the lowest was in the lambs fed with olive oil calcium salts.

In research by Arana *et al*. (2006), investigating the effect of olive fatty acid calcium soaps on adipose tissue growth and fatty acid composition in male lambs during a 35-day fattening period, it was reported that the higher energy consumption in the olive group caused a more significant increase in subcutaneous and perirenal adipose tissue compared to the control group. However, no significant differences were found between the groups in terms of backfat thickness, subcutaneous fat percentage, intermuscular adipose tissue, or intramuscular fat content. In other words, adding 5% oleic acid calcium soaps to the diet for more than 35 days increased internal fat reserves through adipocytes hypertrophy, although the amount of oleic acid in fat and meat did not change.

In this study, there was no significant difference in the cross-section of the longissimus muscle between lambs fed with different diets (Table 5). These results are consistent with the findings of Kazemi *et al*. (2014). The cross-section of the longissimus muscle is positively correlated with live weight. According to this research, Parvar *et al*. (2017) also found no significant differences between treatments regarding carcass weight or carcass percentage in their study of the effects of canola, soybean, and fish oils on the performance and carcass traits of fattening lambs.

The results of this study showed that the use of calcium salts of unsaturated fats (fish and olive oils) and saturated fats over a period of 45 and 90 days had no significant effect on the expression of the FADS1 and FADS2 genes in liver tissue compared to the control group (Table 6). Previous research indicates that the effects of alpha-linolenic acid (ALA) and docosahexaenoic acid (DHA) in the diet on the mRNA expression of $FADS_2$, $FADS_1$, PPARγ, $ACOX_1$ and CPT_1 genes depend on factors such as the type of oils or fatty acids used, the quantity added to the diet, the duration of feeding, and the composition of the basal diet.

Additionally, the expression levels of FADS1, FADS2, and ACOX1 can be influenced by the ratio of DHA to other fatty acids in the experimental diet (Ghoorchi *et al*. 2006; Cherfaoui *et al*. 2013; Iommelli *et al*. 2021). The conversion of ALA to eicosapentaenoic acid (EPA) and DHA, and of linoleic acid to arachidonic acid, occurs through the enzymes delta-5 desaturase (FASD1) and delta-6 desaturase (FASD2) (Glaser *et al*. 2010).

The results further revealed that the use of calcium salts of fish oil and olive oil for both 45- and 90-day periods caused a significant increase in the expression of CPT_1 and $ACOX_1$ liver mRNA in lambs fed unsaturated fats compared to those fed saturated fats or in the control group (Table 6). The CPT1 enzyme is responsible for a mitochondrial transport and plays a crucial role in controlling the oxidation of long-chain fatty acids, thereby promoting mitochondrial β-oxidation (Costa Alvarenga *et al*. 2015). These findings align with previous reports that indicate fish oil-rich diets increase the expression of genes involved in lipid catabolism, including CPT_1 and $ACOX_1$ (Takahashi *et al*. 2002; Dirandeh *et al*. 2016). Clarke (2001) also reported that omega-3 long-chain fatty acids exert their effects on thermogenesis and lipid metabolism by enhancing the expression of genes encoding proteins involved in fatty acid oxidation (such as CPT_1 and $ACOX_1$) while simultaneously regulating the transcription of genes related to fat synthesis (e.g., fatty acid synthase).

Belal *et al*. (2018), found that in cows fed linoleic acid, oleic acid, or their combination, the expression of PPARα gene and other lipid metabolism-related genes such as ACOX and lipoprotein lipase (LPL) significantly increased compared to the control group. Linoleate and oleate also boosted the expression of CPT1 gene, indicating increased beta-oxidation of fatty acids. Polyunsaturated fatty acids reduce the expression of genes responsible for fatty acids and triglyceride synthesis while stimulating the expression of genes involved in fatty acid oxidation (Belal *et al*. 2018; Carranza Martin *et al*. 2018). Oleic acid may act as a signal to create a negative feedback loop to counter excess fatty acids and maintain lipid homeostasis. It enhances fatty acid

oxidation through signaling and transcription mechanisms (Lim *et al*. 2013).

CONCLUSION

The results of this research demonstrated that supplementing lamb rations with calcium salts of unsaturated fatty acids from fish and olive oil improved fattening performance and productivity. This was achieved by increasing weight gain, reducing feed conversion ratio, and lowering carcass fat. Feeding lambs with calcium salts of fish oil, olive oil, and saturated fat had no significant effect on dry matter intake compared to the control group. Additionally, the supplementation enhanced the expression of genes (including CPT_1 and $ACOX_1$) involved in fat lipolysis in liver tissue.

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