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Physicochemical Characteristics and Fatty Acid Profile of Meat and Adipose Tissue from Lambs Fed Diets with Different Levels of Pomegranate Seed Oil

Research Article

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ABSTRACT

Twenty-one male Sanjabi lambs (body weight (BW)=27.5±2.6 kg, 3-month-old), were used to study the effects of diet supplementation with pomegranate seed oil (PSO) on the performance, carcass and meat quality, and fatty acid composition of muscle and subcutaneous fat. Lambs were randomly distributed between three treatments (0, 2, and 4% of dry matter (DM) pomegranate seed oil) and were fed for 90 days before slaughter. Average daily feed intake (g/d) increased by pomegranate seed oil inclusion in diet ($P<0.05$). Hot carcass weight (HCW) and cold carcass weight (CCW), weight of fat depots, subcutaneous fat depth, longissimus thoracis area and weight of carcass cuts, were not affected by pomegranate seed oil. Colour lightness (L*) index and fat content of longissimus thoracis muscle increased by 4% pomegranate seed oil supplementation ($P<0.05$); however, moisture, protein and ash content of muscle did not affect. Addition of pomegranate seed oil to lamb's diet slightly affected fatty acid profile of longissimus thoracis muscle and couldn't change the total amount of polyunsaturated fatty acids (PUFA), and the n-6/n-3 or polyunsaturated fatty acids/saturated fatty acids (SFA) ratios. Pomegranate seed oil supplementation significantly increased total polyunsaturated fatty acids n-3 and n-6 content and significantly decreased n-6/n-3 ratio in subcutaneous fat ($P<0.05$). In conclusion, the results obtained in current study suggest that addition of up to 4% pomegranate seed oil to fattening lambs diet have potential to increase some polyunsaturated fatty acids content and decrease n-6/n-3 ratio of carcass deposited fats without negative impact on fattening performance, carcass traits, and colour stability of meat.

KEY WORDS carcass traits, fatty acid profile, meat quality, performance, pomegranate seed oil, Sanjabi lamb.

INTRODUCTION

Foods derived from animals are rich source of nutrients for humans, especially proteins, iron, zinc, vitamin B12, fat-soluble vitamins and biologically active fatty acids (Białek *et al.* 2021a). Meat is a very important constituent of human diet but in recent years, the fat content and fatty acid composition of meat, especially red meat obtained from ruminants, achieved more attention as consumer awareness and concern about the relationships between dietary fat and human health issues like elevated risk of cardiovascular disease, cancer and obesity have increased (Milićević *et al.* 2014). Increasing ruminant's meat's content of PUFA and conjugated linoleic acid (CLA) isomers and also decreasing its SFA and the n-6:n-3 PUFA ratio proportion is considered extensively as measures to enhance nutritional quality and decrease health issues of ruminant meat consumption (Delgado-Pertíñez and Horcada, 2021). Among all possible factors, feeding strategies, especially inclusion of PUFA rich oils in diet formulation, are known as the most effec-

tive factor on alteration of fatty acid profile and also other meat quality attributes (Boles *et al*. 2005; Białek *et al*. 2021a). Using this method may result in an increased dietary intake of n-3 fatty acids without changing of consumers' dietary habits (Konieczka *et al*. 2017).

CLA and Conjugated linolenic acids (CLnA) isomers consumption have been associated with a range of bioactive properties including anti-cancerogenic, anti-atherosclerotic, anti-obesogenic, anti-diabetogenic effects, along with reports of growth promotion and of the modulation of immune responses, and anti-inflammatory action (Den Hartigh, 2019). Biological activity of CLnA is partially attributed to their endogenous conversion to CLA isomers (Lepionka *et al*. 2019), which makes some authors to call plant oils rich in CLnA indirect dietary sources of CLA or 'super CLA' (Białek *et al*. 2021a). One of CLnA isomers is cis-9, trans-11, cis-13 octadecatrienoic acid (punicic acid, PA), which accounts for more than 70% of PSO fatty acids (Białek *et al*. 2014; Wang *et al*. 2019).

Pomegranate is native to Iran and Iran is known as the center of diversity of pomegranate (Hassani Moghadam *et al*. 2020). Annually about three million tons of pomegranates are produced in the world and Iran is the first producer by producing about nine hundred and forty thousand tons of this fruit (Esmailpour Troujeni *et al*. 2018). Around 55%-60% of pomegranate weight is edible, of which 75%-85% is juice and 15%-25% seed (Seeram *et al*. 2006). Significant amounts of pomaces are generated during juicing, which cause serious environmental hazards. Recently, utilization of these wastes as source of valuable compounds which could be used in food, cosmetic, and pharmaceutical industries has gained a great interest (Durante *et al*. 2017). The most abundant in pomegranate pomaces are skin and seeds, which may be easily separated and used for flavors, pigments, and oil extraction.

PSO constituting about 24% of total seed weight (Khoddami and Roberts, 2015). The oil consists of palmitic acid (C16:0) and stearic acid (C18:0), both SFAs, oleic acid (C18:1), a monounsaturated fatty acid (MUFA), linoleic acid (C18:2), and an isomer of linolenic acid or CLnA (C18:3), called punicic acid (cis-9, trans-11, cis-13 or trichosanic acid), both polyunsaturated fatty acids (Khoddami and Roberts, 2015). Punicic acid (31%-86%) is the dominant fatty acid in pomegranate oil, followed by linoleic acid (0.7%-24.4%) and oleic acid (0.4%-17.4%) (Khodami *et al*. 2014; Khoddami and Roberts, 2015).

Different researches focused on inclusion of lipid sources high in PUFA in ruminants' diets to make any significant change in growth performance or meat's fatty acid content has yielded contradictory results (Castro *et al*. 2015; Quiñones *et al*. 2019). Gao *et al*. (2022) reported that inclusion of 2% linseed oil in Small-tailed Han postweaning diets did not alter growth performance, while the total PUFA content and PUFA/SFA ratio of longissimus dorsi muscle increased in response to the oil supplementation. Administering canola oil to Araucano creole lambs during the finishing diet (150 mL/week) did not show significant differences in live weight gain, however, significantly increased intramuscular fat, monounsaturated fatty acid (MUFA) and PUFA content of longissimus thoracis muscle (Quiñones *et al*. 2019). On the other hand, inclusion of palm kernel oil in to the Santa Inês lambs diet up to 3.44% did not affect longissimus lumborum colour, chemical composition and fatty acids profile, but total CLA and C18:3 n-3 concentrations decreased as a result of increasing the level of palm kernel oil (Castro *et al*. 2022). However, to the best of our knowledge, very little information is available about the effects of PSO inclusion in diets on growth performance and carcass and meat quality of ruminants.

Taking into consideration all abovementioned premises, our hypothesis is that lambs finishing diet supplementation with different (2.0% or 4.0%) levels of pomegranate seed oil will favorably change meat quality attributes and most importantly fatty acids content of muscle and edible adipose tissues, without causing any disturbance in growth performance of lambs. Therefore, this study conducted to investigate the consequences of PSO supplementation on growth performance, carcass characteristics and quality attributes of longissimus thoracis (LT) muscle and also on fatty acid composition of subcutaneous fat in Sanjabi sheep as one of major sheep breeds in Iran.

MATERIALS AND METHODS

Animal management and experimental design

Twenty-one male Sanjabi lambs (3 month-old; BW=27.5±2.6 kg) from single births were used in the experiment. Before starting the experiment, lambs were dewormed by dosing with Dieverm (Albendazole 2.5%, Damloran Pharmaceutical Co., Borujerd, Iran) and vaccinated subcutaneously against enterotoxaemia (Razi Institute, Hesarak, Karaj, Iran). The lambs received similar diet for one week, then were weighed and randomly distributed to individually pens (150×90 cm^2 with soil bed, a door in the front and equipped with movable feeder) and each pen was randomly assigned to one of three experimental diets based on a completely randomized design. After two weeks of nutritional adaptation period, the lambs fed a totally mixed diets in pelleted form (0.5 cm diameter) twice a day (08 00 h and 18 00 h), for more 90 days. PSO was added to the component of respective totally mixed diets and combined by horizontal mixer before pelleting. Distributed feed and its residue were weighed daily for each replicate to measure

feed intake. To ensure *ad libitum* consumption, the amount of daily distributed feed was adjusted to ensure 5 to 10% residue and clean water was always available. Individual lamb weights were recorded every 14 days after 14 h feed withdrawal. Commercially available cold pressed unrefined oil from seeds of pomegranate fruits were purchased from the local market. The oils were stored at 8°C before formulation of experimental diets and administration to lambs (Białek *et al*. 2018). Fatty acid profile of purchased PSO is shown in Table 1. It is common practice in nutrition of ruminants to provide no more than 6-7% of lipids in the diet, thus to make three experimental diets and at the same time comply with this limitation, PSO was supplemented into the concentrate at 0, 2, and 4% of DM levels, so that the ether extract level in the experimental rations reached 2.5, 4.4 and 6.4% of DM, respectively (Table 2). With respect to the recommendations of the NRC (2007), the metabolisable energy (ME) and crude protein (CP) contents of all diets were adjusted as 2.6 Mcal/kg DM and 14.7% DM of diets and forage to concentrate ratio in the control diet was 35 to 65 (diet DM basis).

Slaughter and carcass parameters

After 12 hours feed withdrawal with free access to water, lambs were transported to the slaughterhouse and pre-slaughter live weight was recorded. Following slaughter and after removal of non-carcass components (including kidney and abdominal fat depots), empty body weight, and the HCW were measured. Carcasses were kept at 1-4 ˚C for 24 h and CCW were recorded. Chilling loss was calculated by counting the difference between HCW and CCW for each carcass, as a proportion of HCW. The ratio of hot carcass weight to live and empty body weights were recorded as dressing percentage.

The clod carcasses were cut into different anatomical regions (neck, ribs, loin, foreshank, brisket, flank, long leg, and fat tail) and the weight of each region was recorded (Ezatpoor, 1998). The carcass fat depth over the midpoint of LT muscle at the 12th rib was measured. Fat thickness was assessed at three sites on the location. The LT muscle depth (B), width (A), and area were measured on the cut surface of the LT muscle at the 12th rib. Enough samples of LT muscle and subcutaneous fat were packed under vacuum condition and frozen at −20 ˚C until ensuing determination of meat quality attributes.

Analysis of meat quality

Meat quality attributes were examined in LT muscle. To measure cooking loss (%), samples of LT muscle were cooked in a water bath, which was already hot, at 75 ˚C for 60 min as described by Hoffman *et al*. (2003).

Afterward, the same cooked samples were used to determine shear force value by implementation the steps described by Ekiz *et al.* (2010). A Testometric machine (Model M350-10CT, England) equipped with a Warner Bratzler (WB) shear force apparatus was used to determine shear force value and the average of three sub-samples was recorded as WB shear force value of that sample.

Meat colour on the LT muscle was determined after thawing muscle samples for 48 h at 4 ˚C and considering enough time for blooming at the exposure to the atmosphere (1 h at 21 ˚C). Coordinates a*, b* and L* (CIE, 1986) were measured by a previously calibrated Hunter Lab colorimeter (Konica Minolta Company, Colorimeter model CR-310, Japan), on the cut and fat-free surface of samples with approximately 2.5 cm thickness. Colour measurement repeated three times for each sample and the average of relevant measurements reported as colour coordinate value. Chroma or colour saturation and hue angle indicators were calculated using equations $(a^{*2}+b^{*2})^{1/2}$ and $\tan^{-1}(b^*/a^*)$, respectively (Hunter and Harold, 1987).

Proximate compositions

Moisture, crude protein, fat and ash content of external fat-free LT muscle samples were determined using AOAC (2016) procedures, followed by 24 h thawing samples at 4 ˚C and homogenization.

Analysis of fatty acid

To determine the fatty acid profiles of subcutaneous fat and LT muscle (without any visible connective tissue and external fat) homogenized samples, total lipids extract of each was obtained using a solvent consisting of 2:1 chloroform: methanol (Folch *et al*. 1957). Extracted lipids was dried under N_2 and esterifying and methylation of fatty acids was done using 14% boric trifluoride in methanol, as described method by Metcalfe and Schmitz (1961). Separation and quantifying of the different fatty acids were performed by using a gas chromatography apparatus (Yung lin 6300, South Korea) outfitted with a flame ionization detector and a Cp-Sil 88 fused silica capillary column (length 100 m, 0.25 µm film thickness and 0.25 mm internal diameter, South Korea). Prior to saponification, nonadecanoic acid (19:0, 99%, Sigma, St. Louis, MO, USA) was added as an internal standard.

Helium with a constant flow (1.5 mL/min) was used as carrier gas. The initial oven temperature was 120 ˚C for 5 min, thereafter increased by 2 ˚C/min to 170 ˚C and held for 15 min and increased by 5 ˚C/min to 200 ˚C held for 5 min, and finally increased by 2 ˚C/min to 325 ˚C held for 10 min. The temperatures of injector and detector were held constant at 250 and 300 ˚C, respectively.

Table 1 Fatty acid profile of pomegranate seed oil

Fatty acid	Amount (% of total fatty acids)
C 14:0	0.42
C 16:0	4.85
C 16:1	0.14
C 18:0	3.86
C 18:1 n-9	10.40
C 18:2 n-6	7.38
C 18:3 n-3	0.22
CLnA	72.56
C 20:0	0.17
SFA	9.13
MUFA	10.54
PUFA	80.16
n-3PUFA	72.78
n-6PUFA	7.38
n-6/n-3	0.10

SFA: saturated fatty acids (sum of C14:0+C16:0+C18:0+C20:0); MUFA: monounsaturated fatty acids (sum of C16:1+C18:1n-9); PUFA: polyunsaturated fatty acids (sum of C18:2n-6+C18:3n-3+CLNA) and CLnA: conjugated linolenic acid.

Table 2 Ingredient and chemical composition of experimental diets

Ingredient (% of DM)	Diets		
	0% PSO	2% PSO	4% PSO
Alfalfa hay	35.0	37.0	40.7
Barley grain	26.0	24.0	21.0
Corn grain	21.4	19.0	17.0
Soybean meal	9.1	9.5	9.8
Sugar beet molasses	7.0	7.0	6.0
Mineral-vitamin supplement[1]	1.0	1.0	1.0
Pomegranate seed oil	0.0	2.0	4.0
salt	0.5	0.5	0.5
Chemical composition[2]			
Organic matter (%)	92.1	93.2	93.2
Crude protein (% of DM)	14.7	14.7	14.7
Ether extract (% of DM)	2.5	4.4	6.4
Neutral detergent fiber (% of DM)	25.2	25.7	25.4
Metabolizable energy (Mcal/kg DM)	2.6	2.6	2.7

PSO: pomegranate seed oil.
[1] Containing per kg DM: Calcium: 180 g; Phosphor: 70 g; Magnesium: 30 g; Sodium: 50 g; Manganese: 5000 mg; Iron: 4000 mg; Copper: 300 mg; Iodine: 100 mg; Cobalt: 100 mg; Zinc: 3000 mg; Selenium: 20 mg; Antioxidant: 400 mg; vitamin A: 400000 IU; vitamin D_3: 100,000 IU and vitamin E: 200 IU.
[2] Dry matter (DM), crude protein (CP), ether extract, neutral detergent fiber (NDF) and ash analyzed based on AOAC (2016) methods. Metabolizable energy calculated based on NRC tabular values of ingredients (NRC, 2007).

Peaks of different fatty acids were recognized by comparing retention times with those of their conforming standards (G004263, 37 Component FAME Mix, and cis/trans C18:1, C18:2, and C18:3 FAME isomers on SP-2560, Sigma, St. Louis, MO, USA).

Statistical analysis

These study arranged based on a completely randomized design with three treatments and data were analyzed using a GLM procedure of SAS (2004). Covariate fitted included initial body weight for growth performance traits and HCW for carcass data. Least-square means were compared by Duncan's multiple range tests and significant differences were declared at $P < 0.05$. The following model was used:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:
Y_{ij}: observation value.
μ: population mean.
T_i: effect of treatments.
e_{ij}: experimental error.

RESULTS AND DISCUSSION

FA composition of PSO is shown in Table 1. Content of SFAs was 9.13% of total fatty acids which is in the range reported for eight samples of PSO (15.6% in average) by Białek *et al.* (2021b).

MUFAs accounted for about 10.54% of the total detected fatty acids in PSO which oleic acid was by far the most abundant one. Similarly, Białek *et al*. (2021b) reported that oleic acid was the predominant component of MUFAs in various PSO samples purchased from different suppliers. As expected, PUFAs included the majority of PSO and CLnA by 72.56% of total detected fatty acids was the predominant class in this category. In consistence with our findings, a study of four cultivars of Iranian PSO showed that 7.7% to 8.1% of the fatty acids are saturated and 91.8% to 92.1% unsaturated (Dadashi *et al*. 2013).

Supplementing the diet with 2 and 4% PSO had no significant effect on average daily gain (ADG) and feed conversion ratio (FCR), but caused a significant increase in dry matter intake (DMI; P<0.05; Table 3).

In many researches, oil inclusion in lamb finishing diets had no significant effect on growth performance parameters, including DMI, ADG and feed efficiency (Radunz *et al*. 2009; Sánchez *et al*. 2018; Quiñones *et al*. 2019; Vicente *et al*. 2020; Gao *et al*. 2022). However, Rizzi *et al*. (2002) observed that by increasing the level of ether extract from extruded soybeans and sunflower seeds in diet, DMI and ADG of lambs increased significantly (P<0.05). Kott *et al*. (2003) found no change in DMI as a result of using safflower seeds in diet composition, but ADG and gain:feed slightly increased by inclusion more safflower seeds in diet to increase the level of dietary oil up to 6%. Dutta *et al*. (2008) also stated a quantized raise in ADG when supplemented fat up to 50 g/kg, but, further increase of dietary fat caused growth retardation. In contrast, lambs finishing (high-concentrate) diet supplementation with oil was coordinate with significant reduction (P<0.05) in DMI (Francisco *et al*. 2015) and also significant reduction (P<0.05) in BWG (Bessa *et al*. 2008).

Under the absence of any restriction on net energy consumption, continues inclusion of lipids to the diet will lead to an expected reduction in DMI (Palmquist, 1994). Various factors may be involved in some conflicting results obtained in different studies, such as animal related factors (breed and growth phase), basal diet composition, and the nature and level of lipid supplementation (Francisco *et al*. 2015).

The carcass characteristics of the lambs including HCW, CCW, chilling loss percentage, subcutaneous fat depth, internal fat depots weight (kidney and omental), and LT depth, width and area were not affected by oil supplementation (P>0.05; Table 3). Similar studies in lambs (Kott *et al*. 2003; Boles *et al*. 2005; Dutta *et al*. 2008; Bhatt *et al*. 2011; Francisco *et al*. 2015) also mentioned that oil inclusion in high concentrate diets had no effect on HCW, CCW, percent of chilling loss, kidney fat weight, fat thickness and longissimus area.

Contrary to these findings, some researchers observed higher fat accumulation in carcass and increased carcass yield (Clinquart *et al*. 1995) as a result of fat supplementation into the fattening diet. Indeed, Solomon *et al*. (1992) and Lough *et al*. (1994) reported a significant decrease in carcass muscle proportion, instead of more fat deposition, by inclusion 10% lipid to the sheep's diets. In other species, on the other hand, Engle *et al*. (2000) and Najafi *et al*. (2012) stated that dietary linoleic acid decreased kidney and pelvic fat percentage in Angus steers and Mahabadi goat kids, respectively. This discrepancy may be attributed to the difference in energy intake, because fat accumulation is more related to energy intake than dietary energy concentration (Solomon *et al*. 1992).

The carcass cut weights of lambs were not changed by the oil supplementation at 2 and 4% (P>0.05; Table 3). This result is in agreement with those found in lambs (Boles *et al*. 2005) and kids (Najafi *et al*. 2012) that states various oils supplementation had no significant effects on weights or percentage of carcass cuts. In general, the lack of oil inclusion effect on the weight of commercial cuts was not unexpected, referring to its inability to make any significant difference in lamb's performance and carcass characteristics.

In the present study, PSO inclusion in lamb diet did not make any significant change in dry matter, protein and ash contents of LT muscle (P>0.05; Table 4); although feeding PSO in 4% level caused significantly (P<0.05) higher ether extract percentage in this muscle. Similar findings as a significant increase (P<0.05) in fat content, without any significant change in moisture, protein and ash content of Rambouillet lambs meat as a result of inclusion 50 g soybean oil in the diet are reported (Vicente *et al*. 2020). Total average of moisture, protein, fat and ash in Sanjabi lambs were 71.2, 20.6, 5.7 and 1.0 %, respectively, which are in the range of values reported for sheep meat (Vasta *et al*. 2007; Madruga *et al*. 2008). Bhatt *et al*. (2011) observed coconut oil inclusion in lamb diets did not alter chemical composition in LT muscle which in the case of fat content is in contrast to our finding. Studies on goats also reported lack of differences in moisture, ash, ether extract and protein contents in the longissimus muscle in response to supplementation various vegetable oils (Marinova *et al*. 2001; Najafi *et al*. 2012). These authors proposed that lack of differences may have been due to scrimpy oil supplementation.

Table 3 Performance and carcass traits of Sanjabi lambs received different levels of pomegranate seed oil (PSO)

Performance and carcass traits	PSO (%)			SEM	Significance
	0	2	4		
Initial body weight (kg)	27.5	27.5	27.6	0.57	NS
Final body weight (kg)	47.0	48.8	46.8	0.98	NS
Average daily feed intake (g/day)	1526^{b}	1581^{a}	1585^{a}	25.8	*
Average daily gain (g/day)	235	256	240	5.5	NS
Feed conversion ratio	6.5	6.2	6.6	0.15	NS
Empty body weight (kg)	41.4	44.5	41.6	0.92	NS
Hot carcass weight (kg)	23.7	25.2	24.0	0.56	NS
Cold carcass weight (kg)	23.1	24.6	23.4	0.55	NS
Chilling loss (%)	2.5	2.1	2.3	0.08	NS
Subcutaneous fat depth (mm)	3.9	3.9	3.1	0.31	NS
Kidney fat (g)	98.2	85.8	77.3	8.27	NS
Omental fat (g)	484.5	447.0	539.2	24.49	NS
Longissimus thoracis depth (mm)	31.4	29.7	29.3	0.85	NS
Longissimus thoracis width (mm)	56.1	56.5	55.0	0.83	NS
Longissimus thoracis area (cm^2)	14.1	13.9	13.2	0.42	NS
Carcass cuts (kg)					
Neck	2.1	2.3	2.0	0.04	NS
Fore shank	0.9	0.9	0.8	0.03	NS
Ribs	2.1	2.1	2.2	0.16	NS
Loin	2.1	2.1	2.2	0.10	NS
Long leg	6.0	6.0	5.8	0.16	NS
Brisket	0.7	0.7	0.8	0.02	NS
Flank	1.0	1.2	1.1	0.04	NS
Fat tail	4.9	6.0	5.4	0.35	NS

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05).
SEM: standard error of the means.
NS: non significant.

Table 4 Proximate composition of the longissimus thoracis muscle from Sanjabi lambs received different levels of pomegranate seed oil

On wet weight basis, g/100 g	PSO (%)			SEM	Significance
	0	2	4		
Moisture	73.0	72.0	68.3	0.93	NS
Protein	20.6	21.8	19.5	0.53	NS
Fat	3.2^{b}	3.8^{b}	10.1^{a}	1.40	*
Ash	0.9	1.0	0.9	0.03	NS

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05).
SEM: standard error of the means.
NS: non significant.

Table 5 presents the mean values of WB shear force and color indexes. In the present study, WB shear force and cooking loss in LT muscle were not affected by oil supplementation; however, the cooking loss percentage obtained for lambs in 2% oil supplemented group (37.79%) was slightly higher than lambs in control and 4% oil supplemented groups (34.80 and 30.96%, respectively; P>0.05).

No change in WB shear force and cooking loss values due to oil supplementation supports other findings in goat (Najafi *et al*. 2012) and lambs (Radunz *et al*. 2009; Francisco *et al*. 2015; Güney *et al*. 2021) where high-concentrate diets supplementation with unsaturated oil did not affect these qualities of muscles.

According to Webb *et al*. (2005), WB shear force value is more dependent on fat content of muscle; so that the higher fat content reduces fibrous protein compression in meat and decreases meat resistance to shearing. As shown in Table 4, in the current study intramuscular fat content in 4% oil supplemented group was dramatically higher than 2% oil supplemented and control groups, thus observing similar cooking loss and WB shear force among treatments was not expected.

There were some differences between treatments in muscle colour (Table 5). Longissimus thoracis L* value was significantly higher in lambs received 4% PSO than other 2 groups (P<0.05), possibly due to more intermuscular fat accumulation in this treatment. However, PSO supplementation did not affect a* and b* values (P>0.05).

Table 5 Meat quality parameters of the longissimus thoracis muscle from Sanjabi lambs received different levels of pomegranate seed oil

Item	PSO (%)			SEM	Significance
	0	2	4		
Cooking loss (%)	34.8	37.8	31.0	1.33	NS
Warner Bratzler shear force (kg) (N)	2.6 (25.5)	3.3 (32.4)	3.6 (35.3)	0.42	NS
L* value	39.4^{b}	39.1^{b}	43.2^{a}	0.82	*
a* value	12.5	13.2	15.5	0.96	NS
b* value	8.5	6.4	5.3	0.65	NS
Hue angle	34.4^{a}	26.8^{ab}	19.4^{b}	3.98	*
Chroma value	15.2	14.8	16.4	1.81	NS

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05).
SEM: standard error of the means.
NS: non significant.

Similarly, inclusion of soybean oil up to 50g/kg DM of lambs diet also caused a significant (P>0.05) increase in L* value of meat, while a* and b* values remained unchanged (Vicente *et al.* 2020). In the current study, the hue angle decreased linearly by increasing PSO supplementation level (P<0.05). Velasco *et al.* (2004) suggested that the value of 34 is the critical limit for meat lightness (L*), such a way that consumers usually esteem meats with lower L* values as dark. In the present study, the mean L* values in LT muscle were higher than the mentioned threshold (40.6), which indicates that this muscle was light-coloured and acceptable to consumers. In contrast to the findings of the current study, safflower oil (Boles *et al.* 2005), soybean oil (Bessa *et al.* 2008), soybean and linseed oils (Francisco *et al.* 2015), and oilseed oil (Güney *et al.* 2021) supplementation in lamb's diet or inclusion various fat sources in goats (Najafi *et al.* 2012) and bulls (Oliveira *et al.* 2012) diets did not alter meat colour indexes. Radunz *et al.* (2009), on the other hand, reported chops from lambs received diet with added oil had generally lower value of L* (P<0.001) in comparison to chops from lambs fed no supplemented oil.

Tables 6 and 7 show the fatty acid composition of LT muscle and subcutaneous fat by weight of total lipids, respectively. In general, PSO supplementation did not affect total percentages of SFA, MUFA and PUFA in intramuscular fat. Total PUFA content accounted for about 9.1% of the total fatty acids detected. The fatty acid 18:2n-6 (about 5.4% of total fatty acids detected) was the most abundant PUFAs in intramuscular fat. Using PSO caused a significant increase in the percentages of 14:1, trans-11 18:1, 15:2, 18:3n-3 and other 18:3 fatty acids in intramuscular fat (P<0.05). On the other hand, the proportions of 16:0 in LT muscle reduced by PSO supplementation and gained significantly lower value in 4% oil supplemented group compared to other treatments (P<0.05; Table 6). These results suggest that this oil could be added to the sheep diets at levels that eventuate dietary fat up to 6 % without any negative effect on growth performance and carcass characteristics.

Similarly, Santos-Silva *et al.* (2002) and Boles *et al.* (2005) stated significant decrease in 16:0 content of lamb's meat with inclusion of soybean and safflower oils, respectively. This observation is possibly because *de novo* synthesis of fatty acid extent and the continuity of elongation process is negatively responsive to the share of fatty acids with exogenous or dietary source in the metabolic pool (Santos-Silva *et al.* 2002), although, in the current study, 12:0 percentage was unexpectedly higher in 2% PSO supplemented group (P<0.05; Table 6). However, Scollan *et al.* (2001) observed that adding fish oil to ruminant's diets did not affect the percentage of 16:0 or 18:0 in triacylglycerol and phospholipids of meat which in the case of 16:0 is in conflict with our results. Conversely, Ponnampalam *et al.* (2001) mentioned that inclusion of fish oil in a forage based diet caused a significant reduction in 18:0 share in beef cattle muscle.

Fatty acid composition of animal products is the result not only of their biosynthesis in tissues, but also of the fatty acid composition of ingested lipids (Białek *et al.* 2017b). However, in ruminants the rate of ruminal biohydrogenation as well as the profile of occurring intermediates determine the amount of fatty acids incorporated into tissues (Białek *et al.* 2017a). The conversion of C18:1 isomers into stearic acid is a result of activity of group B ruminal bacteria, (for example *Butyrivibrio proteoclasticus*) for which elevated PUFA concentration is considered to be toxic (Buccioni *et al.* 2012).

Białek *et al.* (2018) findings revealed that incorporation of fish oil, reach source of long chain PUFA, into lambs rations slightly decreased amount of C18:0 in ruminal microbiota which may confirm the impaired activity of ruminal bacteria from group B. Ferreira *et al.* (2016) also reported that increasing level of fish oil incorporated into diet instead of soybean oil caused an increasing duodenal flow of trans-11 18:1 (vaccenic acid) and thus, the enhanced incorporation of this fatty acids in tissues, which is also confirmed by our findings.

Table 6 Fatty acid composition (percentage of total fatty acids) of longissimus thoracis muscle in Sanjabi lambs received different levels of pomegranate seed oil (PSO)

Fatty acids	PSO (%)			SEM	Significance
	0	2	4		
Total SFA[3]	50.52	49.82	47.77	1.203	NS
12:0	0.25[b]	0.73[a]	0.23[b]	0.046	***
13:0	0.22	0.21	0.13	0.035	NS
14:0	3.77	3.13	3.47	0.395	NS
15:0	0.40	0.40	0.30	0.102	NS
16:0	29.80[a]	27.70[a]	25.90[b]	0.528	**
17:0	0.77	0.95	1.13	0.169	NS
18:0	14.90	16.67	15.97	0.734	NS
20:0	0.20	0.30	0.30	0.058	NS
22:0	0.36[a]	0.24[b]	0.33[a]	0.025	*
Total MUFA[3]	42.80	39.78	42.47	1.113	NS
12:1	0.37	0.18	0.17	0.071	NS
14:1	0.13[b]	0.50[a]	0.33[ab]	0.067	*
15:1	0.22	0.13	0.40	0.079	NS
16:1	3.00	2.63	3.17	0.221	NS
17:1	1.13	1.03	0.90	0.159	NS
trans-11 18:1	1.55[b]	2.17[ab]	3.45[a]	0.342	*
cis-9 18:1	35.93	32.60	34.43	1.829	NS
Other 18:1	0.67[a]	0.32[c]	0.47[b]	0.038	**
20:1	0.32	0.27	0.30	0.046	NS
Total PUFA[3]	7.98	9.44	9.98	0.891	NS
15:2	0.20[b]	0.28[ab]	0.35[a]	0.026	*
17:2	1.05	0.73	0.80	0.184	NS
18:2 n-6	4.93	5.87	5.43	0.554	NS
cis-9, trans-11 CLA[3]	1.13	1.50	0.70	0.222	NS
trans-10, cis-12 CLA	0.25	0.33	0.37	0.056	NS
Other 18:2	1.06	0.56	1.07	0.157	NS
18:3 n-3	0.35[b]	1.10[a]	1.47[a]	0.144	**
n-6/n-3	3.07[a]	2.79[ab]	2.01[b]	0.373	**
Other 18:3	0.48[b]	0.83[a]	1.03[a]	0.060	**
Other UFA[3]	0.98	0.85	0.87	0.214	NS

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; CLA: conjugated linoleic acid and UFA: unsaturated fatty acids.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05); ** (P<0.01) and *** (P<0.001).
SEM: standard error of the means.
NS: non significant.

However, Białek *et al.* (2018) did not observe such dependencies in examined tissues of lambs as a results of incorporation of fish oil (10 g/kg) into rations of lambs instead of rapeseed oil.

As was the case for intramuscular fat, PSO supplementation did not affect the total percentages of SFA, MUFA and PUFA in subcutaneous fat (Table 7). Total PUFA compromised about 8.6% of total fatty acids detected in this fat depot and again 18:2n-6 was the most abundant PUFA. In the subcutaneous fat, the proportions of 13:0, 15:1 and 17:1 significantly reduced as a result of adding PSO to the diet (P<0.05), while the oil supplementation caused a significant increase in the proportions of *trans*-11 18:1, major CLA isomers (cis-9, trans-11 and trans-10, cis-12), 18:3n-3, and other 18:3 fatty acids (P<0.05; Table 7).

However, trans-10, cis-12 CLA and other 18:3 isomers experienced two steps significant changes, as proportion of these fatty acids first increased (P<0.05) by 2% PSO supplementation and then reduced (P<0.05) after increasing PSO supplementation level to 4% (Table 7).

In accordance with our findings, Radunz *et al*. (2009) detected no differences in the concentration of palmitic and stearic acids (as the most abundant saturated fatty acids) in lamb's adipose tissue and muscle in response to linseed and soybean oil supplementation (in a ratio of 2:1 and at 3% of diet). In general, results obtained from previous researches showed that answer of individual SFA concentration in muscle fatty acids pool to enrichment high-concentrate diets with oil does not follow necessarily the same pattern; nevertheless, in most studies unsaturated oil supplementation has resulted in no effect or has resulted in a slightly reduction in SFA concentrations of lamb muscle and adipose tissue (Radunz *et al*. 2009), which agrees with the results of current study.

Table 7 Fatty acid composition (percentage of total fatty acids) of subcutaneous fat in Sanjabi lambs received different levels of pomegranate seed oil (PSO)

Fatty acids	PSO (%)			SEM[1]	Significance[2]
	0	2	4		
Total SFA[3]	47.11	49.72	51.61	3.267	NS
10:0	0.33	0.15	0.32	0.098	NS
12:0	0.57	0.33	0.30	0.096	NS
13:0	0.69[a]	0.21[b]	0.23[b]	0.041	***
14:0	3.23	3.53	4.43	0.507	NS
15:0	0.97	0.70	0.63	0.227	NS
16:0	25.83	26.37	26.47	1.264	NS
17:0	1.30	1.17	0.77	0.350	NS
18:0	13.67	16.70	18.00	3.030	NS
20:0	0.40	0.30	0.30	0.081	NS
22:0	0.13	0.27	0.17	0.046	NS
Total MUFA[3]	45.78	43.42	40.58	3.171	NS
12:1	0.15	0.13	0.15	0.044	NS
14:1	1.80	1.27	0.83	0.309	NS
15:1	2.05[a]	0.20[b]	0.22[b]	0.062	***
16:1	3.70	3.23	2.63	0.621	NS
17:1	3.73[a]	1.63[b]	1.87[b]	0.299	**
trans-11 18:1[3]	1.02[b]	1.33[b]	3.23[a]	0.311	**
cis-9 18:1	33.17	34.70	30.23	2.724	NS
Other 18:1	0.73	0.72	1.23	0.328	NS
20:1	0.17	0.20	0.23	0.042	NS
Total PUFA[3]	7.47	8.81	8.45	0.698	NS
15:2	0.40	0.25	0.35	0.081	NS
17:2	0.63	0.85	0.87	0.294	NS
18:2 n-6	1.70	2.83	2.90	0.335	NS
cis-9, trans-11 CLA[3]	0.10[b]	0.40[ab]	0.50[a]	0.101	*
trans-10, cis-12 CLA	0.20[c]	0.71[a]	0.38[b]	0.025	***
Other 18:2	0.70	1.30	1.33	0.263	NS
18:3 n-3	0.37[b]	1.10[a]	1.03[a]	0.159	**
n-6/n-3	2.01[a]	0.90[b]	0.90[b]	0.239	**
Other 18:3	0.47[c]	1.43[a]	1.10[b]	0.062	**
Other UFA[3]	4.03[a]	0.92[b]	1.33[b]	0.342	**

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; CLA: conjugated linoleic acid and UFA: unsaturated fatty acids.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05); ** (P<0.01) and *** (P<0.001).
SEM: standard error of the means.
NS: non significant.

Similar to our results, in other researches, the effect of adding some vegetable oils on the amount of SFA in lamb's muscle or adipose tissues was not significant (Boles *et al.* 2005; Bessa *et al.* 2008; Vicente *et al.* 2020; Güney *et al.* 2021). In the current study, the content of dominant isomer of CLA (cis-9, trans-11 isomer) in muscle remained unchanged among treatments (P>0.05), while its primary precursor, trans-11 18:1, increased significantly in 2 step by PSO supplementation (P<0.05, Table 7). It seems that the conversion of trans-11 18:1 to CLA, as a results of Δ^9 desaturase activity, may be reduced in response to increasing PUFA absorption through the diet, as its considered probable by other researchers (Santora *et al.* 2000; Bolte *et al.* 2002). The effect of adding oils on cis-9, trans-11 CLA concentration in studies was different.

Some previous studies in lamb (Radunz *et al.* 2009) and beef (Engle *et al.* 2000; Beaulieu *et al.* 2002) have also reported that supplementing diets with different vegetable oils could not make a significant change in muscle cis-9, trans-11 CLA concentration. In verification of these findings, Kucuk *et al.* (2004) stated that using different levels of soybean oil in lambs' diet, up to 9.4%, did not affect duodenal entry rate and extent of cis-9, trans-11 CLA. Conversely, however, some evidences support that enrichment high-concentrate diets of lamb (Kott *et al.* 2003; Bessa *et al.* 2005) and beef (Gillis *et al.* 2004) with unsaturated oils has increased cis-9, trans-11 CLA concentration in muscle and milk. Białek *et al.* (2018) also reported a significant increase in cis-9, trans-11 CLA content of longissimus dorsi muscle of lambs as a results of partial (one third) replace-

ment of rapeseed oil by fish oil (with higher content of MUFA and PUFA with more than 18 carbons, especially eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid) in the diet for 35 d.

Regardless of the significance level, the content of *trans*-10, *cis*-12 isomer of CLA, was higher (P<0.05) in muscle and subcutaneous fat from lambs supplemented with PSO. In the few studies reporting this isomer of CLA, higher trans-10, cis-12 CLA concentration in intramuscular fat (Bolte *et al*. 2002; Boles *et al*. 2005) and other adipose tissues (Bolte *et al*. 2002; Radunz *et al*. 2009) were observed as a results of safflower oil supplementation in lambs finishing diets. Moreover, using soybean oil in high-concentrate diet composition increased trans-10, cis-12 CLA flow to duodenum of lambs (Kucuk *et al*. 2004). Milk and adipose tissue of cows received a grain diet have also a greater trans-10, cis-12 CLA concentration (Griinari and Bauman, 1999).

Closer look at the results (Tables 6 and 7) shows slightly higher (P>0.05) proportion of total PUFA in PSO supplemented group fat depots compared to lambs in control group (on average 9.36 *vs*. 7.72, respectively). This result along with the lower n-6/n-3 ratio in all examined fat depots of lambs receiving PSO compared to control group (on average 9.3 *vs*. 3.6, respectively) represent the shift of fatty acid composition towards the ideal state for human health due to PSO supplementation. In some other studies, supplementation lamb's diets with safflower or sunflower seeds and oil (Bolte *et al*. 2002; Rizzi *et al*. 2002; Kott *et al*. 2003; Boles *et al*. 2005) or linseed and soybean oil in the ratio of 2:1, respectively (Radunz *et al*. 2009) also led to an increase in muscle total PUFA content. However, PSO supplementation slightly decreased total MUFA in intramuscular and subcutaneous fats (P>0.05), which manifested mainly in the form of a greater reduction of oleic acid (cis-9 18:1) against the lower rise in 18:1 trans-11. Reduction of cis-9 18:1 in muscle following fed lambs with unsaturated oils is reported by some other researchers (Bolte *et al*. 2002; Boles *et al*. 2005; Bessa *et al*. 2008). Polyunsaturated fatty acids, especially 18:3 fatty acids, consist the main fraction of pomegranate seed oil. Generally, PUFAs undergo biohydrogenation to a large extent in the rumen, however, if absorbed postruminally in intact form, they will be used as essential precursors to longer chain PUFA (Radunz *et al*. 2009). The duodenal flow of these fatty acids could be increased by increasing oilseeds percentage in cattle and lamb diets (Kucuk *et al*. 2004; Scholljegerdes *et al*. 2004).

In the both fat tissues studied, the most abundant fatty acids, in descending order, were oleic (cis-9 18:1), palmitic (16:0) and stearic (18:0) with overall average of 34.3, 27.8 and 15.8% in intramuscular and 32.7, 26.2 and 16.1% in subcutaneous fat, respectively. The mean percentage of MUFA was 41.7% in intramuscular fat, whereas it increased to 43.4% in subcutaneous fat. The reverse trend was observed for PUFA percent, where this percentage was decreased from 9.1% in intramuscular fat to 8.2% in subcutaneous fat. In regard to this difference, Baik *et al*. (2014) reported that intramuscular fat depot has smaller cells than other fat depots. These authors suggested that diverse adipose tissues have obvious differences in the time of hypertrophy and hyperplasia occurrence which makes their fat cell size different from each other (Eguinoa *et al*. 2003). In addition, Smith and Crouse (1984) stated that adipogenesis and lipogenesis, metabolic activities that lead to triglyceride accumulation, occur at slower rates in intramuscular fat cells compared to other adipocytes and Baik *et al*. (2014) reported that among various fat tissues, intramuscular fat has the lowest levels of seven lipid metabolic genes. The net result of these findings is higher proportion of polar lipids in intramuscular fat cells.

CONCLUSION

Inclusion of pomegranate seed oil as a PUFA source in high-concentrate lamb diets had minor effects on meat physical attributes. However, muscle fat content and total 18:3 detected isomers levels in the muscle along with some CLA isomers and total 18:3 fatty acids in subcutaneous fat increased in response to diet supplementation with this source, without any undesirable effects on growth performance or carcass traits. The observed changes in fatty acid profile of edible tissues are towards the ideal state of meat and fat quality for human health.

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