Veterinary Medicine and Science

WILEY

ORIGINAL ARTICLE OPEN ACCESS

Ruminants

The Effect of Dietary Propylene Glycol on the Fatty Acid Composition of Three Fat Depots in Male Akkaraman Lambs

Cafer Tayyar Ateş[1] | Ufuk Kaya[2] | İrem Karaaslan[3] | Hüseyin Özkan[4] | Akın Yakan[4]

[1]Faculty of Veterinary Medicine, Department of Animal Breeding, Hatay Mustafa Kemal University, Hatay, Türkiye | [2]Faculty of Veterinary Medicine, Department of Biostatistics, Hatay Mustafa Kemal University, Hatay, Türkiye | [3]Technology and Research & Development Application Center (MARGEM), Hatay Mustafa Kemal University, Hatay, Türkiye | [4]Faculty of Veterinary Medicine, Department of Genetics, Hatay Mustafa Kemal University, Hatay, Türkiye

Correspondence: Akın Yakan (yakan@mku.edu.tr)

Received: 17 October 2024 | **Revised:** 9 December 2024 | **Accepted:** 30 December 2024

Funding: This study was financially supported by the Scientific and Technological Research Council of Türkiye (TUBİTAK) via project number 119R076.

Keywords: anatomical depot fat location | fatty acid | lamb | propylene glycol

ABSTRACT

This study tested the effects of propylene glycol (PG) on the fatty acid composition of Akkaraman lambs in three different anatomical depot locations (ADLs). Twenty-four lambs were assigned to a randomized complete block design comprising three groups of 8 animals as follows: Con, 1.5%, body weight $(BW)^{0.75}$ (PG1.5) and 3% $BW^{0.75}$ supplemental PG. The animals were slaughtered 90 days after the commencement of feeding. Tail, perirenal and back fat were collected, and their fatty acid compositions were analysed. PG was associated with lower levels of capric acid (C10:0) and lauric acid (C12:0), and higher levels of arachidic acid (C20:0), D-ɣ-linolenic acid (C20:3n6), behenic acid (C22:0), docosadienoic acid (C22:2n6), tyricosylic acid (C23:0) and eicosapentaenoic acid (C20:5n3; $p < 0.05$, $p < 0.01$ and $p < 0.001$). The ADLs differed for all fatty acids except C12:0 and C14:0 (myristic acid). Perirenal fat had the highest SFA levels, while n6/n3 was higher in tail fat than in fat from the other ADLs ($p < 0.001$). The high correlation of Δ9 C16 and Δ9 C18 index values with other sum and index values indicates that desaturation enzyme activity was elevated in the lambs' depot fats ($p < 0.05$, $p < 0.01$ and $p < 0.001$). This suggests that perirenal fats have less favourable fatty acid compositions than the other ADLs.

1 | Introduction

Demand for red meat production has increased due to population growth and increasing welfare levels. This demand is not only quantitative rather people have also become more attentive to meat quality. Consequently, improving meat quality has become an important goal alongside enhancing fattening performance in red meat production (de Lima Valença et al. 2020). One of the most important quality parameters in meat is fatty acid composition, which may be affected by diet or feed supplementation (Alshamiry et al. 2023). While many feed additives and finishing are used to maximize quality, an effective method has not yet been found to replace conventional finishing systems.

Propylene glycol (PG) is used to protect lactating cows from a negative energy balance and treat ketosis. Orally ingested PG increases ruminal volatile fatty acids, especially propionic acid by at least two and three times (Trabue et al. 2007). These increased volatile fatty acids are transferred directly to the liver via the rumino-hepatic cycle and enter gluconeogenesis, leading to glucose formation (Santos et al. 2018; Green 2020). Glucose in the liver may affect fatty acid composition due to its effect on fatty acid synthesis. PG changes the fatty acid composition of intramuscular fat in Akkaraman lambs (Yakan et al. 2024).

In the organism, fatty acid mobilization from metabolically active tissues to adipose tissue is facilitated. Adipocytes in the adipose

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2025 The Author(s). *Veterinary Medicine and Science* published by John Wiley & Sons Ltd.

tissue not only synthesize and store fatty acids but also release free fatty acids when needed, thereby regulating metabolism (Urrutia et al. 2020). Fatty acid composition in diet plays a crucial role in human nutrition. Saturated fatty acids (SFAs) are considered undesirable, whereas polyunsaturated fatty acids (PUFAs) are recommended for inclusion in the diet. PUFAs have many positive roles such as cardiovascular health and immune regulation are important components of the diet for sustainable health (Kapoor et al. 2021). Most fats consumed by humans from meat are depot fats in the carcass (Schumacher et al. 2022). While the fat ratio in lamb carcasses can vary between 10% and 35% depending on factors like age, sex, breed and slaughter weight, the fat content in meat ranges from 1% to 5% (Yakan and Ünal 2010; Lanza et al. 2011). Fatty acid composition can vary between species and breeds and across different parts of the same animal (Kumar et al. 2022).

During the first 4 months of rapid growth in ruminants, the rate of meat production is high, whereas the amount of stored fat is low. From 4 to 5 months of age, however, depot fat accumulation increases as growth slows. Consequently, depot fat rises in line with increasing carcass weight (Gascoigne and Lovatt 2015). The average lamb carcass weight in Türkiye was approximately 23 kg in 2023 (TUIK 2024), which indicates that these carcasses had significant depot fat accumulation. While both inter-muscular and intra-muscular fats are consumed with red meat, depot fats are consumed in much greater quantities. Considering that approximately 10%–35% of a lamb carcass is storage fat, the importance of depot fat consumption becomes apparent. In fat-tailed sheep breeds, tail fat is a significant depot fat that is eaten by humans, while another important source of depot fat is back fat.

The effect of PG on fatty acid composition in ADLs is thought to be related to its stimulation of fatty acid synthesis enzymes (fatty acid synthase and acetyl-CoA carboxylase 1). The different lipogenic enzyme activity in different adipose tissues may differ in the utilization of PG as an energy source. Kong et al. (2022) reported that fatty acids will be found as phospholipids or triglycerides in the structure of adipocytes. Visceral adipocytes have triglyceride structure due to their large volume and dysfunctional nature, while subcutaneous adipocytes have a small volume and phospholipid structure. Their phospholipid structure causes them to have unsaturated fatty acid structure (Ahmad et al. 2020). For these reasons, both visceral adipocytes (perirenal fat) and subcutaneous adipocytes (back fat and tail fat) were selected as materials for fatty acid composition in this study.

Zhang et al. (2020) reported that PG reduces the storage of triglycerides in the liver, causing them to accumulate as storage fat in different anatomical regions of the body. The process of PG participation in phospholipid or triglyceride biosynthesis in storage fat also suggests that the fatty acid composition in the relevant fat region may be different (Kong et al. 2022). Therefore, it was thought that supplemental PG during fattening in lambs has the potential to alter the fatty acid composition in ADLs. Accordingly, the present study investigated the efficacy of different PG doses on fatty acid composition in Akkaraman lambs across several ADLs.

2 | Materials and Methods

2.1 | Animals and Study Design

The animal experiment for this study was conducted at the Small Ruminant Research Unit licensed by Erciyes University Agricultural Research and Application Center (ERUTAM, Kayseri, Türkiye) and the Ministry of Agriculture and Forestry, in accordance with the regulations on the use of experimental animals.

The animal material consisted of male Akkaraman lambs weaned between 2 and 3 months of age and 19.87 ± 0.73 (Con), 20.29 ± 0.71 (PG1.5) and 20.49 ± 0.53 (PG3) kg live weight. The lambs were fed ad libitum with standard lamb finishing feed (10.88 MJ/kg ME and 16% crude protein) and 100 g/head/day of dry hay as roughage. Their access to water was unrestricted. The following three groups were formed: a dose group given PG1.5 mL/kg $BW^{0.75}$/day (PG1.5; $n = 8$), a dose group given PG 3 mL/kg $BW^{0.75}$/day (PG3; $n = 8$) and a control group (Con; $n = 8$) not given PG. In the PG1.5 group, 23.85 mL PG was used daily for a 40 kg lamb, while 47.70 mL PG was presented to lamb weighed 40 kg in PG3 group. The lambs were given PG orally every morning at 08:00 AM using an oral injector. Water was given similarly to the Con group. The dose was calculated according to Kim, Choi, and Myung (2005). After weekly weighing, 1.5 (PG1.5) and 3 mL (PG3) PG doses were calculated for each lamb's kg metabolic body weight. The lambs were subjected to group feeding and housed in six pens, with four lambs per pen, that is, each group had two pens. Each pen's area was 6.6 m^2, comprising 3 m^2 enclosed and 3.6 m^2 open space, providing the lambs with free access to either kind of space.

Following feeding for 90 days, the lambs were slaughtered at 48.28 ± 1.81 (Con), 50.84 ± 1.15 (PG1.5) and 52.48 ± 0.87 (PG3) kg live weight. After slaughter, fat samples of at least 10 g were collected from the following three anatomical storage fat areas: tail, perirenal and back fat. The tail fat was sampled from the centre of the tail, while the back fat was collected from the subcutaneous adipose tissue located between the last thoracic vertebra and the first lumbar vertebra. The samples were stored at −20°C until the analyses.

2.2 | Fatty Acid Analyses

Fat was extracted using the modified Soxhlet method (Horwitz and Latimer 2005). Approximately 5 g of thawed fat was homogenized in 30 mL diethyl ether (purity ≥ 99.9, product no: 309966, Merck, USA) with ultra-turrax (HG-15A, Daihan Sci, Korea) under ice, shaken (SHO-2D, Daihan Sci, Korea) for 15 min and then filtered through Whatman paper (No. 1) in a vacuum. The diethyl ether of the filtrate was evaporated in a rotary evaporator (RE100-Pro, DLAB, China) and fat extraction is completed. Approximately 100 µL of extracted fat was saponified with 2 mL of 0.5 N methanolic NaOH (product no: 567530, Sigma-Aldrich, USA) for 2 min at 90°C. After cooling, 5 mL of boron tri fluoride (product no: B1252, Sigma-Aldrich), 35% in methanol (purity ≥ 99.9, product no: 34860, Sigma-Aldrich), was added and the sample was heated for another 5 min at 90°C. The reaction

tubes were then cooled, 2 mL of *n*-Heptan (purity ≥ 99.9, product no: 34873, Sigma-Aldrich) and 3 mL of saturated NaCl solution (purity ≥ 99.0, product no: S1679, Sigma-Aldrich) were added and mixed for 30 s; further time was allocated to separate the organic phase. The fatty acid methyl esters (FAME) were collected from the top layer, transferred into a vial and kept in the freezer at −20°C until the GC-MS analysis (Yakan et al. 2016). The fatty acids were separated using a Shimadzu GCMS-QP2020NX model GC-MS equipped with a Restek fused silica capillary column (100 m length, 0.25 mm i.d. × 0.20 mm film). The injector, MS detector interface and ion source temperatures were set to 240°C, 270°C and 200°C, respectively. The split was 1:100, and the total injection volume was 1 μL. The oven temperature was programmed to 100°C for 4 min before being ramped to 240°C at a ramp rate of 5°C/min. The total chromatogram time was 60 min. The peaks obtained from the chromatogram were identified using the MS library and confirmed with FAME Mix 37 (Supelco, Merck, USA) as a reference standard to verify the detected fatty acids. Each fatty acid was quantified as a percentage of the total FAME analysed. Helium was used as the carrier gas (flow rate: 1 mL/min; Yakan et al. 2016).

2.3 | Statistical Analysis

Statistical analyses were performed using Stata 15.1 statistical software (StataCorp, College Station, TX, USA). Descriptive statistics for each variable were calculated and presented as mean ± standard error of mean (SEM). The normality of all variables was determined using the D'Agostino–Pearson test. Fatty acid composition and some fatty acid indexes were analysed using a linear mixed model in a nested design. The effects of the PG groups and ADLs as well as their two-way interactions on these parameters were examined using the following model:

$$Y_{ijkl} = \mu + PG_i + ADLs_j + (PG \times ADLs)_{ij} + L_{k(ij)} + e_{ijkl}$$

where Y_{ijkl} are the dependent variables; μ is the overall mean; PG_i is the effect of PG ($i = 3$ classes; Con, PG1.5 and PG3); $ADLs_j$ is the effect of ADLs ($j = 3$ classes; tail, perirenal and back fat); $(PG \times ADLs)_{ij}$ is the interaction between PG_i and $ADLs_j$; $L_{k(ij)}$ is the random effect of the lambs nested within (PG x ADLs)$_{ij}$ and e_{ijkl} is the random error.

In this model, the lambs were assessed as a random effect, while the PG groups, ADLs and interaction term were assessed as the fixed effect. The variance components were used as the covariance structure in the established model to minimize the Akaike information criterion (AIC). When a significant difference was revealed in the model, any significant terms were compared using a simple effect analysis with Bonferroni adjustment for multiple comparisons. The correlation between fatty acids and fatty acid indexes was determined separately in each group using Pearson correlation coefficients and visualized as a heatmap correlogram. $p < 0.05$ was considered significant in all analyses.

3 | Results

A total of 28 fatty acids (10 SFA, 8 MUFA and 10 PUFA) were determined in all the groups by analysing the fatty acid composition of the tail, perirenal and back fat (Table 1). The major fatty acids were C16:0 (19%–24%), C18:0 (9%–25%) and C18:1n9 (37%–46%). PG affected five SFAs (C10:0, C12:0, C20:0, C22:0 and C23:0) and three PUFAs (C20:3n6, C22:2n6 and C20:5n3), while the fat in different regions of the carcass had an effect on any fatty acid except C12:0 and C14:0 ($p < 0.05$, $p < 0.01$ and $p < 0.001$). While it had less effect on mid-chain fatty acids such as C10.0 and C12:0, it increased the levels of long-chain fatty acids like C20:0, C22:0, C23:0, C20:3n6, C22:2n6 and C20:5n3. Finally, four SFAs (C18:0, C20:0, C22:0 and C23:0), one MUFA (C20:1) and four PUFAs (C18:2n6, C18:3n6, C20:3n3 and C20:5n3) differed significantly in the PG × ADLs interaction ($p < 0.05$, $p < 0.01$ and $p < 0.001$).

As with the individual fatty acids, PG had no significant effect on the sum and index values calculated from the fatty acids in Table 2, whereas ADLs had a significant effect on all total ($p < 0.05$) and index values ($p < 0.001$). The SFA and PUFA values in tail (43.397% and 5.417%, respectively) and back fat (43.570% and 4.981%, respectively) were significantly lower than those in perirenal fat (51.415% and 7.045%, respectively; $p < 0.001$). The opposite was true for MUFA ($p < 0.001$). Atherogenic index (AI) and thrombogenic index (TI) values were significantly higher in perirenal fat than those of tail and back fat ($p < 0.001$). The PG × ADLs interaction differed significantly in terms of PUFA ($p < 0.05$), PUFA/SFA ($p < 0.01$), n3 ($p < 0.001$), n6/n3 ($p < 0.05$) and Δ9 C18 ($p < 0.01$) values. The Δ9 C16 and Δ9 C18 values for perirenal fat were significantly lower than those for tail and dorsal fat ($p < 0.001$).

Figure 1 presents the correlations of the sum and index values determined from the individual fatty acids. There were significant correlations between almost all parameters in all three groups (Figure 2). Both n6 and SFA were significantly correlated in the Con group (0.588**) but not in the PG1.5 and PG3 groups. Conversely, n3 and Δ9 C16 were not significantly correlated in the Con group but were in the PG1.5 and PG3 groups (−0.609** and −0.712**). Finally, except for PUFA/SFA and n6/n3, the sum and index values of the n3 fatty acids in the Con group were not correlated. However, they were significantly correlated in the PG1.5 and PG3 groups ($p < 0.05$, $p < 0.01$ and $p < 0.001$).

4 | Discussion

4.1 | PG Effect

In ruminants, the fatty acid composition of reserve lipids depends on the intensity and specificity of endogenous synthesis. Forming more fats during rapid growth corresponds to increased propionate levels in the rumen and is accompanied by changes in the activity of certain adipose tissue enzymes. In particular, acyl-CoA carboxylase and stearoyl-CoA desaturase are strongly influenced by the structure of the animal's diet. As an indirect energy source, PG is first converted to propionic acid in the rumen and then converted to glucose in the liver through the rumino-hepatic cycle (Trabue et al. 2007). Glucose synthesizes fatty acids using acetyl CoA (Botham and Mayes 2015). In the present study, C10:0 and C12:0 were lower in the PG3 group than in the Con group ($p < 0.05$). Thus, for C10:0 and C12:0, it suggested that PG showed a dose-dependent effect. This result is significant

TABLE 1 | Individual fatty acid quantified from total FAME of different anatomical depot location in PG groups (means ± SEM).

Parameters	Group (PG)	Anatomical depot location (ADL)			Overall mean	*p*		
		Tail fat	Perirenal fat	Back fat		PG	ADL	PG×ADL
Capric acid (C10:0)	Con	0.372 ± 0.028	0.237 ± 0.013	0.357 ± 0.035	0.322 ± 0.020[d]	0.021	< 0.001	0.690
	PG1.5	0.378 ± 0.050	0.255 ± 0.023	0.288 ± 0.052	0.307 ± 0.026[d,e]			
	PG3	0.296 ± 0.020	0.191 ± 0.016	0.255 ± 0.021	0.247 ± 0.014[e]			
Overall mean		0.350 ± 0.022[a]	0.229 ± 0.012[b]	0.299 ± 0.024[a]				
Lauric acid (C12:0)	Con	0.377 ± 0.069	0.402 ± 0.037	0.289 ± 0.044	0.356 ± 0.030[d]	0.032	0.459	0.923
	PG1.5	0.414 ± 0.090	0.356 ± 0.070	0.366 ± 0.115	0.379 ± 0.052[d]			
	PG3	0.261 ± 0.016	0.257 ± 0.034	0.202 ± 0.019	0.240 ± 0.015[e]			
Overall mean		0.354 ± 0.041	0.339 ± 0.031	0.289 ± 0.045				
Myristic acid (C14:0)	Con	4.378 ± 0.755	4.812 ± 0.276	4.175 ± 0.361	4.455 ± 0.285	0.101	0.694	0.824
	PG1.5	5.102 ± 0.685	4.283 ± 0.501	4.525 ± 0.807	4.636 ± 0.380			
	PG3	3.924 ± 0.147	3.702 ± 0.345	3.591 ± 0.215	3.739 ± 0.140			
Overall mean		4.497 ± 0.350	4.267 ± 0.240	4.116 ± 0.319				
Myristoleic acid (C14:1)	Con	0.301 ± 0.035	0.208 ± 0.011	0.302 ± 0.024	0.270 ± 0.017	0.843	< 0.001	0.573
	PG1.5	0.295 ± 0.031	0.176 ± 0.003	0.329 ± 0.027	0.266 ± 0.019			
	PG3	0.318 ± 0.035	0.165 ± 0.012	0.355 ± 0.050	0.280 ± 0.027			
Overall mean		0.304 ± 0.018[a]	0.183 ± 0.006[b]	0.329 ± 0.020[a]				
Pentadecanoic acid (C15:0)	Con	1.196 ± 0.163	0.534 ± 0.023	1.202 ± 0.138	0.977 ± 0.098	0.387	< 0.001	0.777
	PG1.5	1.248 ± 0.121	0.542 ± 0.030	1.334 ± 0.130	1.041 ± 0.094			
	PG3	1.357 ± 0.164	0.504 ± 0.041	1.499 ± 0.181	1.120 ± 0.126			
Overall mean		1.266 ± 0.083[a]	0.527 ± 0.018[b]	1.344 ± 0.086[a]				
Pentadecenoic acid (C15:1)	Con	0.273 ± 0.059	0.216 ± 0.017	0.282 ± 0.026	0.257 ± 0.022	0.234	< 0.001	0.191
	PG1.5	0.390 ± 0.058	0.160 ± 0.007	0.391 ± 0.057	0.314 ± 0.035			
	PG3	0.375 ± 0.046	0.155 ± 0.007	0.410 ± 0.072	0.313 ± 0.037			
Overall mean		0.348 ± 0.032[a]	0.176 ± 0.009[b]	0.363 ± 0.033[a]				
Palmitic acid (C16:0)	Con	23.916 ± 0.679	19.713 ± 0.480	23.543 ± 0.664	22.390 ± 0.542	0.204	< 0.001	0.718
	PG1.5	24.110 ± 0.747	19.802 ± 0.663	23.160 ± 1.058	22.357 ± 0.603			
	PG3	22.271 ± 0.580	18.779 ± 0.453	23.309 ± 0.733	21.453 ± 0.544			
Overall mean		23.463 ± 0.415[a]	19.448 ± 0.321[b]	23.329 ± 0.475[a]				
Palmitoleic acid (C16:1)	Con	2.279 ± 0.252	0.975 ± 0.044	2.146 ± 0.106	1.800 ± 0.157	0.911	< 0.001	0.514
	PG1.5	2.378 ± 0.196	1.134 ± 0.066	2.018 ± 0.211	1.843 ± 0.144			
	PG3	2.329 ± 0.186	0.887 ± 0.056	2.355 ± 0.203	1.857 ± 0.177			
Overall mean		2.331 ± 0.117[a]	1.005 ± 0.039[b]	2.166 ± 0.105[a]				
Margaric acid (C17:0)	Con	2.797 ± 0.317	1.913 ± 0.147	2.766 ± 0.291	2.492 ± 0.170	0.386	0.001	0.916
	PG1.5	2.685 ± 0.331	2.061 ± 0.278	3.212 ± 0.444	2.653 ± 0.220			
	PG3	2.908 ± 0.179	2.323 ± 0.204	3.309 ± 0.373	2.847 ± 0.172			
Overall mean		2.792 ± 0.160[a]	2.097 ± 0.128[b]	3.101 ± 0.216[a]				
Heptadecenoic acid (C17:1)	Con	2.015 ± 0.233	0.666 ± 0.067	2.333 ± 0.240	1.671 ± 0.194	0.059	< 0.001	0.285
	PG1.5	2.230 ± 0.310	0.718 ± 0.090	2.112 ± 0.207	1.687 ± 0.188			
	PG3	2.416 ± 0.274	0.738 ± 0.087	2.999 ± 0.238	2.051 ± 0.245			

(Continues)

TABLE 1 | (Continued)

Parameters	Group (PG)	Anatomical depot location (ADL)			Overall mean	*p*		
		Tail fat	Perirenal fat	Back fat		PG	ADL	PG×ADL
Overall mean		2.221 ± 0.157[a]	0.708 ± 0.046[b]	2.465 ± 0.149[a]				
Stearic acid (C18:0)	Con	11.074 ± 0.619[b]	22.297 ± 0.330[a,e]	10.129 ± 0.443[b]	14.500 ± 1.263	0.247	< 0.001	< 0.001
	PG1.5	10.128 ± 0.471[b]	21.078 ± 0.584[a,e]	10.914 ± 0.610[b]	14.040 ± 1.084			
	PG3	9.739 ± 0.963[b]	25.480 ± 0.582[a,d]	9.549 ± 0.976[b]	14.923 ± 1.734			
Overall mean		10.305 ± 0.400[b]	22.866 ± 0.496[a]	10.230 ± 0.406[b]				
Oleic acid (C18:1n9)	Con	45.518 ± 1.052	38.733 ± 1.148	45.070 ± 1.319	43.107 ± 0.947	0.215	< 0.001	0.585
	PG1.5	43.714 ± 1.160	37.297 ± 0.884	44.184 ± 1.096	41.732 ± 0.876			
	PG3	46.245 ± 1.565	36.862 ± 0.504	46.364 ± 1.248	43.157 ± 1.190			
Overall mean		45.093 ± 0.736[a]	37.616 ± 0.517[b]	45.159 ± 0.696[a]				
Linoleic acid (C18:2n6)	Con	3.188 ± 0.164[b]	4.169 ± 0.321[a,d]	2.955 ± 0.183[b]	3.437 ± 0.174	0.821	0.002	0.028
	PG1.5	3.351 ± 0.210	3.565 ± 0.138[d,e]	3.065 ± 0.148	3.327 ± 0.102			
	PG3	3.800 ± 0.340[a]	3.269 ± 0.178[a,b,e]	2.996 ± 0.265[b]	3.355 ± 0.165			
Overall mean		3.442 ± 0.146[a]	3.663 ± 0.145[a]	3.008 ± 0.110[b]				
Arachidic acid (C20:0)	Con	0.092 ± 0.016[b]	0.333 ± 0.138[a,e]	0.087 ± 0.015[b]	0.171 ± 0.051[e]	0.027	< 0.001	0.002
	PG1.5	0.075 ± 0.015[b]	0.842 ± 0.135[a,d]	0.063 ± 0.006[b]	0.327 ± 0.087[d]			
	PG3	0.061 ± 0.015[b]	0.575 ± 0.067[a,e]	0.066 ± 0.006[b]	0.234 ± 0.058[e]			
Overall mean		0.076 ± 0.009[b]	0.595 ± 0.080[a]	0.072 ± 0.006[b]				
ɣ-Linolenic acid (C18:3n6)	Con	1.224 ± 0.100	0.921 ± 0.127[e]	0.917 ± 0.159	1.021 ± 0.079	0.127	< 0.001	0.048
	PG1.5	1.255 ± 0.177[ab]	1.651 ± 0.273[a,d]	0.797 ± 0.019[b]	1.234 ± 0.127			
	PG3	1.236 ± 0.110[a]	1.172 ± 0.130[a,d,e]	0.644 ± 0.039[b]	1.018 ± 0.081			
Overall mean		1.239 ± 0.076[a]	1.266 ± 0.128[a]	0.786 ± 0.055[b]				
Eicosenoic acid (C20:1)	Con	0.508 ± 0.091	0.278 ± 0.113[e]	0.457 ± 0.044[d]	0.415 ± 0.053	0.116	0.002	0.023
	PG1.5	0.673 ± 0.080[a]	0.603 ± 0.085[a,d]	0.302 ± 0.027[b,d,e]	0.526 ± 0.051			
	PG3	0.513 ± 0.125[a]	0.513 ± 0.111[a,d,e]	0.153 ± 0.015[b,e]	0.393 ± 0.065			
Overall mean		0.570 ± 0.057[a]	0.471 ± 0.064[ab]	0.304 ± 0.031[b]				
Eicosadienoic acid (C20:2n6)	Con	0.049 ± 0.007	0.330 ± 0.127	0.111 ± 0.021	0.163 ± 0.049	0.777	0.004	0.102
	PG1.5	0.113 ± 0.018	0.200 ± 0.070	0.116 ± 0.026	0.143 ± 0.026			
	PG3	0.173 ± 0.037	0.167 ± 0.039	0.057 ± 0.004	0.133 ± 0.021			
Overall mean		0.112 ± 0.017[b]	0.231 ± 0.049[a]	0.096 ± 0.013[b]				
Behenic acid (C22:0)	Con	0.044 ± 0.008	0.228 ± 0.102[e]	0.119 ± 0.024	0.130 ± 0.037[e]	0.006	< 0.001	0.044
	PG1.5	0.122 ± 0.023[b]	0.654 ± 0.137[a,d]	0.160 ± 0.025[b]	0.312 ± 0.068[d]			
	PG3	0.127 ± 0.037[b]	0.508 ± 0.078[a,d]	0.111 ± 0.028[b]	0.248 ± 0.050[d]			
Overall mean		0.099 ± 0.016[b]	0.472 ± 0.073[a]	0.131 ± 0.015[b]				
D-ɣ-Linolenic acid (C20:3n6)	Con	0.030 ± 0.007	0.333 ± 0.066	0.117 ± 0.010	0.160 ± 0.036[e]	0.008	< 0.001	0.220
	PG1.5	0.439 ± 0.156	0.577 ± 0.130	0.147 ± 0.017	0.388 ± 0.075[d]			
	PG3	0.345 ± 0.084	0.410 ± 0.088	0.121 ± 0.019	0.292 ± 0.048[d,e]			
Overall mean		0.279 ± 0.071[ab]	0.446 ± 0.060[a]	0.130 ± 0.009[b]				
Erucic acid (C22:1n9)	Con	0.025 ± 0.005	0.792 ± 0.146	0.263 ± 0.026	0.360 ± 0.086	0.964	< 0.001	0.254
	PG1.5	0.135 ± 0.045	0.786 ± 0.169	0.223 ± 0.027	0.381 ± 0.082			
	PG3	0.299 ± 0.137	0.639 ± 0.109	0.186 ± 0.040	0.375 ± 0.071			

(Continues)

TABLE 1 | (Continued)

Parameters	Group (PG)	Anatomical depot location (ADL)			Overall mean	*p*		
		Tail fat	Perirenal fat	Back fat		PG	ADL	PG×ADL
Overall mean		0.152 ± 0.050^{b}	0.741 ± 0.082^{a}	0.224 ± 0.018^{b}				
Eicosatrienoic acid (C20:3n3)	Con	0.021 ± 0.005^{b}	$0.208 \pm 0.054^{b,e}$	$0.595 \pm 0.067^{a,d}$	0.275 ± 0.060	0.626	< 0.001	< 0.001
	PG1.5	0.113 ± 0.032^{b}	$0.656 \pm 0.100^{a,d}$	$0.218 \pm 0.046^{b,e}$	0.329 ± 0.061			
	PG3	0.111 ± 0.035^{b}	$0.668 \pm 0.148^{a,d}$	$0.155 \pm 0.020^{b,e}$	0.311 ± 0.074			
Overall mean		0.083 ± 0.018^{c}	0.517 ± 0.075^{a}	0.318 ± 0.049^{b}				
Arachidonic acid (C20:4n6)	Con	0.030 ± 0.014	0.293 ± 0.063	0.547 ± 0.067	0.290 ± 0.056	0.374	< 0.001	0.137
	PG1.5	0.110 ± 0.020	0.312 ± 0.063	0.326 ± 0.026	0.249 ± 0.031			
	PG3	0.128 ± 0.051	0.502 ± 0.145	0.386 ± 0.148	0.339 ± 0.076			
Overall mean		0.090 ± 0.020^{b}	0.367 ± 0.056^{a}	0.415 ± 0.054^{a}				
Tricosylic acid (C23:0)	Con	0.141 ± 0.022^{b}	$0.195 \pm 0.056^{b,e}$	$0.436 \pm 0.097^{a,e}$	0.257 ± 0.046^{e}	< 0.001	< 0.001	0.002
	PG1.5	0.146 ± 0.035^{c}	$0.575 \pm 0.105^{b,d}$	$0.922 \pm 0.116^{a,d}$	0.548 ± 0.084^{d}			
	PG3	0.163 ± 0.025	0.237 ± 0.031^{e}	0.356 ± 0.084^{e}	0.252 ± 0.034^{e}			
Overall mean		0.150 ± 0.016^{c}	0.347 ± 0.056^{b}	0.587 ± 0.079^{a}				
Docosadienoic acid (C22:2n6)	Con	0.018 ± 0.006	0.068 ± 0.017	0.112 ± 0.008	0.066 ± 0.011^{e}	0.006	< 0.001	0.198
	PG1.5	0.069 ± 0.011	0.173 ± 0.030	0.111 ± 0.012	0.118 ± 0.014^{d}			
	PG3	0.054 ± 0.013	0.179 ± 0.024	0.148 ± 0.054	0.127 ± 0.023^{d}			
Overall mean		0.048 ± 0.007^{b}	0.142 ± 0.018^{a}	0.123 ± 0.017^{a}				
Lignoceric acid (C24:0)	Con	0.026 ± 0.009	0.197 ± 0.044	0.068 ± 0.009	0.097 ± 0.022	0.147	< 0.001	0.309
	PG1.5	0.049 ± 0.009	0.283 ± 0.043	0.079 ± 0.011	0.137 ± 0.026			
	PG3	0.065 ± 0.027	0.200 ± 0.028	0.062 ± 0.009	0.109 ± 0.019			
Overall mean		0.047 ± 0.010^{b}	0.229 ± 0.024^{a}	0.070 ± 0.006^{b}				
EPA (C20:5n3)	Con	0.038 ± 0.013	0.092 ± 0.045^{e}	0.095 ± 0.016	0.075 ± 0.017^{e}	0.002	< 0.001	< 0.001
	PG1.5	0.062 ± 0.011^{b}	$0.352 \pm 0.062^{a,d}$	0.088 ± 0.012^{b}	0.167 ± 0.034^{d}			
	PG3	0.046 ± 0.009^{b}	$0.253 \pm 0.036^{a,d}$	0.036 ± 0.005^{b}	0.112 ± 0.025^{d}			
Overall mean		0.049 ± 0.007^{b}	0.238 ± 0.036^{a}	0.074 ± 0.009^{b}				
Nervoic acid (C24:1)	Con	0.028 ± 0.007	0.694 ± 0.097	0.494 ± 0.085	0.405 ± 0.075	0.765	< 0.001	0.070
	PG1.5	0.141 ± 0.063	0.675 ± 0.142	0.511 ± 0.072	0.442 ± 0.072			
	PG3	0.337 ± 0.149	0.519 ± 0.087	0.303 ± 0.083	0.386 ± 0.064			
Overall mean		0.168 ± 0.057^{b}	0.631 ± 0.065^{a}	0.440 ± 0.048^{a}				
DHA (C22:6n3)	Con	0.042 ± 0.014	0.164 ± 0.058	0.032 ± 0.005	0.079 ± 0.023	0.223	< 0.001	0.255
	PG1.5	0.078 ± 0.016	0.239 ± 0.045	0.038 ± 0.002	0.118 ± 0.024			
	PG3	0.101 ± 0.032	0.143 ± 0.023	0.023 ± 0.002	0.089 ± 0.017			
Overall mean		0.074 ± 0.013^{b}	0.185 ± 0.026^{a}	0.031 ± 0.002^{b}				

[a, b, c]Means with unlike superscript letters in rows differ significantly ($p < 0.05$).

[d,e]Means with unlike superscript letters in columns differ significantly ($p < 0.05$).

because red meat fat composition has been criticized for high SFA content, which is deemed unhealthy for consumers (Daley et al. 2010). da Costa et al. (2020) showed that the use of glycerin in lambs had a dose-dependent effect on C10:0 and C12:0 fatty acid levels. Since glycerin, such as PG, increases propionic acid levels in the rumen, their results support our findings. Although glycerin did not increase the amount of propionic acid in the rumen as much as PG, it was found to have a similar effect because it increased the amount of volatile fatty acids (acetate, propionate, butyrate, valerate and caproate) in general. On the other hand, Carvalho et al. (2015) reported that glycerin use in lambs did change C10:0 and C12:0 fatty acid levels in the meat. This suggests that supplements such as PG and glycerin may have different effects on fatty acids in meat and stored fats. Song et al. (2017) showed that energy restriction in lambs increases C12:0 fatty acid levels. In the present study, high energy was provided to the lambs using PG3, which increased C12:0 levels, thereby contradicting Song et al. (2017). This suggests that C10:0 and

TABLE 2 | Sum and index values obtained from the fatty acids in the ADL for experimental groups (means ± SEM).

Parameters	Group (PG)	Anatomical depot location (ADL)			Overall mean	*P*		
		Tail fat	Perirenal fat	Back fat		PG	ADL	PG×ADL
SFA	Con	44.412 ± 1.336	50.860 ± 0.681	43.171 ± 1.295	46.148 ± 0.980	0.332	< 0.001	0.074
	PG1.5	44.456 ± 1.089	50.728 ± 0.497	45.023 ± 1.263	46.736 ± 0.810			
	PG3	41.172 ± 1.442	52.755 ± 0.548	42.308 ± 1.229	45.412 ± 1.322			
Overall mean		43.397 ± 0.778^{b}	51.415 ± 0.373^{a}	43.570 ± 0.739^{b}				
MFA	Con	50.947 ± 1.340	42.562 ± 1.140	51.347 ± 1.433	48.286 ± 1.155	0.238	< 0.001	0.323
	PG1.5	49.955 ± 0.999	41.547 ± 0.814	50.070 ± 1.211	47.190 ± 1.005			
	PG3	52.833 ± 1.733	40.480 ± 0.405	53.126 ± 1.363	48.813 ± 1.496			
Overall mean		51.186 ± 0.791^{a}	41.531 ± 0.496^{b}	51.449 ± 0.780^{a}				
PUFA	Con	4.641 ± 0.254^{b}	6.578 ± 0.612^{a}	5.481 ± 0.337^{a,b}	5.567 ± 0.294	0.315	< 0.001	0.049
	PG1.5	5.589 ± 0.518^{b}	7.725 ± 0.436^{a}	4.907 ± 0.197^{b}	6.074 ± 0.336			
	PG3	5.995 ± 0.476^{a}	6.764 ± 0.271^{a}	4.566 ± 0.416^{b}	5.775 ± 0.298			
Overall mean		5.417 ± 0.271^{b}	7.054 ± 0.276^{a}	4.981 ± 0.193^{b}				
UFA	Con	55.588 ± 1.336	49.140 ± 0.681	56.829 ± 1.295	53.852 ± 0.980	0.332	< 0.001	0.074
	PG1.5	55.544 ± 1.089	49.272 ± 0.497	54.977 ± 1.263	53.264 ± 0.810			
	PG3	58.828 ± 1.442	47.245 ± 0.548	57.692 ± 1.229	54.588 ± 1.322			
Overall mean		56.603 ± 0.778^{a}	48.585 ± 0.373^{b}	56.430 ± 0.739^{a}				
PUFA/SFA	Con	0.105 ± 0.007^{d}	0.129 ± 0.011	0.127 ± 0.007	0.121 ± 0.005	0.441	0.019	0.008
	PG1.5	0.127 ± 0.013^{a,b,c,d}	0.152 ± 0.008^{a}	0.110 ± 0.006^{b}	0.130 ± 0.006			
	PG3	0.146 ± 0.010^{a,c}	0.129 ± 0.006^{a,b}	0.108 ± 0.010^{b}	0.127 ± 0.006			
Overall mean		0.126 ± 0.007^{a,b}	0.137 ± 0.005^{a}	0.115 ± 0.005^{b}				
UFA/SFA	Con	1.265 ± 0.076	0.968 ± 0.026	1.330 ± 0.078	1.188 ± 0.050	0.207	< 0.001	0.132
	PG1.5	1.258 ± 0.051	0.973 ± 0.019	1.232 ± 0.058	1.154 ± 0.037			
	PG3	1.447 ± 0.088	0.897 ± 0.020	1.377 ± 0.075	1.240 ± 0.066			
Overall mean		1.321 ± 0.044^{a}	0.947 ± 0.014^{b}	1.309 ± 0.041^{a}				
n6	Con	4.541 ± 0.244	6.114 ± 0.507	4.759 ± 0.295	5.138 ± 0.254	0.551	< 0.001	0.154
	PG1.5	5.337 ± 0.467	6.478 ± 0.406	4.563 ± 0.169	5.459 ± 0.262			
	PG3	5.737 ± 0.412	5.700 ± 0.200	4.353 ± 0.421	5.263 ± 0.244			
Overall mean		5.211 ± 0.242^{ab}	6.115 ± 0.228^{a}	4.558 ± 0.169^{b}				
n3	Con	0.100 ± 0.021^{b}	0.464 ± 0.145^{a,d}	0.722 ± 0.070^{a,c}	0.429 ± 0.077	0.068	< 0.001	< 0.001
	PG1.5	0.253 ± 0.056^{b}	1.247 ± 0.145^{a,c}	0.345 ± 0.055^{b,d}	0.615 ± 0.107			
	PG3	0.258 ± 0.069^{b}	1.064 ± 0.162^{a,c}	0.213 ± 0.021^{b,d}	0.512 ± 0.104			
Overall mean		0.206 ± 0.033^{b}	0.940 ± 0.110^{a}	0.423 ± 0.055^{b}				
n6/n3	Con	44.276 ± 5.799^{a}	20.744 ± 6.067^{b}	6.831 ± 0.518^{b}	22.934 ± 4.337	0.130	< 0.001	0.029
	PG1.5	27.258 ± 4.967^{a}	5.646 ± 0.679^{b}	14.879 ± 1.555^{a,b}	15.928 ± 2.491			
	PG3	37.934 ± 11.649^{a}	5.980 ± 0.753^{b}	21.734 ± 3.283^{a,b}	21.883 ± 4.818			
Overall mean		35.679 ± 4.664^{a}	10.556 ± 2.400^{b}	14.499 ± 1.727^{b}				
NV	Con	2.384 ± 0.104	3.113 ± 0.121	2.364 ± 0.117	2.620 ± 0.100	0.146	< 0.001	0.687
	PG1.5	2.255 ± 0.112	2.984 ± 0.161	2.430 ± 0.163	2.557 ± 0.104			
	PG3	2.531 ± 0.120	3.335 ± 0.111	2.421 ± 0.121	2.763 ± 0.112			
Overall mean		2.384 ± 0.067^{b}	3.137 ± 0.081^{a}	2.406 ± 0.076^{b}				
AI	Con	0.529 ± 0.064	0.855 ± 0.025	0.480 ± 0.033	0.621 ± 0.044	0.299	< 0.001	0.204

(Continues)

TABLE 2 | (Continued)

Parameters	Group (PG)	Anatomical depot location (ADL)			Overall mean	*P*		
		Tail fat	Perirenal fat	Back fat		PG	ADL	PG×ADL
	PG1.5	0.566 ± 0.065	0.785 ± 0.036	0.546 ± 0.075	0.632 ± 0.040			
	PG3	0.441 ± 0.033	0.860 ± 0.033	0.420 ± 0.017	0.574 ± 0.048			
Overall mean		0.514 ± 0.033[b]	0.831 ± 0.019[a]	0.485 ± 0.031[b]				
TI	Con	0.782 ± 0.047	1.044 ± 0.030	0.722 ± 0.036	0.849 ± 0.038	0.584	< 0.001	0.075
	PG1.5	0.789 ± 0.042	0.975 ± 0.025	0.774 ± 0.053	0.846 ± 0.030			
	PG3	0.683 ± 0.042	1.074 ± 0.029	0.699 ± 0.033	0.819 ± 0.045			
Overall mean		0.753 ± 0.026[b]	1.029 ± 0.018[a]	0.734 ± 0.025[b]				
Δ9 C16	Con	0.087 ± 0.008	0.047 ± 0.002	0.084 ± 0.006	0.073 ± 0.005	0.632	< 0.001	0.387
	PG1.5	0.089 ± 0.006	0.054 ± 0.002	0.079 ± 0.006	0.074 ± 0.004			
	PG3	0.095 ± 0.007	0.045 ± 0.002	0.091 ± 0.007	0.077 ± 0.006			
Overall mean		0.090 ± 0.004[a]	0.049 ± 0.002[b]	0.085 ± 0.004[a]				
Δ9 C18	Con	0.804 ± 0.011[a]	0.634 ± 0.009[b,c]	0.816 ± 0.010[a]	0.751 ± 0.019	0.957	< 0.001	0.005
	PG1.5	0.812 ± 0.007[a]	0.639 ± 0.006[b,c]	0.802 ± 0.009[a]	0.751 ± 0.017			
	PG3	0.826 ± 0.017[a]	0.591 ± 0.007[b,d]	0.829 ± 0.016[a]	0.749 ± 0.026			
Overall mean		0.814 ± 0.007[a]	0.622 ± 0.006[b]	0.815 ± 0.007[a]				

[a,b] Means with unlike superscript letters in rows differ significantly ($p < 0.05$).
[c,d]Means with unlike superscript letters in columns differ significantly ($p < 0.05$).

C12:0 fatty acids may be directly affected by energy consumption. Velasco et al. (2001) reported that variations in C12:0 levels in subcutaneous and intramuscular fats depended on whether the animals were sourced from pasture or drylot management systems. This indicates that C12:0 metabolism in lambs is directly related to energy consumption. In the present study, while the amount of C12:0 decreased in the PG3 group, the lack of change in the PG1.5 group supports the dose-dependent effect.

C20:0 serves as a precursor to eicosanoids, long-chain fatty acids with anti-inflammatory and anti-clotting properties that contribute to cardiovascular protection. Its intake has been shown to increase long-chain SFAs both in the erythrocyte membrane and systemically, which has been associated with a reduced risk of sudden cardiac arrest (Lemaitre et al. 2014). Moreover, elevated circulating levels of C20:0 and other long-chain SFAs have been linked to a decreased risk of heart failure, arrhythmias, coronary heart disease and sudden cardiac arrest (Fretts et al. 2014). In the present study, C20:0 levels of perirenal fat were significantly higher in the PG1.5 group compared to other groups ($p < 0.05$), a finding that could benefit cardiovascular health and eicosanoid synthesis. Glycerin, which mimics the effects of PG in the rumen, has been reported to influence or reduce C20:0 levels in lamb meat (Cunha et al. 2016; de Lima Valença et al. 2020). However, the findings from Cunha et al. (2016) and de Lima Valença et al. (2020) were derived from studies on the longissimus muscle, and the feeding regimens of the lambs differed, potentially explaining variations in their results.

C23:0, found in glycosphingolipids and brain tissue, can be derived from very long-chain fatty acids, which can be shortened to produce propionyl-CoA. Following conversion to succinyl-CoA, propionyl-CoA can replenish the citric acid cycle with anaplerotic intermediates, thereby enhancing mitochondrial energy metabolism (Palacios, Starai, and Escalante-Semerena 2003). We found that PG supplementation had a positive effect on C23:0 as levels were higher in the PG1.5 group than the Con group, while the PG3 group had similar levels to the Con group. The lack of effect in the PG3 group may be attributed to metabolic homeostasis.

Our findings regarding C20:3n6 were generally similar to those in the literature and were higher with PG1.5 supplementation (Nuernberg et al. 2005; Vacca et al. 2008). The lower C20:3n6 levels reported in some previous studies (de Lima Valença et al. 2020) and the lack of difference in other reports (Terré et al. 2011; Cunha et al. 2016) suggest that PG and glycerin may have differing effects on C20:3n6 synthesis. In addition, it is evident that they may have different effects in muscle and adipose tissue. In our study, PG supplementation was associated with a higher C22:2n6 levels. When PG is given orally, it is converted to glucose in the liver, which appears to suppress ghrelin secretion. This in turn stimulates C22:2n6 biosynthesis from long-chain fatty acids (Trabue et al. 2007). Lu et al. (2012) reported that inhibition of gastric ghrelin secretion increased the C22:2n6 biosynthesis in mice. On the other hand, Song et al. (2017) reported that energy restriction decreased C22:2n6 levels in lambs. These findings suggest that Lu et al. (2012) report that suppression of gastric ghrelin secretion stimulates C22:2n6 biosynthesis is also valid in ruminants.

Long-chain PUFAs are now known to play important roles in human health. In particular, C20:5n3 appears to be a protective agent in a range of pathologies, including cardiovascular disease, Type 2 diabetes and metabolic syndrome (Sayanova and Napier 2004). In our study, another fatty acid affected by PG

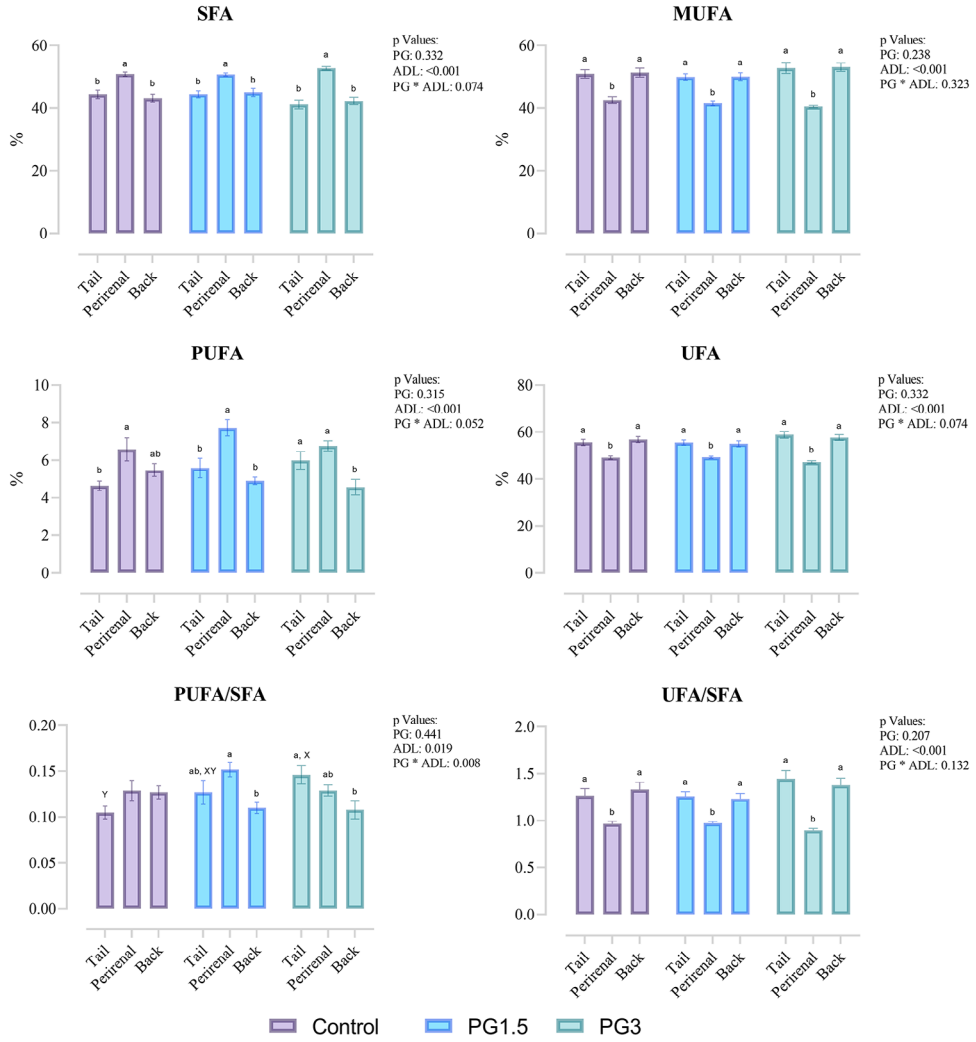

FIGURE 1 | Sum and index values obtained from the individual fatty acid in the ADL for experimental groups (means ± SEM). a and b signify that means with unlike superscript letters among ADL in PG groups are significantly different ($p < 0.05$). X and Y signify that means with unlike superscript letters among PG groups in ADL are significantly different ($p < 0.05$).

supplementation was C20:5n3, which is biosynthesized through two basic mechanisms: desaturation and chain elongation (Khozin-Goldberg et al. 2002). PG appears to increase C20:5n3 levels by using these mechanisms. Many studies using glycerin as a food supplement in lambs reported no change in C20:5n3 levels in the meat (Terré et al. 2011; Carvalho et al. 2015; Cunha et al. 2016; da Costa et al. 2020). This difference from our findings may be due to their use of glycerin or their use of muscle tissue instead of adipose tissue.

4.2 | ADLs Effect

Our study found no significant effect of ADLs on individual fatty acids, except C12:0 and C14:0. Juárez et al. (2008) reported that the ADLs affected all fatty acids except C16:0 in lambs. In our study, the major fatty acids (C16:0 and C18:1) had lower levels in perirenal fat than the other regions, whereas the opposite was true for C18:0. This difference among ADLs is thought to be due to phospholipids or triglycerides present in the structure of adipocytes because the fatty acids in phospholipids and triglycerides are structurally different. Phospholipids generally contain unsaturated fatty acids while triglycerides are known to contain SFAs. In order to pack more triglycerides, adipocytes also differ in size (Halim et al. 2022; Zhang et al. 2020). Thus, the fatty acid composition in ADLs may be different. These findings are consistent with previous reports (Song et al. 2017). Together, these findings indicate that fatty acid composition in lambs differs depending on the specific fat depots in different parts of the body.

C18:1 is generally obtained either directly from dietary fat sources or by stearoyl-CoA desaturase 1–mediated conversion of C18:0 (Reddy et al. 2019). MUFA perirenal fat levels were probably low in the present study because the conversion of the C18:0 to C18:1 was higher in perirenal than tail or back fat. These findings are confirmed by the Δ9 C16 and Δ9 C18 data, which were lower for perirenal fat than the other two depots. This indicates that C16:0 and C18:0 SFAs cause less desaturation enzyme activity in the perirenal fat, while the level of formed MUFAs is low (Carvalho et al. 2015). This difference was detected in different adipose tissues and is consistent with a previous study on lambs (Juárez et al. 2008).

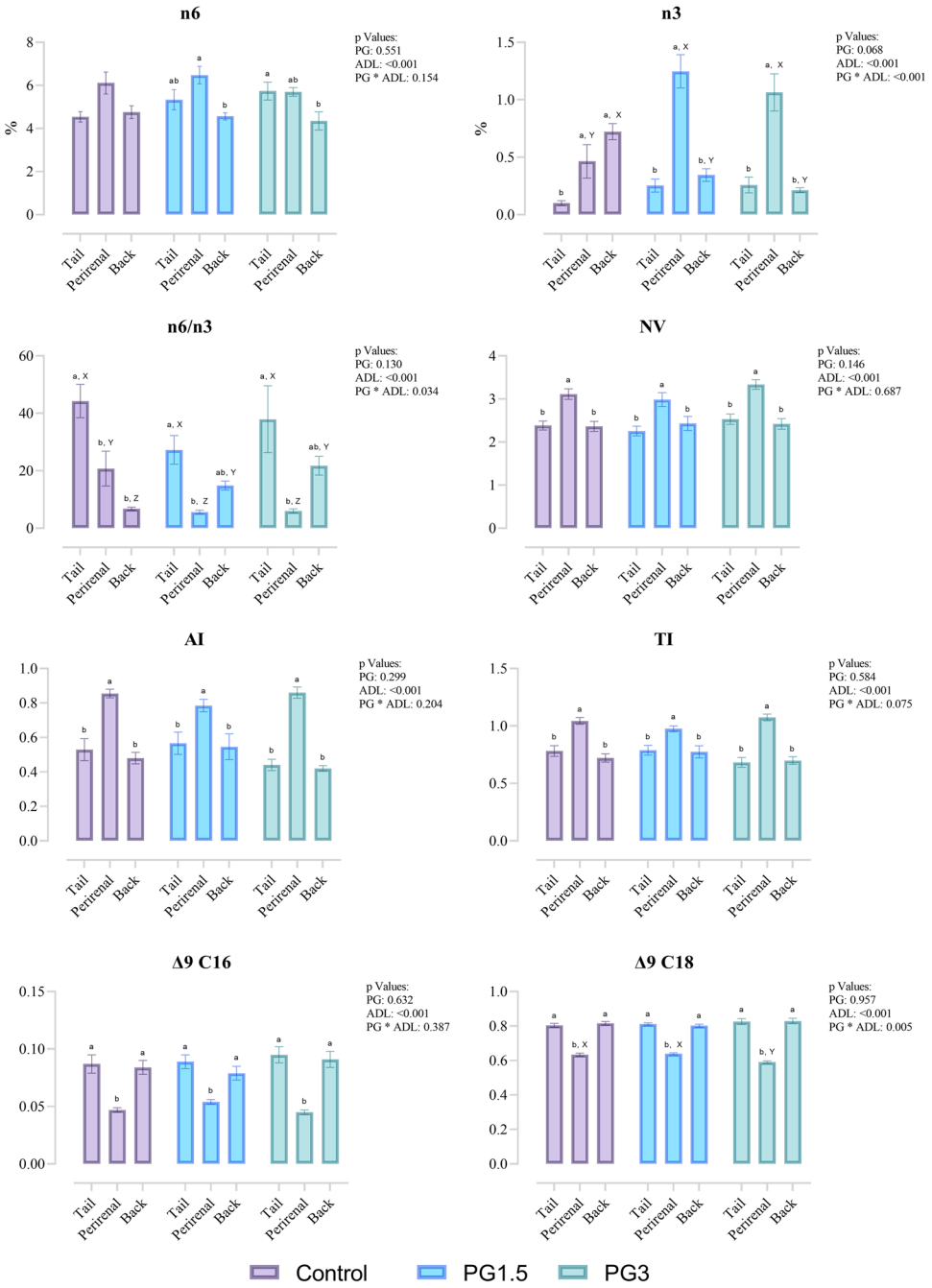

FIGURE 1 | (Continued)

C20:4n6, which is synthesized from C18:2, an essential fatty acid, has diverse important functions in various physiological processes, such as growth, reproduction, stress resistance, pigmentation, immunity, lipid accumulation and bone development (Xu et al. 2022). In our study, C20:4n6 levels were higher in perirenal and back than tail fat, which may be due to the higher number of cells in the first two depots (Juárez et al. 2008). C20:4n6 is mainly found in cell membranes. Urrutia et al. (2016) investigated the diameter of adipocytes in tissues of Navarra breed lambs at 26.7 kg slaughter weight and reported differences in adipocyte size between fat depots from subcutaneal and intra-muscular fat.

In the present study, all sum and index values calculated from individual fatty acids were significantly affected by ADLs, with changes occurring in the perirenal adipose tissue, except for the n6/n3 ratio. This tissue, which is located in the abdominal cavity, differs from tail and back fat. Hence, the main reason for the difference appears to be location. The perirenal fat SFA values observed in our study agree with previous reports of fatty acid profiles of different tissue adipose depots in sheep (Song et al. 2017). The main reason for high SFA values in perirenal fat is high C18:0 levels. Although health professionals recommend reducing consumption of SFA, trans-fatty acids and cholesterol, this was not considered a significant disadvantage in our study because the

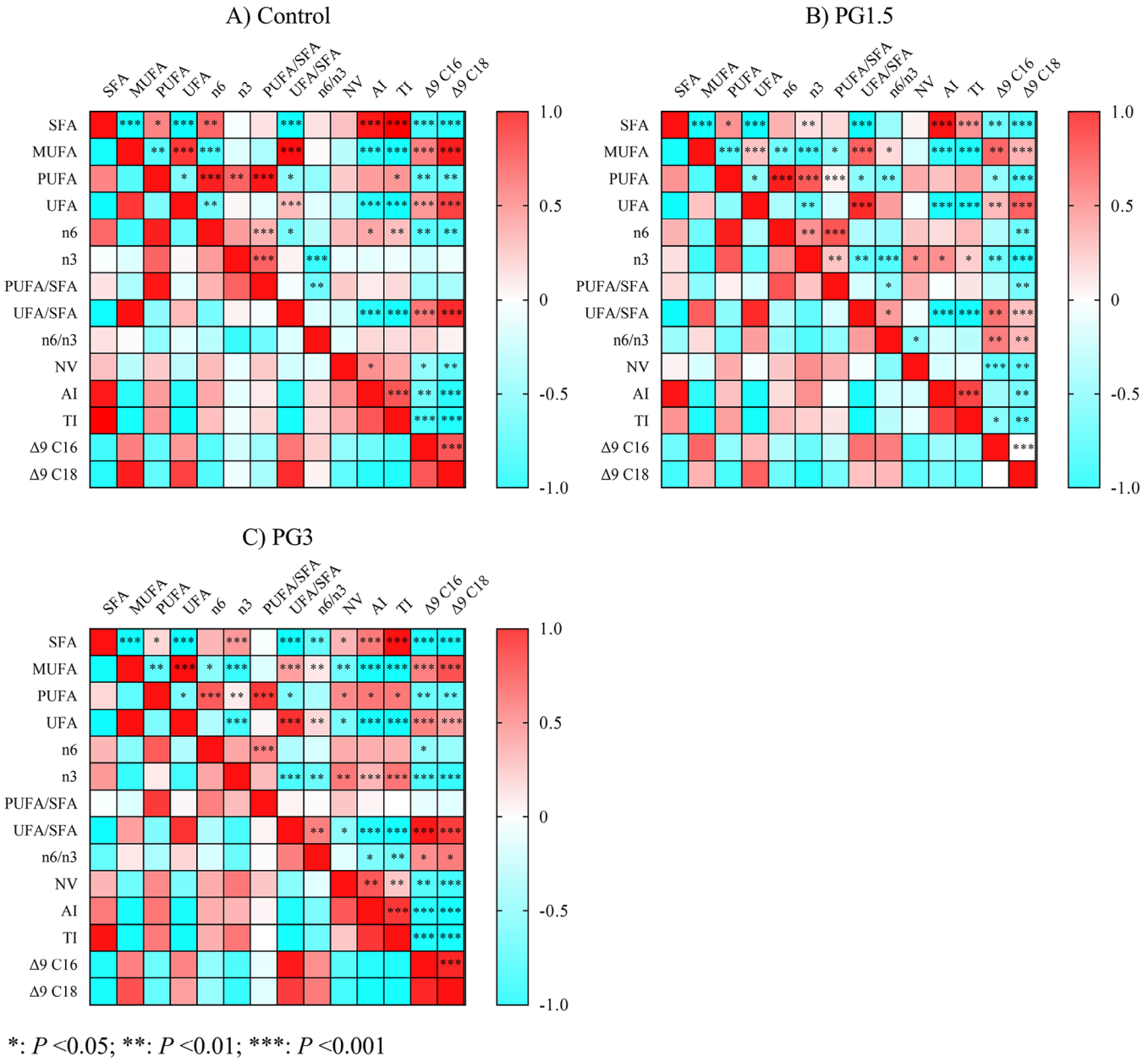

FIGURE 2 | Correlations of among 14 sum and index values in different experimental groups.

reason for the high SFA was C18:0, which is a soft waxy solid with a melting point of 69.4°C. Epidemiological and clinical studies have shown that C18:0 is associated with lower LDL cholesterol than other SFAs (Hunter, Zhang, and Kris-Etherton 2010). In our study, MUFA levels in the lambs' tail and back fat were lower than the high SFA levels in their perirenal fat.

The n6/n3 index value is a very important parameter in fatty acid composition, with < 4 being desirable (British Department of Health 1994). The n6/n3 values in our study were well above 4 (10.5–35.6) in all three ADLs and significantly higher in tail fat than the other two regions. Previous studies on lambs have also reported a wide range of n6/n3 values above 4 (Juárez et al. 2008; Carvalho et al. 2015). This suggests that factors like feed, breed and age, along with ADLs, strongly affect the n6/n3 ratio, which is also significantly higher in tail fat than other fat regions.

AI and TI values were higher in perirenal than tail or back fat but below the critical value of 1 (Ulbricht and Southgate 1991). These results are compatible with those for intramuscular fat in lambs (Vacca et al. 2008; Yakan et al. 2024). The higher levels in perirenal fat are thought to be due to the anatomical region (Juárez et al. 2008).

4.3 | PG × ADLs Interaction

The PG × ADLs interaction subgroups showed significant differences in certain fatty acids (C18:0, C18:2n6, C18:3n6, C20:0, C20:1, C20:3n3, C22:0, C23:0 and C20:5n3). The presence of interactions for the PG groups in perirenal fat may be because perirenal fat is metabolized differently due to being located in the abdomen (Wood et al. 2008). Several studies have reported

that, in ruminants, internal adipose tissue grows faster than subcutaneous adipose tissue (Poulos, Hausman, and Hausman 2010; Hausman et al. 2014). We found higher levels of SFAs such as C18:0, C20:0, C22:0 and C23:0 in perirenal fat, which is an internal adipose tissue, for the PG groups than the Con group. This appears to be related to the animal's high energy intake due to PG supplementation.

The significant PG × ADLs interaction may also be due to the different enzymatic activities of particular adipose tissues (e.g., intra-muscular and subcutaneous). The activity of lipogenic enzymes (NADP-malate dehydrogenase, phosphogluconate-6-dehydrogenase and glycose-6-phosphate dehydrogenase) is higher in subcutaneous adipose tissue than in other adipose tissues (Martins et al. 2018). In our study, levels of certain fatty acids, especially C18:0, C20:0, C22:0, and C23:0, were higher in perirenal adipose tissue, which can be related to a higher lipogenic enzyme activity in this tissue than in other non-internal adipose tissues. In support of this interpretation, Xin et al. (2018) reported that PG induces lipogenic enzyme activity.

The PG × ADLs interaction was significant for n3, PUFA/SFA, n6/n3 and Δ9 C18 from the sum and index values calculated from the fatty acids ($p < 0.05$, $p < 0.01$ and $p < 0.001$) (Table 2). The interactions of the total and index values also showed significant differences in the perirenal region structures as in individual fat sites. Since these values were determined by calculation, it is normal to find similar results.

4.4 | Correlations of Sum and Index Values in the Fatty Acids

As shown in Figure 2, there were correlations between the sum and index values as calculated from the individual fatty acids in almost all parameters. However, these correlations are expected because the values were obtained by calculation. However, no significant correlations were found between n3 and the other parameters (UFA/SFA, NV, AI, TI, Δ9 C16 and Δ9 C18) in the Con group, whereas significant negative correlations were found in the PG1.5 and PG3 groups for UFA/SFA, Δ9 C16 and Δ9 C18, while positive correlations were found for NV, AI and TI. Fatty acids are synthesized by de novo, desaturation and chain elongation systems (Halim et al. 2022). Our results suggest that, in lambs, n3 is synthesized by desaturation or/and chain elongation systems rather than de novo synthesis in depot fats. In the case of the de novo synthesis, no correlation with the other parameters would be expected. The fact that Δ9 C16 and Δ9 C18 showed significant negative correlations in all three groups (Con, PG1.5 and PG3) with a few exceptions (e.g., the correlation between Δ9 C16 and PUFA/SFA) suggests that, in lambs, the desaturation system is involved in fatty acid metabolism in depot fat. Juárez et al. (2008) also reported the effect of ADLs on desaturase enzyme activity observed in our study.

As adipocytes have different structural and functional properties in ADLs, there may be differences in fatty acid composition when PG is used as an energy source (Trabue et al. 2007; Zhang et al. 2020). The main reason for this is thought to be that adipocytes in the perirenal region react differently to PG because they are smaller and dysfunctional than back fat and adipocytes of adipose fat.

5 | Conclusion

The use of PG in lamb meat production has shown the potential to provide a significant benefit by dose, dependently reducing C10:0 and C12:0 SFAs. PG is thought to be an effective food supplement for lambs because it impacts different mechanisms of fatty acid in ADLs. However, this effect does not appear to radically alter overall fatty acid composition. Rather, fatty acid composition in lambs varies across specific ADLs. In particular, perirenal fats are richer in SFAs. Therefore, it is necessary, in order to protect human health, to pay attention to fatty acid composition when consuming abdominal fats.

5.1 | Limitations of the Study

As the lambs used in this study were also controlled for fattening performance, routine rumen contents were not analysed for volatile fatty acids. This was considered as a limiting factor for the study. Another limiting factor is that individual fatty acids could not be absolute quantification content. Therefore, it is recommended that these issues should be taken into consideration in future studies.

Author Contributions

Cafer Tayyar Ateş: writing–review and editing, formal analysis. **Ufuk Kaya**: data curation, formal analysis, investigation, methodology, writing–review and editing. **İrem Karaaslan**: methodology, formal analysis. **Hüseyin Özkan**: writing–review and editing. **Akın Yakan**: methodology, project administration, writing–original draft.

Acknowledgements

This study was financially supported by the Scientific and Technological Research Council of Türkiye (TUBİTAK) via project number 119R076.

Ethics Statement

The study was approved by the Erciyes University's Local Ethics Committee for Animal Experiments (date: 06.03.2019; decision number: 19/044).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data are available from the corresponding author.

References

Ahmad, S., L. K. Lyngman, M. Mansouryar, et al. 2020. "Depot and Sex-Specific Implications for Adipose Tissue Expandability and Functional Traits in Adulthood of Late Prenatal and Early Postnatal Malnutrition in a Precocial Sheep Model." *Physiological Reports* 8: e14600. http://doi.org/10.14814/phy2.14600.

Alshamiry, F. A., A. S. Alharthi, H. H. Al-Baadani, R. S. Aljumaah, and I. A. Alhidary. 2023. "Growth Rates, Carcass Traits, Meat Yield, and Fatty

Acid Composition in Growing Lambs Under Different Feeding Regimes." *Life* 13: 409. http://doi.org/10.3390/life13020409.

Botham, K. M., and P. A. Mayes. 2015. "Biosynthesis of Fatty Acids & Eicosanoids." In *Harper's Illustrated Biochemistry*, edited by M. Weitz and R. Y. Brown, 196–208. New York: McGraw-Hill Education.

British Department of Health. 1994. *Nutritional Aspects of Cardiovascular Disease*. Report on Health and Social Subjects No. 46. London, UK: Her Majesty's Stationery Office (HMSO), The National Archives.

Carvalho, V. B., R. F. Leite, M. T. C. Almeida, et al. 2015. "Carcass Characteristics and Meat Quality of Lambs Fed High Concentrations of Crude Glycerin in Low-Starch Diets." *Meat Science* 110: 285–292. https://doi.org/10.1016/j.meatsci.2015.08.001.

Cunha, C. M., A. R. M. Fernandes, T. C. Cornélio, et al. 2016. "Tissue Composition, Quality Traits and Fatty Acid Profile of Longissimus Muscle of Lambs Fed Increasing Levels of Crude Glycerin." *Boletim de Indústria Animal* 73: 310–318. https://doi.org/10.17523/bia.v73n4p310.

da Costa, C. A., G. P. de Andrade, M. do Vale Maciel, et al. 2020. "Meat Quality of Lambs Fed Crude Glycerin as a Replacement for Corn." *Small Ruminant Research* 192: 106245. https://doi.org/10.1016/j.smallrumres.2020.106245.

Daley, C. A., A. Abbott, P. S. Doyle, G. A. Nader, and S. Larson. 2010. "A Review of Fatty Acid Profiles and Antioxidant Content in Grass-Fed and Grain-Fed Beef." *Nutrition Journal* 9: 1–2. https://doi.org/10.1186/1475-2891-9-10.

de Lima Valença, R., A. G. da Silva Sobrinho, E. P. Romanzini, et al. 2020. "Peanut Meal and Crude Glycerin in Lamb Diets: Meat Quality and Fatty Acid Profile." *Small Ruminant Research* 185: 106076. https://doi.org/10.1016/j.smallrumres.2020.106076.

Fretts, A. M., D. Mozaffarian, D. S. Siscovick, et al. 2014. "Plasma Phospholipid Saturated Fatty Acids and Incident Atrial Fibrillation: The Cardiovascular Health Study." *American Heart Journal* 3: e000889. https://doi.org/10.1161/JAHA.114.000889.

Gascoigne, E., and F. Lovatt. 2015. "Lamb Growth Rates and Optimizing Production." *In Practice* 37: 401–414. https://doi.org/10.1136/inp.h4537.

Green, M. H. 2020. "Are Fatty Acids Gluconeogenic Precursors?" *Journal of Nutrition* 150: 2235–2238.

Halim, N. F. A. A., M. S. M. Ali, A. T. C. Leow, and R. N. Z. R. A. Rahman. 2022. "Membrane Fatty Acid Desaturase: Biosynthesis, Mechanism, and Architecture." *Applied Microbiology and Biotechnology* 106: 5957–5972. http://doi.org/10.1007/s00253-022-12142-3.

Hausman, G. J., U. Basu, S. Wei, D. B. Hausman, and M. V. Dodson. 2014. "Preadipocyte and Adipose Tissue Differentiation in Meat Animals: Influence of Species and Anatomical Location." *Annual Review of Animal Biosciences* 2: 323–351.

Horwitz, W., and G. W. Latimer. 2005. *Official Methods of Analysis of AOAC International*, 18th ed. Rockville, MD: AOAC International.

Hunter, J. E., J. Zhang, and P. M. Kris-Etherton. 2010. "Cardiovascular Disease Risk of Dietary Stearic Acid Compared With Trans, Other Saturated, and Unsaturated Fatty Acids: A Systematic Review." *American Journal of Clinical Nutrition* 91: 46–63. https://doi.org/10.3945/ajcn.2009.27661.

Juárez, M., A. Horcada, M. J. Alcalde, M. Valera, A. M. Mullen, and A. Molina. 2008. "Estimation of Factors Influencing Fatty Acid Profiles in Light Lambs." *Meat Science* 79: 203–210. https://doi.org/10.1016/j.meatsci.2007.08.014.

Khozin-Goldberg, I., S. Didi-Cohen, I. Shayakhmetova, and Z. Cohen. 2002. "Biosynthesis of Eicosapentaenoic Acid (EPA) in the Freshwater Eustigmatophyte *Monodus subterraneus* (Eustigmatophyceae)." *Journal of Phycology* 38: 745–756. https://doi.org/10.1046/j.1529-8817.2002.02006.x.

Kim, Y. K., H. Choi, and K. H. Myung. 2005. "Effects of Propylene Glycol on Carcass Traits and Its Related Gene Expression in Korean Native Steers." *Journal of Animal Science* 83: 344–349. https://doi.org/10.2527/2005.832344x.

Kong, Y., Z. Yuan, X. Liu, F. Li, and X. Yue. 2022. "A Novel SNP Within LIPE Gene Is Highly Associated With Sheep Intramuscular Fat Content." *Small Ruminant Research* 209: 106658. http://doi.org/10.1016/j.smallrumres.2022.106658.

Kapoor, B., D. Kapoor, S. Gautam, R. Singh, and S. Bhardwaj. 2021. "Dietary Polyunsaturated Fatty Acids (PUFAs): Uses and Potential Health Benefits." *Current Nutrition Reports* 10: 232–242.

Kumar, S., P. S. Banakar, A. K. Tyagi, and H. Sharma. 2022. "Intra-Species Variation in Fatty Acid Profile and Nutritional Indices of Cattle (*Bos indicus*), Buffalo (*Bubalus bubalis*) and Goat (*Capra hircus*) Ghee Deciphered Using GC-FID and FT-IR Spectroscopy." *International Dairy Journal* 129: 105342.

Lanza, M., C. Fabro, M. Scerra, et al. 2011. "Lamb Meat Quality and Intramuscular Fatty Acid Composition as Affected by Concentrates Including Different Legume Seeds." *Italian Journal of Animal Science* 10: e18. https://doi.org/10.4081/ijas.2011.e18.

Lemaitre, R. N., I. B. King, K. Rice, et al. 2014. "Erythrocyte Very Long-Chain Saturated Fatty Acids Associated With Lower Risk of Incident Sudden Cardiac Arrest." *Prostaglandins, Leukotrienes, and Essential Fatty Acids* 91: 149–153.

Lu, X., X. Zhao, J. Feng, et al. 2012. "Postprandial Inhibition of Gastric Ghrelin Secretion by Long-Chain Fatty Acid Through GPR120 in Isolated Gastric Ghrelin Cells and Mice." *American Journal of Physiology-Gastrointestinal and Liver Physiology* 303: G367–376. https://doi.org/10.1016/j.plefa.2014.07.010.

Martins, T. S., M. V. A. Lemos, L. F. Mueller, et al. 2018. "Fat Deposition, Fatty Acid Composition, and Its Relationship With Meat Quality and Human Health." In *Meat Science and Nutrition*, edited by M. S. Arshad, 17–38. London, UK: IntechOpen.

Nuernberg, K., G. Nuernberg, K. Ender, et al. 2005. "Effect of Grass vs. Concentrate Feeding on the Fatty Acid Profile of Different Fat Depots in Lambs." *European Journal of Lipid Science and Technology* 107: 737–745. https://doi.org/10.1002/ejlt.200501141.

Palacios, S., V. J. Starai, and J. C. Escalante-Semerena. 2003. "Propionyl Coenzyme A Is a Common Intermediate in the 1,2-propanediol and Propionate Catabolic Pathways Needed for Expression of the prpBCDE Operon During Growth of *Salmonella enterica* on 1,2-propanediol." *Journal of Bacteriology* 185: 2802–2810. https://doi.org/10.1128/jb.185.9.2802-2810.2003.

Poulos, S. P., D. B. Hausman, and G. J. Hausman. 2010. "The Development and Endocrine Functions of Adipose Tissue." *Molecular and Cellular Endocrinology* 323: 20–34.

Reddy, M. R. G., M. S. Kumar, V. Acharya, S. M. Venkata, U. K. Putcha, and S. M. Jeyakumar. 2019. "Vitamin A Deficiency Increases the Oleic Acid (C18: 1) Levels in the Kidney of High Fructose Diet-Fed Rats." *Indian Journal of Medical Research* 150: 620–629. https://doi.org/10.4103/ijmr.IJMR_1574_17.

Santos, A., F. J. Giráldez, E. Trevisi, L. Lucini, J. Frutos, and S. Andrés. 2018. "Liver Transcriptomic and Plasma Metabolomic Profiles of Fattening Lambs Are Modified by Feed Restriction During the Suckling Period." *Journal of Animal Science* 96: 1495–1507. https://doi.org/10.1093/jas/sky029.

Sayanova, O. V., and J. A. Napier. 2004. "Eicosapentaenoic Acid: Biosynthetic Routes and the Potential for Synthesis in Transgenic Plants." *Phytochemistry* 65: 147–158.

Schumacher, M., H. DelCurto-Wyffels, J. Thomson, and J. Boles. 2022. "Fat Deposition and Fat Effects on Meat Quality—A Review." *Animals* 12, no. 12: 1550.

Song, S. Z., J. P. Wu, S. G. Zhao, et al. 2017. "The Effect of Energy Restriction on Fatty Acid Profiles of Longissimus Dorsi and Tissue Adipose Depots in Sheep." *Journal of Animal Science* 95: 3940–3948. https://doi.org/10.2527/jas.2016.1235.

Terré, M., A. Nudda, P. Casado, and A. Bach. 2011. "The Use of Glycerine in Rations for Light Lamb During the Fattening Period." *Animal Feed*

Science and Technology 164: 262–267. https://doi.org/10.1016/j.anifeedsci.2010.12.008.

Trabue, S., K. Scoggin, S. Tjandrakusuma, M. A. Rasmussen, and P. J. Reilly. 2007. "Ruminal Fermentation of Propylene Glycol and Glycerol." *Journal of Agricultural and Food Chemistry* 55: 7043–7051. https://doi.org/10.1021/jf071076i.

TUIK. 2024. Statistical of Red Meat Production. https://data.tuik.gov.tr/Bulten/Index?p=Kirmizi-Et-Uretim-Istatistikleri-2022-49696.

Ulbricht, T. L. V., and D. A. T. Southgate. 1991. "Coronary Heart Disease: Seven Dietary Factors." *Lancet* 338: 985–992. https://doi.org/10.1016/0140-6736(91)91846-M.

Urrutia, O., J. A. Mendizabal, L. Alfonso, B. Soret, K. Insausti, and A. Arana. 2020. "Adipose Tissue Modification Through Feeding Strategies and Their Implication on Adipogenesis and Adipose Tissue Metabolism in Ruminants." *International Journal of Molecular Sciences* 21, no. 9: 3183.

Urrutia, O., J. A. Mendizabal, K. Insausti, B. Soret, A. Purray, and A. Arana. 2016. "Effects of Addition of Linseed and Marine Algae to the Diet on Adipose Tissue Development, Fatty Acid Profile, Lipogenic Gene Expression, and Meat Quality in Lambs." *PLoS ONE* 11, no. 6: e0156765. https://doi.org/10.1371/journal.pone.0156765.

Vacca, G. M., V. Carcangiu, M. L. Dettori, et al. 2008. "Productive Performance and Meat Quality of Mouflon×Sarda and Sarda×Sarda Suckling Lambs." *Meat Science* 80: 326–334. https://doi.org/10.1016/j.meatsci.2007.12.016.

Velasco, S., V. Cañeque, C. Pérez, et al. 2001. "Fatty Acid Composition of Adipose Depots of Suckling Lambs Raised Under Different Production Systems." *Meat Science* 59: 325–333. https://doi.org/10.1016/S0309-1740(01)00135-8.

Wood, J. D., M. Enser, A. V. Fisher, et al. 2008. "Fat Deposition, Fatty Acid Composition and Meat Quality: A Review." *Meat Science* 78: 343–358. https://doi.org/10.1016/j.meatsci.2007.07.019.

Xin, M., Y. Sun, H. Chen, et al. 2018. "Propylene Glycol Guluronate Sulfate (PGGS) Reduces Lipid Accumulation via AMP-Activated Kinase Activation in Palmitate-Induced HepG2 Cells." *International Journal of Biological Macromolecules* 114: 26–34.

Xu, H., X. Meng, Y. Wei, Q. Ma, M. Liang, and G. M. Turchini. 2022. "Arachidonic Acid Matters." *Reviews in Aquaculture* 14: 1912–1944. https://doi.org/10.1111/raq.12679.

Yakan, A., C. T. Ates, S. Alasahan, et al. 2016. "Damascus Kids' Slaughter, Carcass and Meat Quality Traits in Different Production Systems Using Antioxidant Supplementation." *Small Ruminant Research* 136: 43–53.

Yakan, A., H. Özkan, U. Kaya, et al. 2024. "The Effects of the Feeding Duration of Propylene Glycol on Major Meat Quality Parameters and Substantial Proteins in the Muscle of Akkaraman Lambs." *Meat Science* 217: 109615.

Yakan, A., and N. Ünal. 2010. "Meat Production Traits of a New Sheep Breed Called Bafra in Turkey 1. Fattening, Slaughter, and Carcass Characteristics of Lambs." *Tropical Animal Health and Production* 42: 751–759.

Zhang, F., X. Nan, H. Wang, Y. Zhao, Y. Guo, and B. Xiong. 2020. "Effects of Propylene Glycol on Negative Energy Balance of Postpartum Dairy Cows." *Animals* 10: 1526. https://doi.org/10.3390/ani10091526.