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Crude glycerin and waste sesame seed in the diets of growing lambs: impacts on growth performance, nutrient digestibility, ruminal fermentation, carcass characteristics, and meat fatty acid profile

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Abstract: The aim of the present study was to investigate the effects of a combination of crude glycerin and waste sesame seed on the growth performance, rumen fermentation, carcass characteristics, and meat fatty acid profile of lambs. Twenty-four male Karayaka lambs with initial body weights of 26.5 ± 0.53 kg were randomly divided into four groups and raised in individual pens. For 70 days, lambs were fed ad libitum the one of four concentrates: concentrate without CG and WSS (control), concentrate with 10% CG (G), concentrate with 10% WSS (W), and concentrate with 5% CG and 5% WSS (GW). All diets consisted of 85% concentrate and 15% forage (wheat straw). The dietary treatments did not affect dry matter intake, average daily gain, or feed efficiency. The rumen parameters and carcass measurements did not differ among the treatments. Greater levels of linoleic acid, total polyunsaturated fatty acids, and total n-6 fatty acids ($p < 0.001$) and lesser levels of margaric acid ($p = 0.008$) were detected in the W diet compared to the other diets. Overall, a combination of CG and WSS did not effectively increase the beneficial fatty acid content of lamb meat. However, both byproducts can partially replace grains as alternative energy sources in lambs' concentrates up to 10% without affecting growth performance, ruminal fermentation, and carcass characteristics.

Key words: Alternative feeds, fattening performance, glycerol, lamb meat, ruminal biohydrogenation, oilseed

1. Introduction

Unsaturated fatty acids (UFAs) have well-known positive effects on health, whereas saturated fatty acids (SFAs) are associated with diseases, such as cancer and cardiovascular diseases [1,2]. Many consumers consider ruminant meat to be unhealthy because of its high SFA content (e.g., palmitic and myristic acid), and this perception overshadows the health benefits of n-3 and n-6 polyunsaturated fatty acids (PUFAs) found in ruminant-derived fat. The high SFA levels in meat are partially related to ruminal biohydrogenation (BH), which is responsible for the saturation of UFAs and polyunsaturated fatty acids (PUFAs) in the rumen [3]. Nutrition is the main factor regulating the fatty acid profile of ruminants [4]. Dietary modifications, such as increasing UFAs and PUFAs in the diet, can enrich meat with beneficial fatty acids [5,6]. Several researchers have used PUFA-rich vegetable oils in ruminant diets to exceed the BH capacity and increase duodenal flow of PUFAs [7,8]. However, this nutritional strategy may not always be successful because ruminal fermentation and dry matter intake (DMI) could be adversely affected when the total dietary lipid exceeds 60–70 g/kg of dry matter [9]. These negative effects primarily

result from the toxicity of UFAs to ruminal microbiota, particularly to cellulolytic bacteria [9,10].

Inhibiting ruminal BH with glycerol is another strategy to increase the flow of PUFAs and UFAs to the duodenum without harming ruminal fermentation. Previous studies have reported that glycerol reduced rumen BH by partially inhibiting rumen bacterial lipases [11,12] and modulated the release of UFAs [13] in the rumen. Crude glycerin (CG) is a byproduct of biodiesel production that is mainly composed of glycerol. For each liter of biodiesel produced, 0.1 L of crude glycerin is generated [14]. According to OECD-FAO [15], global biodiesel production was 40 billion liters in 2020 and is expected to reach 46 billion liters by 2029. Additionally, Turkey's biodiesel production has increased 112% in the last five years, reaching 134 million liters in 2019 [16]. The oversupply of CG in the global market has led to a drop in CG prices from \$0.55 per liter to \$0.15 per liter [17]. Recently, CG has been applied as a cost-effective alternative energy source in ruminant diets [18,19].

Some researchers hypothesized that combining vegetable oils with CG could increase the flow of UFAs and

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PUFAs to the duodenum, thereby increasing the levels of beneficial fatty acids in meat [8,13]. Notably, the majority of these researchers used vegetable oils (e.g., soybean oil), which are expensive and potentially harmful to rumen microorganisms. In contrast, oilseeds are less detrimental to rumen microorganisms and tend to release their fatty acid content slower than vegetable oils [20]. Furthermore, oilseeds have been reported to bypass the rumen because of their seed coat, which partially protects them from ruminal BH [21].

Several studies have used oilseeds, such as soybeans, sunflower seeds, cottonseeds, rapeseeds, and linseeds to improve meat quality [6,22]. However, these oil sources are also used in human diets and may not be cost-effective in ruminant feeding. Thus, the use of local agro-industrial byproducts in ruminant diets gains importance [23]. Waste sesame seed (WSS) is a byproduct obtained during the sieving and sorting processes of sesame, and it is mainly composed of low-quality sesame seeds and some harvest residues. According to FAOSTAT¹, 200,000 t of sesame seeds are processed in Turkey annually. During the sesame processing, 2%–4% of the total weight is separated as WSS. The market price of WSS is currently 0.10–0.15 cents per kilogram, which is quite affordable compared to grain prices. Sesame seed contains 44%–58% oil on average, which consists of 45.4% and 36.1% monounsaturated fatty acids (MUFAs) and PUFAs, respectively [24]. Because WSS contains a high percentage of low-quality sesame seeds (e.g., broken or small-sized seeds), it has significant levels of n-6 PUFA such as linoleic acid (C18:2n-6), which may be advantageous to ruminant nutrition as an alternative lipid source [25].

We hypothesized that combining WSS with CG would reduce ruminal BH and increase the PUFA content of lamb meat. Therefore, we investigated whether a combination of CG and WSS in lambs' diet could effectively increase the duodenal flow of UFAs and PUFAs and thereby improve the fatty acid profile of lamb meat. We also explored whether CG or WSS could partially replace grains in lambs' diet without affecting growth performance, ruminal fermentation, and carcass characteristics.

2. Materials and methods

This study was conducted at a private farm located in Samsun Province, Turkey (41°41'50.3"N 35°52'13.5"E). The procedures and protocols used in this study were approved by the Local Ethics Committee of Ondokuz Mayıs University for Experimental Animals (HADYEK, approval number: 2019/11).

2.1. Animals, diets, and experimental design

Twenty-four male Karayaka lambs with average initial body weights of 26.5 ± 0.53 kg and aged 3–4 months were

used for the study. The animals were kept in individual pens (1.1 m²) with suspended slatted floors, and they were randomly assigned to one of the following four groups: concentrate without the inclusion of CG and WSS (control), concentrate with 10% CG (G), concentrate with 10% WSS (W), and concentrate with 5% CG and 5% WSS (GW). To adapt lambs to the high-concentrate diets, a 20-day step-up protocol was followed, gradually increasing concentrate levels from 30% to 85%. The study was then performed for 70 days.

All diets were formulated to have similar protein and energy contents to meet or to exceed the NRC [26] requirements for growing lambs (2.8 ME Mcal/kg DM, 16% CP of DM). The experimental diets consisted of 15% wheat straw and 85% concentrate. All diets were given as a total mixed ration and offered twice daily ad libitum at 8 a.m. and 6 p.m., respectively, with free access to fresh water. The CG was obtained from biodiesel production based on sunflower oil and was purchased from Aydınlar Kimya (İstanbul, Turkey), and contained 85.44% glycerol, 3.11% salt, <0.009% methanol, and 9.63% moisture. The wheat straw used in the study contained 96.6% dry matter, 3.78% crude protein, 1.17% ether extract (EE), and 78% neutral detergent fiber (NDF). The WSS was provided by a sesame seed processing factory that produced tahini. Tables 1 and 2 present the nutrient composition and fatty acid profile of the WSS, respectively.

2.2. Dry matter intake and growth performance

The amount of feed offered to the animals was calculated based on the previous days' intake, and, when necessary, adjustments were made to ensure that the refused feed did not exceed 10% of the daily intake. Feed refusals for each lamb were collected and weighed daily to calculate the total DMI. The feed refusals were then passed through 5 mm pore sieve to determine straw and concentrate intake separately. Grab samples of feed and feed refusals were collected throughout the experiment and stored at –20 °C until analysis. The lambs were weighed every 14 days after 12 h of fasting, and average daily weight gain (ADG) and feed efficiency (FE) were calculated.

2.3. Rumen fluid analysis

On the final day of the experiment, 100 mL of rumen fluid was sampled 2 h after the morning feeding with an esophageal probe and ruminal pH was measured immediately using a digital pH meter (S220-K, Mettler Toledo, Switzerland). The rumen liquid was then filtered through four layers of gauze, and two different subsamples (10 mL each) were placed in separate glass tubes. For the subsequent ammonia-nitrogen (NH₃-N) analysis, 0.2 mL of 50% (v/v) sulfuric acid was added to one subsample. For the volatile fatty acid (VFA) analysis, 2 mL of 25% metaphosphoric acid was added to the other

¹ FAOSTAT (2020) Food and Agriculture Data. Website <http://www.fao.org/faostat/en/#data/TP>. [accessed 30 March 2022].

Table 1. Ingredients and chemical composition of concentrates and waste sesame seed.

Items	Concentrates ^A				WSS ^B
	Control	G	W	GW	
<i>Ingredients, %</i>					
Corn	42	29	15	20	
Barley	24	20	33.5	30	
Sunflower meal	28	29.5	18.5	24	
Wheat bran	-	5.5	17	10	
Crude glycerin	-	10	-	5.0	
Waste sesame seed (WSS)	-	-	10	5.0	
Molasses	3.2	3.2	3.2	3.2	
Salt	0.7	0.7	0.7	0.7	
CaCO ₃	1.5	1.5	1.5	1.5	
Monocalcium phosphate	0.5	0.5	0.5	0.5	
Mineral-vitamin premix ^C	0.1	0.1	0.1	0.1	
<i>Chemical composition, % DM</i>					
Dry matter	88.74	87.01	89.96	89.03	95.4
Organic matter	91.86	92.73	91.30	92.02	84.2
Crude protein	17.91	17.69	17.70	17.87	16.9
Ether extract	2.31	2.08	5.61	3.75	35.8
Neutral detergent fiber (aNDF)	25.35	22.75	25.34	23.81	25.6
Acid detergent fiber (ADF)	12.35	12.06	12.28	11.96	22.5
Metabolisable energy ^D (Mcal/kg DM)	2.70	2.72	2.73	2.75	3.62

^A Control: Concentrate without crude glycerin and waste sesame seed; G: Concentrate with 10% crude glycerin; W: Concentrate with 10% waste sesame seed; GW: Concentrate with 5% crude glycerin + 5% waste sesame seed. ^B Waste sesame seed. ^C Each kg contained 50,000 mg Mn, 50,000 mg Fe, 50,000 mg Zn, 10,000 mg Cu, 150 mg Co, 150 mg Se, 800 mg I, 8,000,000 IU vitamin A, 2,000,000 IU vitamin D3, 20,000 mg vitamin E. ^D Calculated according to NRC [26].

subsample. The ammonia-nitrogen levels were determined according to the method of Markham [27], and the concentrations of the VFAs (mmol/L) were determined by gas chromatography (GC-17A, Shimadzu Co., Japan) equipped with a flame ionization detector according to the method of Erwin et al. [28]. The gas chromatography column (TRB-FFAP, Teknokroma, Spain) dimensions were 30 m × 0.53 mm i.d. × 0.5 µm film thickness, and the carrier gas was nitrogen at a constant flow rate of 5.05 mL/min. The gas chromatography oven temperature program was as follows: the initial temperature of 60 °C was maintained for 1 min and then ramped to 200 °C at a rate of 5 °C/min. The injector temperature was 250 °C, and the detector temperature was 300 °C. Samples were centrifuged at 3000 g for 10 min at 0 °C, and the resulting supernatant was transferred to vials after 0.22 µm filtration. A volume of 1 µL was injected in splitless mode with an autosampler (AOC-5000, Shimadzu Co., Japan). The identification of the VFAs

was accomplished by comparing the peak retention times of the samples to those of the analytical reference standards (Sigma-Aldrich GmbH, USA) of acetic acid (CAS 64-19-7), propionic acid (CAS 79-09-4), butyric acid (CAS 107-92-6), isobutyric acid (CAS 79-31-2), valeric acid (CAS 109-52-4), and isovaleric acid (CAS 503-74-2).

2.4. Carcass measurements

The slaughter weights of the lambs were measured after 16 h of fasting, and the lambs were slaughtered according to standard commercial procedures [29]. Immediately after dressing, hot carcass weights (HCW) was recorded with the kidneys included. The carcasses were chilled at 4 °C for 24 h, and then cold carcass weights (CCW) were measured. The chilling losses were calculated as the difference between hot (HCW) and cold carcass weights (CCW). Hot and cold carcass yields were calculated by dividing the HCW and CCW by the fasted body weights and multiplying by 100, respectively.

Table 2. Fatty acid composition of concentrates and waste sesame seed (% of total fatty acids).

Fatty acids	Concentrates ^A				WSS ^B
	Control	G	W	GW	
Myristic (C14:0)	0.14	0.40	0.24	0.26	-
Palmitic (C16:0)	13.6	16.0	17.2	16.4	12.7
Palmitoleic (C16:1)	0.11	0.15	0.17	0.22	-
Stearic (C18:0)	4.34	7.48	13.5	11.6	18.2
Oleic (C18:1)	31.5	28.5	24.7	25.6	29.4
Linoleic (C18:2)	46.6	44.3	40.1	42.3	38.2
α -Linolenic (C18:3)	2.17	2.28	2.62	2.43	1.07
Arachidic (C20:0)	0.45	0.28	0.30	0.27	-
SFA ^C	18.6	24.2	31.3	28.6	31.1
UFA ^D	80.4	75.3	67.6	70.5	68.7
MUFA ^E	31.6	28.7	24.9	25.8	29.5
PUFA ^F	48.8	46.6	42.7	44.7	39.2

^A Control: Concentrate without crude glycerin and waste sesame seed; G: Concentrate with 10% crude glycerin; W: Concentrate with 10% waste sesame seed; GW: Concentrate with 5% crude glycerin + 5% waste sesame seed. ^BWSS: Waste sesame seed. ^CSFA: Saturated fatty acids. ^DUFA: Unsaturated fatty acids. ^EMUFA: Monounsaturated fatty acids. ^FPUFA: Polyunsaturated fatty acids.

The initial pH of meat was measured with a digital pH-meter (Testo 205, Testo SE & Co., Germany) between the 12th and 13th ribs in the center of *longissimus dorsi* (LD) within 45 min after slaughter. The final pH of meat was measured at the same place after 24-h chilling at 4 °C. A cross-section cut was made between the 12th and 13th ribs of each carcass to expose the LD. Subcutaneous fat thickness measurements were taken at 3/4 of the length ventrally over the LD using a digital caliper with a precision of 0.01 mm (16EX, Mahr, Germany). *Longissimus dorsi* were sampled between the 12th and 13th ribs, vacuum-packed, and stored at -20 °C until analysis.

2.5. Chemical analysis

The composite samples of feed and feed refusals were dried at 55 °C for 72 h in a forced-air oven (UNB 100, Memmert, Germany) and then ground to pass through a 1-mm screen. The dry matter (DM) content was determined by drying the samples in an oven at 105 °C for 24 h [30] (method 925.09). Ash content and organic matter were determined by combusting the samples at 550 °C for 4 h in a muffle furnace (ELF 11/14B, Carbolite, UK) [30] (method 923.05). The crude protein was determined by the Kjeldahl procedure [30] (method 991.20). A Soxhlet extractor (B-811, BUCHI, Switzerland) was used to determine the EE content [30] (method 920.29). The neutral detergent fiber was determined using heat-stable alpha-amylase and sodium sulfite as described by Mertens [31]. Acid detergent

fiber (ADF) was determined according to AOAC [31] (method 973.18). The analysis of both NDF and ADF was performed using the fiber analyzer (Ankom 200, Ankom Technology Corporation, USA) and expressed inclusive of residual ash.

2.6. Fatty acid analysis

Lipids were extracted from the meat and feed samples as described by Bligh and Dyer [32], and fatty acid methyl esters (FAMES) were obtained according to the UNE-EN ISO 5509 [33] method. The FAMES were analyzed with a gas chromatography-mass spectrometry instrument (HP 6890, Agilent, USA). The column (HP-88, Agilent, USA) dimensions were 100 m \times 0.25 mm i.d. \times 0.20 μ m. The carrier gas was helium at a constant flow rate of 1 mL/min. The gas chromatography oven temperature program began at 150 °C, held this temperature for 1 min, and then ramped to 240 °C at 3°C/min. The injector temperature was 250 °C, and the detector temperature was 270 °C. A volume of 1 μ L was injected automatically in a split mode (1:50). The methyl esters of the fatty acids were identified by comparing the sample peak retention times with those of FAME standard mixtures (Supelco 37 Component FAME-Mix, Sigma-Aldrich GmbH, USA).

2.7. Statistical analysis

The data were analyzed with SPSS 14.01 (IBM, USA). Considering type I error (alpha to be 0.05) and power (beta error to be 0.90), it was calculated to use 6–7

animals per group (PS Sample Size and Power Calculator, Vanderbilt University, TN). In a randomized complete block design, an animal within an individual pen served as the experimental unit ($n = 6$ per treatment). All results were expressed as mean \pm SEM (the standard error of the mean). Data were checked for normality and homogeneity using the Shapiro-Wilk and Levene's tests. The parametric data were analyzed using the ANOVA test with Tukey's multiple comparison. Nonparametric data (rumen volatile fatty acid concentrations) were analyzed by the Kruskal-Wallis test with Bonferroni correction. Differences were considered significant at $p < 0.05$, and $0.05 < p < 0.10$ were interpreted as tendencies.

3. Results

3.1. Dry matter intake and performance

The inclusion of CG and/or WSS in the lambs' diets did not affect DMI, final body weight, and ADG ($p \geq 0.350$; Table 3). Likewise, feed efficiency and intakes were similar ($p > 0.05$) for all dietary treatments (Table 3).

3.2. Ruminal fermentation

The ruminal pH were similar for all dietary treatments ($p = 0.660$; Table 4). The ruminal NH₃-N concentration tended to decrease in lambs fed W and GW diets ($p = 0.058$). The ruminal VFA concentrations, including the levels of acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate ($p \geq 0.580$), as well as the acetate-to-propionate (C2/C3) ratio ($p = 0.496$) were not affected by dietary treatments.

3.3. Carcass measurements

Carcass weight, carcass yield, and subcutaneous fat thickness were similar for all dietary treatments ($p \geq 0.429$; Table 5). The inclusion of WSS or CG did not affect the initial ($p = 0.191$) and final pH ($p = 0.279$) of LD.

3.4. Fatty acid profile

The GW group had greater levels of pentadecanoic acid (C15:0) ($p = 0.046$) than control group. The W group had a greater concentration of linoleic acid ($p < 0.001$) and a lesser concentration of margaric acid ($p = 0.008$) compared to the other groups (Table 6). All other fatty acids detected in the LD were not affected by the dietary treatments ($p > 0.05$).

The inclusion of CG or WSS in the lambs' diets did not affect the total proportions of SFA, MUFA, or UFA ($p \geq 0.181$; Table 7). The W group had greater total PUFA ($p < 0.001$) and total n-6 fatty acid ($p < 0.001$) levels as well as a greater PUFA/SFA ratio ($p = 0.006$) than the control group.

4. Discussion

4.1. Dry matter intake and performance

Studies have often reported that lipid supplementation in ruminant diets negatively affected DMI and performance, depending on the type and amount of the lipid [7,10]. Unsaturated fatty acids, for instance, could harm cellulolytic bacteria in the rumen and suppress fiber digestion [3]. Fiorentini et al. [8] reported that a combination of CG and soybean oil in the diet of Nellore bulls increased FE and ADG, but they also noted that DMI decreased significantly with the addition of soybean oil due to the high dietary EE level (88.7 g EE/kg DM). On the other hand, we observed that the addition of WSS to lambs' diets did not affect DMI and performance, which could be explained by the reasonable amount of the lipid added to the diet (<56.1 g EE/kg DM) and the seed form of the WSS. Similarly, Parvar et al. [34], who supplemented lambs' diet with rapeseed oil, soybean oil, and fish oil (54 g EE/kg DM), and Ghafari et al. [35], who supplemented lambs' diets with sesame oil (< 50 g EE/kg DM), have

Table 3. Feed intake and growth performance of lambs fed concentrates containing waste sesame seed and/or crude glycerin.

Item	Diets ^A				P
	Control	G	W	GW	
Average initial weight, kg/day	26.4 \pm 1.12	26.5 \pm 1.22	26.6 \pm 1.13	26.6 \pm 1.12	0.989
Average final weight, kg/day	41.0 \pm 1.74	41.3 \pm 1.68	42.2 \pm 1.74	42.8 \pm 1.48	0.859
Average daily weight gain, g/day	208 \pm 10.90	211 \pm 10.90	224 \pm 10.90	231 \pm 20.50	0.706
Concentrate intake, g of DM/day	1178 \pm 43.80	1131 \pm 43.80	1194 \pm 43.80	1256 \pm 43.80	0.277
Straw intake, g of DM/day	91.9 \pm 5.50	88.6 \pm 5.50	84.9 \pm 5.50	91.7 \pm 5.50	0.780
Total dry matter intake, g of DM/day	1270 \pm 48.90	1219 \pm 48.90	1279 \pm 48.90	1347 \pm 48.90	0.350
Feed efficiency (g feed/g gain)	6.11 \pm 0.37	5.77 \pm 0.37	5.72 \pm 0.37	5.83 \pm 0.37	0.824

^A Control: Concentrate without crude glycerin and waste sesame seed; G: Concentrate with 10% crude glycerin; W: Concentrate with 10% waste sesame seed; GW: Concentrate with 5% crude glycerin + 5% waste sesame seed.

Table 4. Ruminal fermentation parameters of lambs fed concentrates containing waste sesame seed and/or crude glycerin.

Item	Diets ^A				p
	Control	G	W	GW	
Rumen pH	5.76 ± 0.08	5.81 ± 0.04	5.90 ± 0.06	5.88 ± 0.06	0.660
Ammonia-N, mg/dL	21.7 ± 1.8	23.4 ± 1.1	20.6 ± 0.6	19.3 ± 1.7	0.058
VFA ^B concentration (mM/L)					
Acetate (C2)	18.4 ± 5.87	14.2 ± 1.93	13.9 ± 2.27	12.9 ± 1.29	0.960
Propionate (C3)	10.3 ± 2.59	10.7 ± 2.66	7.81 ± 1.66	7.63 ± 1.13	0.824
Butyrate	3.04 ± 1.38	2.25 ± 0.67	2.35 ± 0.85	1.87 ± 0.25	0.845
Isobutyrate	0.59 ± 0.2	0.55 ± 0.08	0.47 ± 0.12	0.44 ± 0.14	0.992
Valerate	0.51 ± 0.17	0.37 ± 0.04	0.56 ± 0.18	0.31 ± 0.02	0.580
Isovalerate	0.39 ± 0.13	0.33 ± 0.45	0.42 ± 0.13	0.24 ± 0.01	0.676
Total VFA	33.3 ± 9.4	28.5 ± 5.05	25.6 ± 4.43	23.4 ± 2.18	0.660
Molar proportions, % of total VFA					
Acetate (C2)	53.5 ± 2.31	51.3 ± 2.33	55.0 ± 0.84	55.2 ± 1.86	0.484
Propionate (C3)	33.1 ± 3.47	36.3 ± 3.11	30.8 ± 3.08	31.9 ± 2.82	0.632
Butyrate	8.69 ± 1.82	7.65 ± 1.87	8.77 ± 2.12	8.54 ± 1.76	0.973
Isobutyrate	1.66 ± 0.16	2.02 ± 0.21	1.72 ± 0.27	1.80 ± 0.48	0.845
Valerate	1.78 ± 0.40	1.42 ± 0.20	2.06 ± 0.40	1.40 ± 0.17	0.428
Isovalerate	1.21 ± 0.18	1.21 ± 0.12	1.58 ± 0.29	1.09 ± 0.10	0.321
C2:C3 ^C	1.74 ± 0.31	1.48 ± 0.19	1.89 ± 0.26	1.81 ± 0.12	0.496

^A Control: Concentrate without crude glycerin and waste sesame seed; G: Concentrate with 10% crude glycerin; W: Concentrate with 10% waste sesame seed; GW: Concentrate with 5% crude glycerin + 5% waste sesame seed.

^BVFA: Volatile fatty acids. ^C C2:C3: Acetate to propionate ratio.

reported that DMI and performance were not affected by oil supplementation. Nevertheless, lipid supplementation could cause negative effects on the DMI when dietary EE content exceeds 70 g/kg of DM [10,36].

Glycerol regulates the release of UFAs in the rumen, thereby reducing the toxic effects of fatty acids on cellulolytic bacteria, especially when added to diets with high fat contents [13,37]. We did not observe any difference in DMI and other performance parameters when using CG with or without WSS. On the other hand, many studies have reported that the addition of CG to ruminant diets negatively affected ruminal fermentation and, consequently, can decrease DMI and fattening performance [38,39]. However, these adverse effects are generally observed when the CG concentration exceeds 10% of the dietary DM.

4.2. Rumen fermentation

The average ruminal pH of all groups remained below the optimum range (pH > 6.0) for the activity of cellulolytic bacteria and ciliated protozoa [40]. This was due to a high concentrate level in experimental diets. Our findings agree

with those of other studies indicating that high concentrate levels in ruminants' diet result in a low ruminal pH [41,42].

In all experimental groups, the ruminal NH₃-N levels were in the range of 15–29 mg/dL, which is considered to be ideal for ruminal fermentation [43]. Notably, W and GW groups tended to present lesser NH₃-N levels compared to the other dietary treatments. This could be related to the inhibition of ciliated protozoa activity in the rumen (i.e. defaunation) due to the increased levels of dietary fat, which also improves nitrogen utilization by rumen bacteria [44]. Similarly, Granja-Salcedo et al. [36] reported that animals fed diets containing both CG and oil had the lowest NH₃-N levels. The authors claimed that the combination of CG and oil provided better nitrogen and energy synchronization in the rumen, which could result in increased nitrogen assimilation by the ammonia-utilizing species.

Several studies indicated that the addition of glycerol to ruminant diets increased the propionate concentration and decreased the acetate/propionate ratio [39,42] which was not observed in the present experiment. In the case

Table 5. Carcass measurements of lambs fed concentrates containing waste sesame seed and/or crude glycerin.

Item	Diets ^A				p
	Control	G	W	GW	
Hot carcass weight (kg)	20.71 ± 0.89	20.73 ± 0.91	21.48 ± 0.96	21.9 ± 0.67	0.712
Cold carcass weight (kg)	20.32 ± 0.87	20.34 ± 0.9	21.03 ± 0.93	21.5 ± 0.64	0.710
Hot carcass yield (%)	50.49 ± 0.39	50.1 ± 0.6	50.78 ± 0.51	51.19 ± 0.31	0.429
Cold carcass yield (%)	49.57 ± 0.4	49.16 ± 0.6	49.72 ± 0.54	50.26 ± 0.37	0.462
Carcass chilling loss (kg)	0.38 ± 0.03	0.39 ± 0.01	0.45 ± 0.06	0.4 ± 0.04	0.603
Subcutaneous fat thickness (mm)	6.66 ± 0.31	6.61 ± 0.44	6.92 ± 0.42	7.21 ± 0.49	0.736
Initial pH	6.12 ± 0.09	6.2 ± 0.13	6.06 ± 0.16	6.43 ± 0.07	0.191
Final pH	5.59 ± 0.02	5.64 ± 0.02	5.62 ± 0.02	5.66 ± 0.03	0.279

^A Control: Concentrate without crude glycerin and waste sesame seed; G: Concentrate with 10% crude glycerin; W: Concentrate with 10% waste sesame seed; GW: Concentrate with 5% crude glycerin + 5% waste sesame seed.

of dietary lipid supplementation, vegetable oils generally reduce the molar proportions of acetate, butyrate, and total VFA by inhibiting the activity of cellulolytic bacteria [45,46]. Nevertheless, the structure and fatty acid content of the lipids may differ in their effects on ruminal fermentation and VFA proportions. Oilseeds, for instance, have limited effects on rumen fermentation and VFA concentration owing to their naturally protected form in the rumen. In our study, we found that the addition of WSS did not affect ruminal VFA concentrations. These results might be attributed to two possible reasons; the dietary EE levels were held below 7%, which is the maximum recommended fat level for ruminants, and the seed form of the WSS. Finally, the lack of differences in ruminal fermentation parameters might be explained by all groups being fed diets with similar protein and energy content and consuming comparable amounts of roughage.

4.3. Carcass measurements

Carcass yields can vary depending on factors such as diet, slaughter weight, carcass fat, noncarcass portions, sex, age, and breed [47]. For example, adding oil sources to ruminant diets could increase carcass fatness and yield [48]. On the other hand, adding crude glycerin to ruminant diets could increase noncarcass components by further developing organs such as the liver and thereby reducing the carcass yield [49]. However, we did not observe those effects when CG or WSS was added to diets possible due to the feeding level. On the other hand, carcass yields for all groups were greater than those of other studies that evaluated the carcass yields of Karayaka lambs at similar slaughter weights [50,51]. The observed improvement in carcass yields could be attributed to the high concentrate levels in the diets [52].

Postmortem glycogen content is quite influential on the final pH of the meat [8]. If animals are subjected to

preslaughter stress, the glycogen stores in the meat could be depleted [49]. In our study, meat acidification occurred as expected (pH 5.4–5.8), indicating that all animals had sufficient glycogen reserves and did not experience preslaughter stress.

4.4. Meat fatty acid profile

Odd chain fatty acids (OCFAs) (e.g., C15:0 [pentadecanoic acid] and C17:0 [margaric acid]) are mainly produced by rumen microorganisms and are considered to be beneficial to human health [53]. Glycerol may stimulate the production of OCFAs by increasing the available primers (e.g., propionate) for rumen microorganisms [54]. Similarly, we found that the GW group had greater C15:0 levels, and the G group had greater C17:0 levels. Our findings agreed with those of other studies indicating that CG increases C15:0 and C17:0 levels [49,55]. The decrease in C17:0 levels in the W group may be due to some rumen bacteria involved in C17:0 synthesis being adversely affected by the high EE level. Likewise, Parvar et al. [34] and Ghafari et al. [35] showed that C17:0 levels decreased with increasing EE levels in lambs' diet.

In the current study, palmitic (C16:0), stearic (C18:0), and myristic (C14:0) acids were the most abundant SFAs in the meat. Among SFAs, palmitic and myristic acids attract the most attention because of their hypercholesterolemic effect [56]. Therefore, these fatty acids should be reduced in meat to support human health. On the contrary, stearic acid does not have this characteristic, and it is rapidly converted by Δ^9 - desaturase to oleic acid, which exhibits hypocholesterolemic activity. Edwards et al. [12] hypothesized that glycerol could reduce the SFA concentration in meat by inhibiting lipolysis and ruminal BH. In accordance with that hypothesis, Carvalho et al. [49] and Costa et al. [18] reported that the addition

Table 6. Fatty acid composition in the *longissimus dorsi* of lambs fed concentrates containing waste sesame seed and/or crude glycerin (% of total fatty acids).

Fatty acids		Diets ^A				p
		Control	G	W	GW	
<i>SFA</i> ^B						
Capric	C10:0	0.17 ± 0.01	0.20 ± 0.01	0.15 ± 0.02	0.17 ± 0.01	0.140
Lauric	C12:0	0.20 ± 0.04	0.21 ± 0.04	0.24 ± 0.03	0.27 ± 0.05	0.689
Myristic	C14:0	3.44 ± 0.37	3.44 ± 0.34	3.54 ± 0.29	3.44 ± 0.48	0.996
Pentadecanoic	C15:0	0.45 ± 0.03 ^a	0.54 ± 0.03 ^{ab}	0.54 ± 0.05 ^{ab}	0.63 ± 0.04 ^b	0.046
Palmitic	C16:0	25.2 ± 0.43	23.4 ± 0.59	24.7 ± 0.78	25.1 ± 0.84	0.183
Margaric	C17:0	1.51 ± 0.05 ^a	1.77 ± 0.07 ^a	1.11 ± 0.12 ^b	1.58 ± 0.11 ^{ab}	0.008
Stearic	C18:0	12.7 ± 0.34	11.8 ± 0.6	13.2 ± 0.88	12.9 ± 0.66	0.457
<i>MUFA</i> ^C						
Myristoleic	C14:1	0.25 ± 0.03	0.25 ± 0.03	0.36 ± 0.04	0.34 ± 0.03	0.078
Palmitoleic	C16:1	3.61 ± 0.07	3.48 ± 0.1	3.36 ± 0.1	3.39 ± 0.12	0.257
Heptadecenoic	C17:1	1.39 ± 0.1	1.38 ± 0.08	0.98 ± 0.12	1.31 ± 0.03	0.094
Oleic	C18:1	44.6 ± 0.83	45.7 ± 0.93	42.1 ± 1.35	43.7 ± 1.2	0.260
Eicosenoic	C20:1	0.08 ± 0.06	0.15 ± 0.02	0.11 ± 0.03	0.09 ± 0.03	0.431
<i>PUFA</i> ^D						
Linoleic	C18:2	4.10 ± 0.05 ^a	4.99 ± 0.45 ^{ac}	6.84 ± 0.23 ^b	5.12 ± 0.24 ^c	< 0.001
α-Linolenic	C18:3	0.89 ± 0.02	1.03 ± 0.03	1.25 ± 0.13	1.18 ± 0.17	0.120
γ-Linolenic	C18:3	0.26 ± 0.04	0.45 ± 0.13	0.39 ± 0.04	0.38 ± 0.06	0.133
Eicosadienoic	C20:2	0.07 ± 0.03	0.13 ± 0.02	0.15 ± 0.03	0.1 ± 0.02	0.302
Eicosatrienoic	C20:3	0.06 ± 0.03	0.12 ± 0.02	0.1 ± 0.04	0.1 ± 0.02	0.505
Arachidonic	C20:4	0.51 ± 0.05	0.44 ± 0.06	0.42 ± 0.1	0.51 ± 0.12	0.760
^E EPA	C20:5	0.1 ± 0.05	0.23 ± 0.05	0.12 ± 0.04	0.22 ± 0.08	0.326
^F DPA	C22:5	0.06 ± 0.03	0.07 ± 0.02	0.11 ± 0.06	0.11 ± 0.05	0.824
^G DHA	C22:6	0.07 ± 0.02	0.08 ± 0.01	0.11 ± 0.06	0.15 ± 0.07	0.685

a, b, c: means within a row with different superscript differ significantly ($p < 0.05$). ^AControl: Concentrate without crude glycerin and waste sesame seed; G: Concentrate with 10% crude glycerin; W: Concentrate with 10% waste sesame seed; GW: Concentrate with 5% crude glycerin + 5% waste sesame seed.

^BSFA: Saturated fatty acids. ^CMUFA: Monounsaturated fatty acids. ^DPUFA: Polyunsaturated fatty acids.

^EEPA: Eicosapentaenoic acid. ^FDPA: Docosapentaenoic acid. ^GDHA: Docosahexaenoic acid.

of CG to lambs' diet reduced the concentrations of palmitic, myristic, and stearic acids and increased the concentration of oleic acid in meat by inhibiting ruminal BH. Meanwhile, feeding neither CG nor WSS altered the concentrations of palmitic, stearic, myristic, and oleic acid in meat. Additionally, all experimental groups presented low ruminal pH, which could result in decreased BH and lipolysis. Therefore, low pH might have hindered glycerol's effect on BH and lipolysis. Indeed, glycerol may be more effective as a BH inhibitor, particularly in a forage-based diet, which presents a higher ruminal pH.

Essential fatty acids (e.g., linoleic acid and α-linolenic acid) cannot be synthesized by the animals and must be received via diet [56]. Notably, most of the dietary essential fatty acids are converted to MUFAs and SFAs by ruminal microorganisms. Thus, only a small proportion can be incorporated into tissue lipids [57]. To increase the beneficial fatty acids in meat, we used CG as a BH inhibitor and WSS as a PUFA source in our experimental diets. Indeed, linoleic acid concentration increased in both the W and GW groups compared to the control group, suggesting that WSS was the main factor in increased

Table 7. The proportions of meat fatty acids in the *longissimus dorsi* of lambs fed concentrates containing waste sesame seed and/or crude glycerin.

Fatty acids	Diets ^A				p
	Control	G	W	GW	
Total SFA ^B	43.8 ± 0.94	41.4 ± 1.08	43.5 ± 1.58	43.2 ± 1.76	0.482
Total MUFA ^C	50.0 ± 0.85	51.0 ± 0.85	46.9 ± 1.42	48.8 ± 1.23	0.181
Total PUFA ^D	6.12 ± 0.21 ^a	7.54 ± 0.44 ^a	9.49 ± 0.37 ^b	7.85 ± 0.61 ^{ab}	<0.001
Total UFA ^E	56.1 ± 0.94	58.5 ± 1.08	56.4 ± 1.58	56.7 ± 1.76	0.482
PUFA/ SFA	0.14 ± 0.01 ^a	0.18 ± 0.01 ^{ab}	0.22 ± 0.02 ^b	0.18 ± 0.02 ^{ab}	0.006
UFA/SFA	1.29 ± 0.05	1.42 ± 0.06	1.31 ± 0.09	1.33 ± 0.09	0.488
Total n-6	5.06 ± 0.18 ^a	6.2 ± 0.37 ^a	8.01 ± 0.29 ^b	6.29 ± 0.38 ^a	<0.001
Total n-3	1.06 ± 0.04	1.34 ± 0.08	1.48 ± 0.14	1.56 ± 0.31	0.076
n-6/n-3	4.76 ± 0.11	4.64 ± 0.17	5.63 ± 0.5	4.48 ± 0.53	0.398

a, b, c: means within a row with different superscript differ significantly ($p < 0.05$).

^AControl: Concentrate without crude glycerin and waste sesame seed; G: Concentrate with 10% crude glycerin; W: Concentrate with 10% waste sesame seed; GW: Concentrate with 5% crude glycerin + 5% waste sesame seed. ^BSFA: Saturated fatty acids. ^CMUFA: Monounsaturated fatty acids. ^DPUFA: Polyunsaturated fatty acids.

^EUFA: Unsaturated fatty acids.

linoleic acid concentration. Therefore, increased linoleic acid levels in these groups could be attributed to the high n-6 PUFA levels in WSS rather than BH inhibition by CG. Similarly, Fiorentini et al. [8] reported that supplementing ruminant diets with PUFA-rich oil (soybean oil) increased linoleic acid concentration (C18:2n-6). On the other hand, α -linolenic acid concentrations were similar for all dietary treatments, possibly because the concentrates contained comparable amounts of α -linolenic acids [8].

The long-chain PUFAs (C:20 - C:22) are derived from linoleic acid and α -linolenic acid by the activity of desaturase and elongase enzymes. Adding n-3 PUFA-rich ingredients, such as fish oil or flaxseed, to ruminant diets could increase the concentration of long-chain fatty acids in meat [34]. We found that the concentrations of eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid were not affected by the dietary treatments. This may be explained by the low content of n-3 PUFA in all diets, and these findings are consistent with studies that added glycerol [49,58] and oil [7,22] to lambs' diet.

The PUFA/SFA and n-6/n-3 ratios are commonly used as indices to assess the nutritional quality of fat for consumer health. Lamb meat contains high levels of SFA and has a low PUFA/SFA ratio, and the addition of oilseeds to lambs' diet generally increases the PUFA/SFA ratio by increasing the PUFA concentration [22]. Similarly, we observed that WSS increased the PUFA, although we also found that the PUFA/SFA ratios for all groups were below 0.45, which is the minimum value recommended by WHO

[59] for human diets. While increasing the levels of PUFAs in ruminant meat is an important objective, this increase should be in favor of n-3 PUFAs. WHO [59] recommends achieving a ratio of n-6/n-3 fatty acids between 4:1 and 5:1 in the human diet. In this study, we noted that the n-6/n-3 ratios were within the recommended range for all groups except the W group, which had a greater ratio because of the higher levels of linoleic acid in the meat. The addition of linoleic acid-rich oil sources (e.g., soybean oil and sunflower oil) to ruminant diets generally increases the n-6/n-3 ratio [5,6].

5. Conclusion

The combined use of WSS and CG in lambs' diet did not effectively improve the fatty acid profile of lamb meat. However, the inclusion of WSS at 10% in lambs' diet might be a good nutritional strategy to improve their meat fatty acid profile, as indicated by the increases in the proportion of linoleic acid (C18:2n-6), total PUFA and the PUFA/SFA ratio. Additionally, both byproducts can partially replace cereals as an alternative energy source in lambs' diet without affecting growth performance, ruminal fermentation, and carcass characteristics.

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Conflict of interest

The authors declare no conflicts of interest. This study is a part of the first author's ongoing PhD dissertation.

Informed consent

The procedures and protocols used in this study were approved by the Local Ethics Committee of Ondokuz Mayıs University for Experimental Animals (HADYEK, approval number: 2019/11).

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