Utilizing Grape Pomace in Fattening Lamb: Impacts on Growth, Blood Chemistry and Meat Quality

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SUMMARY

Although grape pomace has been used in ruminant feeding for a long time, further research is needed to determine the method of administration, application amounts, feeding duration, species tolerance, assimilation capacity, and its relationships with other feed ingredients. Various plant materials used in ruminant feeding are sources of phenolic compounds that can be considered natural antioxidants to preserve and improve meat quality. This study investigated the effect of adding different levels of grape pomace to lamb diets on fattening performance, blood biochemical parameters and meat quality. In the study, 20 Akkaraman male lambs with an average age of 96.4 ± 2.98 days (mean±standard error) and an average live weight of 43.3 ± 1.15 kg were divided into four groups and fed with total mixed rations (TMR) containing 0% (control), 5%, 10%, and 15% grape pomace (determined from the average feed intake during the adaptation period). The addition of grape pomace did not affect the dry matter intake of the lambs ($p > 0.05$), while the highest final live weight ($p < 0.05$) was observed in the control group. No differences were found between the groups in average daily live weight gain and feed efficiency ($p > 0.05$). No differences were found between the groups in blood biochemical parameters, blood fatty acid profile, carcass weights, carcass yield, pH, color, or meat quality characteristics measured at different storage (days 1, 4, 8, 12) times ($p > 0.05$). The addition of 15% grape pomace to the ration increased the total polyunsaturated fatty acid content of the Longissimus dorsi muscle ($p < 0.05$). The results indicate that 15% grape pomace can be used as a feed ingredient in lamb rations without adverse effects on fattening performance, blood parameters, and meat quality characteristics, providing a strategy to reduce production costs and utilizing by-products that have negative environmental impacts.

KEY WORDS

Grape pomace; lamb fattening; meat quality; fatty acid profile; biochemical parameters.

INTRODUCTION

Red meat is a source of high-quality protein, minerals, vitamins, and essential fatty acids necessary for human dieatary [1]. Although different factors such as genetics and breeding methods can affect meat quality, the consumed feed may provide the expected characteristics for a good product [2]. Various plant materials used in ruminant feeding are sources of phenolic compounds that can be considered natural antioxidants to preserve and improve meat quality [3, 4].

Rations rich in bioactive compounds show the ability to manipulate ruminal biohydrogenation by leading to changes in the rumen microorganism population [5]. Specifically, flavonoids, phenolic acids and tannins found in grape by-products can inhibit the effect of microorganisms involved in the biohydrogenation of PUFAs, causing their accumulation and increased rumen extravasation [6, 7]

Grape (*Vitis vinifera*) is one of the most produced fruits in the world [8] with a production of more than 100 million tons [9]. Turkey produces more than 4 million tons and ranks 6th in the world with this production [10]. Grapes are used for table, drying and wine. Accordingly, a large amount of by-products are produced. Grape pomace, a significant by-product of the wine industry, is formed during the pressing of grapes and contains skins, seeds, and stems. Approximately 20% of the weight of processed grapes is extracted as grape pomace [11]. Grape pomace contains oil characterized by high linoleic acid content and the presence of - and -tocotrienols and tocopherols, which exhibit strong antioxidant activity [12]. Including grape pomace in lamb feed can improve the production system by reducing producer costs and utilizing by-products that negatively impact the environment. Additionally, grape pomace can modulate fatty acid accumulation in meat and enhance the oxidative stability of the meat [13].

In studies on the use of grape pomace in lambs [14-15-16], more emphasis has been placed on performance, while meat quality and fatty acid profile have been addressed to a limited extent. Furthermore, to the best of our knowledge, the effect of grape pomace use on blood fatty acid and biochemistry parameters has not been studied. Therefore, in this study, it was

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hypothesized that feeding grape pomace to fattening lambs may affect performance and that fatty acid composition of grape pomace may alter blood and meat fatty acid composition. This study aimed to determine the effect of adding grape pomace to the ration on fattening performance, blood biochemical parameters, and meat quality.

MATERIALS AND METHODS

The experiment was conducted in the semi-open, slatted floor pen at the Small Ruminant Breeding Unit of Niğde Ömer Halisdemir University Ayhan Şahenk Agricultural Research and Application Center (37°52'38"N 34°35'02"E and 1174 m altitude). The animal study protocol was approved by the Niğde Ömer Halisdemir University Ayhan Şahenk Agricultural Research and Application Center Animal Experiments Local Ethics Committee (protocol code: 2023/04 and date of approval: 17 January 2023). The grape pomace was obtained from the Sergi Karası genotype with dark red color, which is one of the local genotypes (Kemerhisar/Niğde/Turkey) and dried in an environment without sunlight. Akkaraman lambs were used in the study. Akkaraman lambs can be fattened at approximately 2.5 - 3.0 months of age with a live weight of 20 - 25 kg [17]. Fattening period is 80-85 days and daily live weight gain is 250 - 255 g [17].

Animal Housing and Feeding

In the study, 20 Akkaraman male lambs with an average age of 96.4 ± 2.98 days (mean ± standard error) and an average live weight of 43.3 ± 1.15 kg were used. All animals were treated against clostridial infections (Coglavax Ceva-Phylaxia, Hungary) and internal-external parasites (Dektomax, Zoetis, Guarulhos, Sao Paulo, Brazil).

The lambs were housed in individual stalls of 2.16 m² during a 7-day adaptation period and a 38-day experimental period. After the adaptation period, the lambs were fed with total mixed rations (TMR) containing 0% (control), 5% (GP5), 10% (GP10) and 15% (GP15) grape pomace. The grape pomaces were added to the ration at levels determined from the average feed intake during the adaptation period. The TMR had a roughage: concentrate ratio of 25:75, using alfalfa hay and lamb fattening feed (16.8% crude protein and 2850 ME kcal/kg). Water was provided as ad-libitum and TMRs were given three times a day (morning, noon, and evening) ensuring that 5-10% of the feed remained in the feeders.

Animal Performance and Rations

Dry matter intake was determined on a daily basis. After the adaptation period, the lambs were weighed to determine their initial live weights.

The lambs were weighed weekly to determine individual weight gains. Daily live weight gain was obtained by dividing the total live weight gain by the 38-day experimental period. Feed efficiency was calculated by dividing individual dry matter intake by weight gain.

Nutrient composition of the rations used in the study is presented in Table 1.

Table 1 - Ingredients, proximate composition, and fatty acid profile of concentrate, alfalfa hay, and grape pomacea[a]

Ingredients Concentrate	%		
Corn	15.0		
Barley	40.0		
Wheat	15.9		
Soybean Meal	10.0		
Sunflower Seed Meal	15.0		
Limestone	3.0		
Di calcium phosphate (DCP)	0.5		
Vitamin Premix	0.1		
Mineral Premix	0.1		
Salt	0.4		
Nutrient composition	Concentrate	Alfalfa hay	Grape pomace
Dry matter	90.4	91.0	87.4
Crude protein	16.8	17.3	2.1
Starch	43.6	-	-
Neutral detergent fibre (NDF)	21.3	48.5	8.2
Acid detergent fibre (ADF)	11.6	35.8	7.2
Acid detergent lignin (ADL)	3.7	10.6	3.5
Ether extract	2.5	1.6	0.16
Crude ash	6.3	8.5	2.7
Total phenolic compound (mg gallic acid/kg)	-	-	3686.95
Antioxidant capacity (%)	-	-	43.62
Fatty acid profile g/100 g FA[b]			
Palmitic acid (C16:0)	12.80	16.21	9.27
Palmitoleic acid (C16:1)	-	-	0.26
Stearic acid (C18:0)	2.32	3.97	5.26
Oleic acid (C18:1n9c)	28.70	2.83	17.11
Elaidic acid (C18:1n9t)	-	-	0.63
Linoleic acid (C18:2n6c)	53.34	22.67	66.44
Arachidic acid (C20:0)	-	-	0.55
Linolenic acid (C18:3n3)	2.84	54.33	-
γ-Linolenic acid (C18:3n6)	-	-	0.48

[a]Ingredients and proximate analysis results were given dry-matter basis. [b] FA = Fatty acid

Slaughter and Carcass Characterization

At the end of the study period, lambs were taken to a private slaughterhouse and slaughtered. Slaughter was performed after a 12-hour fasting period and unlimited water supply. Lambs were slaughtered in accordance with animal welfare standards and using standard commercial procedures. Following evisceration after slaughter, carcasses were weighed to determine hot and cold carcass weights. The hot carcass yield was calculated by the ratio of hot carcass weight to slaughter weight (Hot carcass yield = (Hot carcass weight / Slaughter weight) x 100). After a 24-hour cooling period at +4°C, the carcasses were re-weighed to determine the cold carcass weight and cold carcass yield (Cold carcass yield = (Cold carcass weight / Slaughter weight) x 100). The lamb carcasses were cut according to the Standard Mediterranean Carcass Cutting method [18]. To determine meat quality, the M. Longissimus dorsi (eye muscle) was extracted from the region between the 12th and 13th ribs. pH was measured in the right butt of the carcass within the first 45 minutes after slaughter using a portable pH meter (Testo 205 SE & Co. KGaA, Titisee-Neustadt, Germany). pH measurement was repeated at the right butt of the carcass at the end of the 24th hour. Additionally, pH measurement was also done at the end of the 24th hour in the M. Longissimus dorsi muscle extracted from the region between the 12th and 13th ribs. Color measurement was performed at two different points in the M. Longissimus dorsi muscle at the end of the 24th hour, as described by Miltenburg et al. [19]. Color measurement results were evaluated in three different color coordinate planes, specified as L* brightness value (0-100), a* redness value (+red; -green), and b* yellowness value (+yellow and -blue). For subsequent analyses, meat samples approximately 10 cm x 8 cm x 2 cm in size were taken from the right and left sides of the lamb carcasses, frozen at -20°C, and analyzed for dry matter, ash, protein, and fat content according AOAC [20]. Meat samples were thawed at 4°C for 24 hours before analysis. To determine the effect of grape pomace on meat shelf life, the meat samples were taken from -20°C conditions and thawed in a refrigerator at 4°C. pH (VWR pH enomenal pH 1100L, Avantor, VWR International Ilc., USA) and color measurements (ensuring the same time as the first measurement on each analysis day) were performed on days 1, 4, 8, and 12 (Minolta CR-300, Konica Minolta, Japan). Approximately 2 cm thick pieces cut from each lamb carcass were placed on trays and randomly assigned to different storage periods (1, 4, 8, and 12 days). The trays were randomly placed in a refrigerator at 4°C simulating retail storage conditions. The trays with meat samples were rotated once a day to minimize differences in light intensity and potential temperature changes.

Evaluation of Blood Parameters

During the fattening period, blood samples were taken three times - at the beginning, middle, and end of the trial - to analyze blood biochemistry parameters. Blood was drawn from the Vena jugularis into sterile blood tubes (Hema&Lab, Ankara, Turkey) at 8:00 am before feeding the lambs. The blood samples were analyzed for glucose, urea, total protein, total cholesterol, triglycerides, creatinine, iron (Fe), albumin (ALB), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), white blood cells (WBC) and lymphocyte (LYM) levels using species-specific kits.

Determination of Fatty Acids in Meat and Blood

Samples taken from the lambs were dried using a lyophilizer (freeze-dryer) (TRS-2-2, Teknosem, Istanbul, Turkey), and then fat extraction was performed. A dried sample of 0.375 g was mixed with 15 ml chloroform-methanol (2:1 vol/vol) and 375 L pure water [21]. The resulting extract was filtered and 2.2 ml pure water was added, followed by centrifugation at 4000 rpm. After centrifugation, the upper layer of the sample was taken, re-washed (with 30 ml chloroform, 480 ml methanol, and 470 ml NaCl solution (7.3 g/L water)) and centrifuged again to purify the fat. An aliquot of 0.1 - 0.3 g of the extracted fat was taken, and 0.5 ml of 2N methanolic KOH solution was added. The solution was completed to 10 ml with hexane and then centrifuged at 4000 rpm for 10 minutes. 1 ml was taken from the upper part of the solution in the centrifuge tube and placed in vials [22].

Fat extraction was performed using n-octane for blood samples. The triacylglycerol (TAG) fraction was separated using a silica column (Bond Elut SI, 500 mg, 3 mL; Varian Inc., Walnut Creek, CA, USA) and a hexane-methyl tert-butyl ether mixture (96:4 vol/vol). The TAG fraction in the solution was evaporated, and the fatty acids were separated into methyl esters by heating at 80°C for 10 minutes with 0.4 mL 0.5 N methanolic $NaOCH_3$, followed by 0.5 mL 14% boron trifluoride for another 10 minutes at 80°C. Fatty acid esters were converted with 100 L hexane and 1 ml was taken and placed into vials.

All extracted and esterified fat samples were analyzed using a gas chromatography (GC) device, injecting 1 L solution from each vial to determine the fatty acid profiles. The FAMEs and KLA (AOAC 996.06 Standard, FAME Mix cat. # 35077, and KLA standard cat # 16413) standards were applied. Fatty acid analysis was performed using a flame ionization detector (FID) and capillary column (60 m x 0.25 mm ID x 0.250 µm (cat. # 13199)) on a Shimadzu GC 2010 Plus device. Injection parameters were: 2.0 L split (split ratio 200:1), 4 mm inlet liner (cat. # 20814), injection temperature: 225°C, carrier gas: Hydrogen, flow rate: 1.2 mL/min, and oven temperature: from 100°C (4 min) to 240°C (10 min) at 3°C/min. Fatty acid composition was expressed as g/100g based on the analysis results from the gas chromatography device.

Feed Analysis

Dry matter, crude protein, crude fat and crude ash analyzes of fattening feed, alfalfa straw and grape pomace used in the feeding of lambs were analyzed according to the methods as described in AOAC [20]. Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), and Acid Detergent Lignin (ADL) analyses were performed according to Van Soest et al. [23]. Starch content was determined using a polarimetric method [24].

The extraction of phenolic compounds in grape pomace was done by mixing 5 g of the sample with 5 ml of 80% methanol, followed by centrifugation at 4000 rpm for 20 minutes. 100 L was taken from the clear part of the mixture than 100 L Folin-Ciocalteau, 3000 L pure water were added and left for 10 minutes. 50 L of 20% Na_2CO_3 was added to the solution and kept in a dark for 2 hours. Blank reading was performed on a spectrophotometer (Perkin Emler Lambda 25 UV/VIS, Massachusetts, USA, 2005) at a wavelength of 765 nm. The amount of phenolic compounds equivalent to the absorbance values determined in the samples in terms of gallic acid was calculated using the standard curve equation prepared with gallic acid.

Total phenolic content was determined as "mg gallic acid/kg" [25].

The antioxidant activity of the grape pomace was evaluated using the DPPH assay. This activity was assessed following the method previously described in reference [26]. Briefly, an 80% methanol solution was added to 5 ml of grape pomace sample, mixed with a vortex then centrifuged at 4000 rpm for 20 minutes at 40°C. After this process, 100 L of the sample was taken and 2460 L of 1,1-diphenyl-2-picrylhydrazyl (DPPH) was added. For control samples, 100 L distilled water was used. Absorbance against 80% methanol was read at 0, 5, 10, 30, 45, and 60 minutes using a Perkin Elmer Lambda 25 UV/VIS spectrophotometer at a wavelength of 515 nm. Data obtained at the 5th minute were taken as the baseline. Antioxidant activity in grape pomace was expressed as the percentage inhibition of DPPH (AA (%) = (Acontrol - Asample) / Acontrol x 100) [27]. The increase in the % value means that the antioxidant capacity increases [28].

Statistical Analysis

Statistical analyses were performed using the SAS package program (Version 9.3, SAS Institute Inc. 2011) with a mixed model for repeated measurements (PROC MIXED). The mathematical model included treatment (control and levels of grape pomace), week, and treatment x week interaction as fixed effects. Lambs within treatments were included as random effects and week was taken as repeated measure. The autoregressive covariance structure was chosen according to Littell et al. [29], Cetin and Bek [30]. Initial and final body weights were used as covariates in meat nutrient composition and fatty acid profiles of meat and blood. A p value of <0.05 was considered significant, and a value of $0.05 \leq p \leq 0.10$ was considered to indicate a tendency towards increase or decrease. Tukey test was used to compare means. The results are given as least squares means and the weighted standard error of these means.

RESULTS AND DISCUSSION

The nutrient compositions and fatty acid profiles of the concentrate, alfalfa hay and grape pomace used in the experiment are given in Table 1. Total phenolic matter and antioxidant levels of grape pomace used in the experiment were 3686.95 mg gallic acid/kg and 43.62% antioxidant capacity, respectively. It was also determined that grape pomace contained 66.44% linoleic acid. The nutrient content of grapes varies widely depending on the variety, origin, and fertilization conditions [31, 32]. Various studies have shown that the total content, profile, and tissue distribution of polyphenols in grapes significantly vary between varieties according to their ripening stages and climatic conditions [33, 34]. In a study by Gungor et al. [35], the dry matter content of grape pomace was determined to be 93.77%, crude protein content 12.15%, crude fat 7.63%, crude fiber 33.52%, crude ash 10.90%, ADF content 53.45%, and ADL content 34.46% on a dry matter basis. Grape pomace generally has a high ADF and lignin content, resulting in a lignocellulosic structure that leads to low energy content and low digestibility [31]. The chemical structure of polyphenols can be altered by digestive processes and metabolism, which may also change their antioxidant capacities and bioavailability [36].

Fattening Performance

The effects of using grape pomace in the diet on lamb fattening performance are presented in Table 2. The addition of grape pomace to the diet did not affect the dry matter intake of lambs ($p > 0.05$). However, the addition of grape pomace did affect the final live weight of the lambs ($p < 0.05$), with the control group having a higher live weight (54.9 kg) compared to the groups fed with grape pomace. Since there was no difference in dry matter intake and average daily live weight gain between the experimental groups ($p > 0.05$), the addition of grape pomace in the ration did not affect the feed efficiency ratio ($p > 0.05$).The higher the lignin content, the less dry matter is expected to be consumed by the animal [37]. Reduced digestibility results in the undigested portion of fiber remaining in the rumen longer and reduces dry matter intake. The general quadratic trend in dry matter intake with increasing levels of grape pomace can be attributed to the nutritional profile of the feed [38], particularly lignin and tannin content [39]. In this study, dry matter intake values did not differ between the trial groups ($p > 0.05$).

This may be explained by the fact that the potential decrease in ruminal nutrient digestibility and digestive transit rate is not significant enough to reduce dry matter intake. [40]. The effect of tannins is reported to depend not only on their content but also on their source and structure [41].

The decrease in dry matter intake with increasing levels of polyphenolic compounds in the diet is also associated with their astringent taste [42]. In a study where grape seed tannin extract obtained from wine grapes was added to lamb diets [43], no differences were found in live weight, daily live weight gain, and dry matter intake between the control group and groups containing different levels of grape seed tannin extract, similar to our study results.

Table 2 - Effect of grape pomace used in the diet on fattening performance.

	Treatment (T)[1]					P		
Variable	Control	GP5	GP10	GP15	SEM[2]	T	Week (W)	TxW
Initial age (day)[3]	99.2	95.0	98.0	93.4	2.98	0.512	-	-
Initial live weight (kg)[3]	43.66	43.14	43.16	43.24	1.149	0.984	-	-
Final live weight (kg)[3]	54.50	52.50	54.10	54.00	0.507	0.022	-	-
Average daily gain (g/d)	295.3	264.7	284.2	280.3	27.04	0.152	<.0001	0.102
Dry matter intake (g/day)	2160.10	2163.32	2145.48	2157.40	18.910	0.917	<.0001	0.337
Feed efficiency	5.29	5.99	5.79	5.62	0.391	0.818	<.0001	0.573

[1] Control: TMR without grape pomace (GP); GP5: TMR containing 5% GP; GP10: TMR containing 10% GP; GP15: TMR with 15% GP. GP levels were expressed as % of the dry matter intake by lambs during the adaptation period. [2] SEM: Standard error of means. [3] Since these three variables were subjected to one-way analysis of variance, the effects of Week and Trt x Week were not included in the statistical model.

Table 3 - Effect of grape pomace used in the diet on some blood biochemistry and hemogram parameters.

	Treatment (T)[1]					P		
Variable	Control	GP5	GP10	GP15	SEM[2]	T	Week (W)	TxW
Glucose (mg/dl)	80.5	81.3	81.5	86.0	2.97	0.557	0.001	0.588
Urea (mg/dl)	51.3	50.6	47.1	44.1	2.14	0.099	0.013	0.650
Total protein (g/dl)	5.87	5.95	6.10	5.95	0.126	0.633	<0.01	0.190
Total cholesterol (mg/dl)	63.2	69.5	68.2	60.5	4.04	0.387	0.001	0.654
Triglycerides (mg/dl)	17.4	15.7	14.9	14.0	1.27	0.334	0.004	0.310
Creatinine (mg/dl)	0.623	0.646	0.675	0.630	0.0295	0.626	0.304	0.853
Fe (ug/dl)	254.22	226.73	210.25	215.50	24.479	0.601	<0.01	0.526
ALB (g/dl)	4.11	4.14	4.13	4.18	0.067	0.910	<0.01	0.854
ALP (U/L)	360.05	416.18	323.54	390.57	56.366	0.686	0.221	0.124
GGT (U/L)	65.2	70.5	80.3	74.7	7.08	0.506	<0.01	0.338
AST (U/L)	137.74	117.61	141.30	139.28	12.062	0.494	<0.01	0.047
ALT (U/L)	26.1	31.1	26.1	27.3	3.05	0.631	0.133	0.707
WBC (10^9 /L)	24.8	20.0	29.6	25.7	4.19	0.467	<0.01	0.588
LYM (10^9 /L)	21.7	17.4	26.1	22.8	4.47	0.607	<0.01	0.416

[1] Control: TMR without grape pomace (GP); GP5: TMR containing 5% GP; GP10: TMR containing 10% GP; GP15: TMR with 15% GP. GP levels were expressed as % of the dry matter intake by lambs during the adaptation period. [2] SEM: Standard error of means. Fe=Iron, ALB=Albumine; ALP= Alkaline phosphatase; GGT= Gamma glutamyl transferase; AST= Aspartate aminotransferase; ALT= Alanine aminotransferase; WBC= White blood cells; LYM= Lymphocyte.

Blood Parameters

The profiles of blood metabolites are indicators of the digestion results of nutrients used by the animal [44]. The addition of grape pomace to the diet did not affect the blood biochemical parameters of the lambs ($p > 0.05$, Table 3) except urea ($p = 0.099$). Blood urea is an indirect indicator of the protein composition of the diet. High serum urea concentrations are associated with greater protein degradation in the rumen, with a simultaneous increase in ammonia production [45]. Creatinine is formed in skeletal muscle by the breakdown of phosphocreatine for energy production. Serum creatinine concentration is observed to be proportional to muscle mass [46, 47]. Parameters other than creatinine and ALT were found to vary depending on the weeks ($p < 0.01$ for all variables). Limited studies have evaluated the effects of using grape pomace in ruminant diets on liver function. Nudda et al. [48] reported that the addition of grape seeds to the diet did not affect liver function, including AST and ALT activities or plasma urea nitrogen concentration in lactating sheep. Mu et al. [49] found that the total protein and albumin concentrations in peripheral blood increased early in their study using 0 (control), 10, 20, and 40 mg/kg levels of grape seed proanthocyanidin in lamb diets, while AST and ALT activities decreased linearly with increasing grape seed proanthocyanidin levels. Chedea et al. [50] reported that the addition of 15% dried grape pomace to the diet did not affect AST, ALP, and GGT concentrations in dairy cows. Similarly, Iannaccone et al. [51] reported that the addition of 10% dried grape pomace had no significant effect on AST and ALT values.

This differential effect on liver enzyme levels may be attributed to the polyphenols present in grape pomace. Polyphenols are known for their antioxidant properties, which can influence liver function by reducing oxidative stress and modulating inflammatory responses [48]. This unique response underscores the complexity of polyphenol interactions with liver enzymes and highlights the need for more extensive research to fully understand these mechanisms. Further studies could elucidate how specific polyphenols contribute to these effects and determine the long-term implications of grape pomace supplementation on liver health.

Table 4 - The effect of grape pomace used in the diet on the blood fatty acid composition.

	Treatment (T)[1]					P		
Variable	Control	GP5	GP10	GP15	SEM[2]	T	Week (W)	TxW
Butyric acid (C4:0)	4.10	4.00	3.91	4.03	0.313	0.980	0.717	0.600
Caproic acid (C6:0)	1.80	1.73	1.74	1.63	0.136	0.863	<.0001	0.206
Caprylic acid (C8:0)	2.54^{b}	2.67^{a}	2.64^{a}	2.73^{a}	18.386	<.0001	0.999	0.897
Lauric acid (C12:0)	2.95	2.61	2.84	2.70	0.218	0.706	<.0001	0.487
Myristic acid (C14:0)	3.05^{a}	2.53^{ab}	2.77^{ab}	2.26^{b}	0.170	0.016	0.437	0.409
Palmitic acid (C16:0)	23.4	22.0	22.9	23.3	0.88	0.686	<.0001	0.840
Palmitoleic acid (C16:1)	3.61	4.02	3.72	3.74	0.368	0.878	0.028	0.732
Stearic acid (C18:0)	12.5	11.9	12.5	12.9	0.77	0.801	0.0002	0.120
Oleic acid (C18:1n9c)	7.34	5.00	5.54	4.55	1.369	0.528	0.440	0.269
Linoleic acid (C18:2n6c)	5.62	6.71	6.62	5.89	0.643	0.571	0.001	0.631
γ-Linolenic acid (C18:3n6)	3.90	4.22	3.95	4.12	0.290	0.864	<.0001	0.769
α-Linolenic acid (C18:3n3)	2.13	2.39	1.88	2.10	0.178	0.286	0.001	0.167
Eicosatrienoic acid (C20:3n3)	2.91	3.16	3.40	3.51	0.210	0.233	0.0002	0.326
Eicosapentaenoic acid (C20:5n3)	3.39	3.76	3.62	3.75	0.233	0.669	0.006	0.256
Heneicosanoic acid (C21:0)	5.38	5.84	5.54	5.44	0.338	0.786	0.0003	0.213
Docosahexaenoic acid (C22:6n3)	2.78	2.99	2.98	3.21	0.280	0.761	0.227	0.771
Nervonic acid (C24:1n9)	6.01	7.09	6.64	7.13	0.405	0.238	0.011	0.541

[1] Control: TMR without grape pomace (GP); GP5: TMR containing 5% GP; GP10: TMR containing 10% GP; GP15: TMR with 15% GP. GP levels were expressed as % of the dry matter intake by lambs during the adaptation period. [2] SEM: Standard error of means. Means with different letters in the same row are statistically different ($p < 0.05$).

Table 5 - Effects of grape pomace on carcass characteristics.

Variable	Treatment (T)[1]					P[2]
	Control	GP5	GP10	GP15	SEM[3]	T
Hot carcass weight (kg)	31.6	31.2	30.5	30.3	0.32	0.175
Cold carcass weight (kg)	31.2	30.7	30.2	29.9	0.34	0.489
Hot carcass yield (%)	52.5	53.4	51.6	51.1	0.79	0.264
Cold carcass yield (%)	51.8	52.7	51.0	50.5	0.81	0.301
pH (45th min. right butt)	6.65	7.00	6.81	6.78	0.112	0.438
pH (24th hour right butt)	5.71	6.00	5.61	5.93	0.121	0.207
pH (24th hour Longissimus dorsi)	5.61	5.59	5.60	5.67	0.047	0.595
$L^*_{24th\ hour}$[4]	48.1	45.5	47.6	47.1	1.02	0.347
$a^*_{24th\ hour}$[4]	11.3	10.6	10.0	9.1	1.16	0.606
$b^*_{24th\ hour}$[4]	14.1	13.0	12.9	12.0	1.05	0.586

[1] Control: TMR without grape pomace (GP); GP5: TMR containing 5% GP; GP10: TMR containing 10% GP; GP15: TMR with 15% GP. GP levels were expressed as % of the dry matter intake by lambs during the adaptation period. [2] Initial live weight was used as covariate [3] SEM: Standard error of means [4]Lightness (L*, higher numbers indicate brighter color); Redness (a*, higher numbers indicate more intense red color); Yellowness (b*, higher numbers indicate more intense yellow color).

Table 6 - Effects of grape pomace on meat quality.

Variable	Treatment (T)[1]					P[2]		
	Control	GP5	GP10	GP15	SEM[3]	T	Day (D)	TxD
L	49.7	48.6	49.2	48.6	0.75	0.645	0.001	0.317
a	8.6	8.3	8.9	9.7	0.38	0.201	0.001	0.536
b	15.4	14.6	14.8	15.0	0.23	0.166	0.275	0.824
pH	5.62	5.62	5.60	5.61	0.033	0.968	0.202	0.693

[1] Control: TMR without grape pomace (GP); GP5: TMR containing 5% GP; GP10: TMR containing 10% GP; GP15: TMR with 15% GP. GP levels were expressed as % of the dry matter intake by lambs during the adaptation period. [2] Initial and final live weights were used as covariate. [3] SEM: Standard error of means.

Fatty Acid Composition

The addition of grape pomace in the ration resulted in an increase in serum caprylic acid (C8:0) concentration ($p < 0.0001$) and a decrease in myristic acid (C14:0) concentration ($p = 0.016$). The ration had no effect ($p > 0.05$, Table 4) on the concentration of serum stearic acid (C18:0) and oleic acid (C18:1n9c). Other fatty acids were not affected by the addition of grape pomace to the diet. Since there is very limited data on the serum fatty acid profile of lambs, it is difficult to compare the findings of this study with those of others. Additionally, information on the impact of diet on the blood fatty acid profile and metabolic pathways of ruminant animals is limited. The levels of fatty acids in the blood are largely dependent on the sources of fatty acids in the ration, the bacterial synthesis of fatty acids in the rumen and biohydrogenation [52, 53].

Supplements that can minimize oxidative rancidity during storage and extend the product's shelf life are needed. The fact that there is no difference in the color and pH values of the meat during storage may be an indication that the antioxidant capacity of the grape pomace is affected by the digestive processes and metabolism of the lambs and changes occur in the chemical structures of polyphenols. At the same time, the similarity of meat color parameters between groups suggests that glycolysis in muscles creates a regular trend [66].

The effects of grape pomace addition on the dry matter, protein, fat and ash contents of lamb meat were not significant ($p > 0.05$, Table 7). Flores et al. [67] found similar results for moisture, protein and ash contents. Fatty acids are related to various meat quality parameters, such as color, texture and appearance [68]. However, unsaturated fatty acids are highly susceptible to rapid oxidation due to the presence of two or more double bonds, making them a significant determinant of meat shelf life [69, 70].

The fatty acid profile of the Longissimus dorsi muscle is given in Table 8. The addition of grape pomace in lamb diets did not cause significant changes in the overall fatty acid profile of the Longissimus dorsi muscle. The addition of grape pomace in lamb rations increased the PUFA content in the Longissimus dorsi muscle ($p = 0.039$). The addition of grape pomace to the ration resulted in an increase in n-3 fatty acid content ($p < 0.05$).

Table 7 - Effects of grape pomace on meat composition.

Variable	Treatment (T)[1]					P[2]
	Control	GP5	GP10	GP15	SEM[3]	T
Protein (%)	21.4	21.8	21.7	21.5	1.08	0.404
Fat (%)	3.14	2.79	3.17	2.41	0.847	0.898
Ash (%)	1.03	1.08	1.19	1.14	0.082	0.484
Dry Matter (%)	25.6	25.6	25.8	25.1	0.60	0.789

[1] Control: TMR without grape pomace (GP); GP5: TMR containing 5% GP; GP10: TMR containing 10% GP; GP15%: TMR with 15% GP. GP levels were expressed as % of the dry matter intake by lambs during the adaptation period. [2] Initial and final live weights were used as covariate. [3] SEM: Standard error of means.

Table 8 - Effects of grape pomace on fatty acid composition (% total FA) of the longissimus dorsi muscle.

Variable	Treatment (T)[1] Control	GP5	P[2] GP10	GP15	SEM[3]	T
Capric acid (C10:0)	0.258	0.273	0.278	0.290	0.0475	0.966
Lauric acid (C12:0)	0.217	0.202	0.152	0.193	0.0558	0.827
Myristic acid (C14:0)	3.26	3.39	3.14	3.63	0.383	0.786
Myristoleic acid (C14:1)	0.416	0.318	0.334	0.375	0.0583	0.739
Palmitic acid (C16:0)	28.8	29.0	28.5	28.6	1.25	0.989
Palmitoleic acid (C16:1)	1.86	1.89	1.91	2.00	0.263	0.979
Heptadecanoic acid (C17:0)	1.42	1.01	1.15	1.12	0.164	0.571
Stearic acid (C18:0)	12.0	14.2	13.5	13.1	1.36	0.832
Vaccenic acid (C18:1)t11	2.19	2.56	2.93	2.98	0.424	0.515
Oleic acid (C18:1n9c)	43.4	42.3	42.7	41.6	2.24	0.950
Linoleic acid (C18:2n6c)	3.32	2.77	3.12	3.40	0.196	0.243
γ-Linolenic acid (C18:3n6)	0.374	0.328	0.355	0.427	0.0523	0.562
α-Linolenic acid (C18:3n3)	0.569	0.667	0.599	0.769	0.0890	0.432
Eicosatrienoic acid (C20:3n3)	0.541[a]	0.280[b]	0.323[b]	0.510[a]	0.0496	0.029
Arachidonic acid (C20:4n6)	0.190	0.043	0.062	0.122	0.0270	0.053
Eicosapentaenoic acid (C20:5n3)	0.103	0.070	0.087	0.085	0.0077	0.231
Docosahexaenoic acid (C22:6n3)	0.132	0.086	0.105	0.141	0.0190	0.266
Total SFA[4]	46.0	48.1	46.7	46.9	2.49	0.972
Total UFA[4]	54.0	51.9	53.3	53.1	2.49	0.972
Total MUFA[4]	48.8	47.7	48.6	47.6	2.35	0.981
Total PUFA[4]	5.23[ab]	4.24[b]	4.65[ab]	5.45[a]	0.234	0.039
Total n-3	1.34[ab]	1.10[c]	1.11[c]	1.51[a]	0.069	0.012
Total n-6	3.89	3.14	3.53	3.95	0.218	0.153
n-3/n-6	0.348	0.345	0.316	0.383	0.0261	0.343
n-6/n-3	2.94	2.91	3.16	2.64	0.202	0.329

[1]Control: TMR without grape pomace (GP); GP5: TMR containing 5% GP; GP10: TMR containing 10% GP; GP15: TMR with 15% GP. GP levels were expressed as % of the dry matter intake by lambs during the adaptation period. [2] Initial and final live weights were used as covariate. [3] SEM: Standard error of means. Means with different letters in the same row are statistically different ($p < 0.05$). [4]SFA: Saturated fatty acids; UFA: Unsaturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids.

Similar to our findings, Kafantaris et al. [71] reported an increase in n-3 fatty acid content with the addition of grape pomace in lamb rations. The tannins and polyphenols in grape pomace may support ruminal accumulation by altering the synthesis of endogenous enzymes and microflora in the rumen through digestion [6, 72]. Another study found that the direct addition of grape pomace and grape seed to lamb rations did not affect the fatty acid profile of meat, similar to our study [73]. Similar to our study findings, addition of grape pomace to the rations of lactating sheep did not effect the fatty acid profile of lamb meat [66].

CONCLUSIONS

The present study showed that 15% grape pomace can be used in lamb diets without any negative effect on fattening performance, blood parameters and meat quality characteristics. Thus, grape pomace can be a new feed material for ruminants in our planet struggling with climate change and it can be beneficial for human health by increasing PUFA and n-3 fatty acids. However, it should be taken into account that the number of samples was limited and the variation in fruit pomace may be large in the evaluation of the results of the study.

Author Contributions

Conceptualization, O.B. and U.S.; methodology, O.B. and U.S.; formal analysis, U.S. and S.C.D.; writing-original draft preparation, O.B.; writing-review and editing, O.B.,U.S. and S.C.D.; project administration, S.C.D. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The animal study protocol was approved by the Ni de Ömer Halisdemir University Ayhan Şahenk Agricultural Research and Application Center Animal Experiments Local Ethics Committee (protocol code: 2023/04 and date of approval: 17 January 2023).

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

Conflicts of Interest

The authors declare no conflicts of interest.

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