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Effect of Feeding Pomegranate Byproduct on Fatty Acid Composition of Ruminal Digesta, Liver, and Muscle in Lambs

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ABSTRACT: This work investigated the effects of feeding whole pomegranate byproduct (WPB) to lambs on ruminal, liver, and intramuscular fatty acids (FA). Seventeen lambs, divided into two groups, were fed for 36 days with a cereal-based concentrate diet (CON) or with a concentrate diet containing 200 g/kg DM of WPB to partially replace barley and corn (WPB). The dietary treatment did not affect the final body and carcass weight, the dry matter intake, or the average daily gain. However, total polyunsaturated FA (PUFA), linolenic, rumenic (RA), and vaccenic (VA) acid were increased in liver (+15%, +32%, +344%, and +118%, respectively) and muscle (+46%, +38%, +169%, and +89%, respectively) of WPB lambs ($P < 0.05$). Punicic acid and three isomers of conjugated linolenic acid were detected exclusively in the rumen and tissues of WPB-lambs. The C18:1 *t*10/*t*11 ratio in rumen digesta or in tissues was reduced by feeding WPB (−791%, −690%, and −456%, respectively, in rumen, liver and muscle; $P < 0.001$), suggesting that the WPB prevented the *t*10-shift rumen biohydrogenation pathway. In conclusion, the inclusion of WPB into a concentrate-based diet can be a strategy to improve the FA composition of meat, without effects on the animal performances.

KEYWORDS: *pomegranate, rumen, liver, meat quality, fatty acid metabolism, conjugated linoleic and linolenic acids*

INTRODUCTION

In the past decade, there has been an enormous increase in the demand of pomegranate fruits (*Punica granatum* L.),[1] especially destined to juice production, because of the recognition of its beneficial effects on human health.[2] The current increasing trend in the industrial production of pomegranate juice has led to great amounts of residual biomasses. The primary byproduct is the whole pomegranate byproduct (WPB), which contains peels, membranes, seeds, and residual arils' pulp. The WPB is produced in massive amounts in many parts of the world, and if not intended for other uses, its disposal as a waste represents a cost for the processing industries. However, WPB is a natural source of bioactive compounds. Specifically, pomegranate peel is rich in phenolic compounds, such as flavonoids, anthocyanidins, and tannins, with the latter being mostly represented by ellagitannins.[2] These compounds have been shown to possess antimicrobial, antioxidant, anti-inflammatory, antitumoral, and immunomodulatory properties both *in vivo* and *in vitro*.[3] Also, pomegranate seeds contain variable amounts of oil ranging from 6 to 24%, and approximately 80% of the fatty acids (FA) in pomegranate oil are conjugated linolenic acids (CLnA), characterized by the occurrence of three double bonds conjugated together. Punicic acid (C18:3 *c*9 *t*11 *c*13; PA), in particular, is the predominant CLnA in the pomegranate seed oil, where it can represent more than 70% of total fatty acids.[4] Other CLnA isomers, such as *α*-eleostearic acid (C18:3 *c*9 *t*11 *t*13; *α*-ESA), catalpic acid (C18:3 *t*9 *t*11 *c*13; CA), and *β*-eleostearic acid (C18:3 *t*9 *t*11 *t*13; *β*-ESA), occur at lower concentrations in pomegranate seed oil.[5,6] All these fatty acids are among the few CLnA found in nature. *In vivo* and *in vitro* studies showed that CLnA isomers can exert several health benefits in humans.[7] Tsuzuki et al.[8] reported that CLnA would have a stronger antitumor propriety than conjugated linoleic acid (CLA). Furthermore, studies have demonstrated that CLnA isomers can be converted to *c*9 *t*11 CLA (rumenic acid; RA) in rat tissues[8,9] and in *in vitro* cell cultures.[10]

The fatty acid composition of ruminant meat and milk is mostly determined by complex interactions between dietary factors and rumen metabolism.[11] Several studies have focused on possible feeding strategies to increase the content of polyunsaturated fatty acids (PUFA) and CLA in meat and milk, via the manipulation of the ruminal biohydrogenation (BH). Diets rich in PUFA are certainly among the most effective feeding strategies to achieve this objective. Indeed, it has been well documented that diets enriched of unsaturated FA increase the amount of PUFA and other BH intermediates escaping the complete saturation in the rumen.[12] Also, the inclusion of phenolic compounds in the ruminant diets is being investigated, as it has been demonstrated that dietary phenolic compounds affect the ruminal microbial community[13] and lead

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to alterations of the ruminal BH with the consequent accumulation of unsaturated FA.

In light of the above, WPB could be usefully included in the animal diet to increase the deposition of desirable fatty acids in meat. Indeed, some studies have demonstrated that feeding ruminants with pomegranate byproducts, such as seed pulp, increased the amount of PUFA and RA in meat[14] and milk.[15,16] To our knowledge, only ensiled WPB was tested in milk[17] and meat production,[18] with controversial results on fat composition, and no studies have investigated in depth the effects of pomegranate byproducts on ruminant lipid metabolism. As WPB contains both CLnA and tannins, different mechanisms might explain its effect in increasing the deposition of PUFA and RA in meat. Such mechanisms, alone or in combination, may involve the reduction of the ruminal BH extent, as well as the direct conversion of punicic acid into RA both in the rumen and in muscle. For these reasons, it would be of interest to get a deeper insight on the effects of pomegranate byproducts on the lipid metabolism in ruminants. Therefore, here we have investigated, for the first time, the effect of dietary dried whole pomegranate byproduct on the composition of fatty acids in lamb ruminal digesta, liver, and muscle, which represent the main organ and tissue sites involved in lipid metabolism.

MATERIALS AND METHODS

The trial was conducted between October and December 2016 at the experimental farm of the University of Catania (37°24′35.3″N 15°03′34.9″E). All experimental procedures were accomplished following the European Union Guidelines (2010/63/EU Directive) and according to the protocol approved by the Universities of Catania and Perugia.

Whole Pomegranate Byproduct. Fresh WPB was obtained from a local juice manufacturing company (Catania, Sicily, Italy). At the factory, the pomegranate fruits (Wonderful variety) were halved and squeezed mechanically. The residual part containing peel, seeds, membranes, and portion of arils was collected and dried in a ventilated oven set at 40 °C for approximately 36 h until constant weight. Approximately 300 kg of dried biomass was obtained for the use in the feeding trial. This product is referred to as "whole pomegranate by-product" (WPB) throughout the article.

Experimental Design. Seventeen Comisana male lambs, born within 10 days, were selected. At 60 days of age, lambs were weighed (initial body weight 14.82 ± SD 2 kg) and individually penned indoor. Animals were randomly assigned to two dietary treatments balanced for the body weight. After 8 days of adaptation period, where pre-experimental concentrate was gradually replaced with the experimental diets, lambs were fed for 36 days with barley-corn based concentrate diet (CON, 8 lambs) or a concentrate diet containing 200 g/kg DM of WPB to partially replace barley and corn (WPB, 9 lambs). Table 1 reports the ingredients and the chemical composition of the experimental diets, which were planned to provide similar levels of energy and nitrogen. All the ingredients were ground (5 mm screen), mixed, and pelleted (at 40 °C) using a pelleting machine (CMS-IEM - Colognola ai Colli, Verona, Italy) to avoid selection. During the experimental period, all the lambs were fed *ad libitum* with their respective diets (10% minimum amount of residual allowed). For each lamb, offered and refused feed was recorded every day to calculate dry matter intake (DMI). Water was continuously available. From the beginning of the trial, the body weight was measured weekly in order to calculate average daily gain (ADG).

Slaughter Procedure and Samplings. At the end of the trial, all the lambs were slaughtered on the same day at a commercial slaughterhouse according to the European Union welfare guidelines. The experimental diet and water was available to lambs until around 3 h before slaughter. Lambs were first stunned by a captive bolt and then exsanguinated. Individual ruminal content was sampled within 15 min from butchery. After cutting the ruminal wall with a scalpel, the whole rumen content was placed into a 4-L-plastic beaker and homogenized with a ladle. Ruminal pH measurement was performed by a pH meter (HI-110; Hanna Instruments, Padova, Italy). An aliquot of approximately 120 mL of rumen content was immediately placed in dry ice prior to storage at −80 °C. Liver samples were collected immediately after slaughter, vacuum-packed, and stored at −80 °C. Each carcass was immediately weighed and stored at 4 °C for 24 h. Thereafter, the entire *longissimus thoracis et lumborum* muscle (LTL) was removed from the right side of each halved carcass, packed under vacuum, and stored at −80 °C.

Analyses of Feedstuffs. Samples of the feedstuffs were collected at the beginning, middle, and end of the experimental period, vacuum-packed, and stored at −30 °C. Feed sample for analysis was obtained by mixing equal amounts of the collected subsamples during the trial. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined according to the method described by Van Soest et al.[19] Furthermore, crude protein, crude fat

Table 1. Ingredients and Chemical Composition of the Experimental Diets

	experimental diet[a]	
	CON	WPB
Ingredient, g/kg of Dry Matter		
corn	226	116
barley	226	116
alfalfa hay	198	198
wheat bran	200	200
soybean meal	120	140
whole pomegranate byproduct	0	200
molasses	9	9
mineral mix[b]	21	21
Chemical Composition, g/kg DM		
dry matter (DM), g/kg as fed	887	892
crude protein	176	178
NDF	233	263
ADF	129	155
ADL	29.8	27.0
ash	58.7	44.0
crude fat	21.1	25.1
metabolizable energy[c]	10.6	10.3
total phenols[d]	3.04	18.9
total tannins[d]	1.41	17.0
vitamin E (α-tocopherol)	7.82	16.8
total fatty acids (FA)	13.1	15.5
Individual FA, g/100 g Total FA		
C14:0	0.28	0.17
C16:0	17.3	14.0
C18:0	2.36	2.11
C18:1 *c*9	17.6	12.5
C18:2 *c*9 *c*12	42.1	32.5
C18:3 *c*9 *c*12 *c*15 (α-LnA[e])	5.11	5.09
C18:3 *c*9 *t*11 *c*13 (PA[e])	-	11.1
C18:3 *c*9 *t*11 *t*13 (α-ESO[e])	-	1.03
C18:3 *t*9 *t*11 *c*13 (CA[e])	-	1.38
C18:3 *t*9 *t*11 *t*13 (β-ESO[e])	-	0.62

[a]CON: control barley-corn based concentrate diet. WPB: diet including 20% of whole pomegranate byproduct. [b]Containing: 25% calcium carbonate, 25% sodium bicarbonate, 25% bicalcic phosphate and 25% sodium chloride. [c]Mj/kg DM Estimated using Feed Tag (University of California, Davis, CA, U.S.A.). [d]Expressed as g tannic acid equivalents/kg DM. [e]α-LnA, α-linolenic acid; PA, punicic acid; α-ESO, α-eleostearic acid; CA, catalpic acid; β-ESO, β-eleostearic acid.

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(ether extract), and ash were analyzed according to the AOAC methods.[20]

Total phenolic compounds and total tannins in the feeds were analyzed following the procedure originally described by Makkar et al.,[21] with modifications as follows. Briefly, finely ground feeds (200 mg) were first extracted with acetone 70% (v/v) in a sonicating water-bath at 4 °C for 15 min. Following centrifugation, the supernatant was collected, and the residual solid pellet was re-extracted using methanol 80% (v/v) in a sonicating water-bath at 4 °C for 15 min. The supernatants were combined, solvents were evaporated using a rotary evaporator system (Rotavapor R-114, Büchi, Flawil, Switzerland), and the residue was dissolved in methanol 70% (v/v). As described by Makkar et al.,[21] total phenolic compounds were determined using Folin-Ciocalteu reagent (1N) and sodium carbonate 20% (w/v) via a spectrophotometer (model UV-1601; Shimadzu Corporation, Milan, Italy). Non-tannin phenolics were determined with the same procedure, after removal of the tannins from the extract with insoluble polyvinylpyrrolidone (PVPP). Tannin concentration was calculated as difference between total phenols and total non-tannin phenols. Standard solutions of tannic acid (TA) were used to prepare an external calibration curves in order to quantify phenolic compounds, which were expressed as g TA equivalents/kg dry matter.

Vitamin E (α-tocopherol) was extracted and quantified as reported by Valenti et al.[22] Briefly, α-tocopherol was extracted three times using hexane/ethyl acetate (9/1, v/v), after saponification with KOH (60%) at 70 °C for 30 min. The extracted solution was dried under nitrogen and dissolved with acetonitrile, and 50 μL was injected via an autosampler (Jasco, model AS 950−10, Tokyo, Japan) into a HPLC system (pump model PerkinElmer series 200, Norwalk, CT, U.S.A.). A Synergy Hydro-RP column (4 μm, 4.6 × 100 mm; Phenomenex, Bologna, Italy) was fitted to the HPLC. The sample was run in isocratic condition with a flow rate of 2 mL/min and a mobile phase prepared with acetonitrile/methanol/tetrahydrofuran/1% ammonium acetate (68/22/7/3, v/v/v/v). α-tocopherol was identified using a fluorescence detector (Jasco, model FP-1525, Tokyo, Japan) set at excitation and emission wavelengths of 295 and 328 nm, respectively. Quantification was achieved through an external calibration curves with increasing amounts of pure standard compounds (Sigma-Aldrich, Bornem, Belgium).

Fatty Acid Analyses. Fatty acids were extracted from 200 mg of freeze-dried sample of the experimental diets and converted to fatty acid methyl esters (FAME) with a 1-step procedure using chloroform and 2% (v/v) sulfuric acid in methanol[23] and nonadecanoic acid (Sigma-Aldrich, St. Louis, MO, U.S.A.) as an internal standard.

Rumen digesta FA were directly converted to fatty acid methyl esters (FAME) by combining basic and acid methylation as described by Alves et al.,[24] with some modifications to prevent the isomerization of CLnA. Briefly, rumen digesta samples were freeze-dried (Christ, alpha 2−4 LD plus, Osterode am Harz, Germany) and 500 mg was incubated at 50 °C for 10 min with 4 mL of sodium methoxide in methanol (0.5 M). After the solution was cooled room temperature, 6 mL of 3 N HCl solution in methanol was added, and the solution was allowed to react for 15 min at 50 °C. Once cooled, 8 mL of 6% aqueous potassium carbonate was added to prevent excessive effervescence, followed by addition of 4 mL of hexane. Following centrifugation, the organic phase was collected, and the extraction step was repeated twice. The final solution was dried over anhydrous sodium sulfate, evaporated under nitrogen at room temperature and resuspended in 1 mL of hexane. The internal standard used was methyl nonadecanoate (1 mg/mL).

Liver fat was extracted from 10 g of tissue with a mixture of chloroform and methanol (2:1, v/v) and 50 mg of lipids were converted to FAME by base catalyzed transesterification, using 1 mL of sodium methoxide in methanol 0.5 M and 2 mL of hexane containing C19:0 as an internal standard.[22]

LTL muscle, at the level of the 13th thoracic rib, was deprived of any visible external fat, and 10 g was used to obtain the FAME as described above for the liver.

Fatty acid methyl esters, obtained from feeds, rumen digesta, liver, and muscle samples, were separated and quantified using a gas chromatograph (ThermoQuest, Milan, Italy) equipped with a flame ionization detector (FID) and a 100-m high-polar fused silica capillary column (25 mm i.d., 0.25-μm film thickness; SP. 24056; Supelco Inc., Bellefonte, PA). One microliter of sample was injected, split at a ratio of 1:80, and carried by a constant flow (1 mL/min) of helium. The oven temperature was set at 50 °C and held for 4 min, then increased to 120 °C at 10 °C/min, held for 1 min, then increased up to 180 °C at 5 °C/min, held for 18 min, then increased up to 200 °C at 2 °C/min, held for 15 min, and then increased up to 230 °C at 2 °C/min, held for 19 min. The temperature of injector and detector was set at 270 and 300 °C, respectively. FAME was identified by comparison with the retention times of a commercial mixture of standard FAME (GLC-674, Nu-Chek Prep Inc., Elysian, MN, U.S.A.), individual standard FAMEs (21-1615; 21-1614; 21-1413; 21-1412; 20-1823; Larodan Fine Chemicals, Malmo, Sweden) and with the chromatograms published by Alves and Bessa[25] and Kramer et al.[26] Punicic acid was identified by comparing retention time with the pure standard compound (10-1875; Larodan Fine Chemicals, Malmo, Sweden). The identification of the other CLnA isomers was based on the comparison between published chromatograms of pomegranate seed oil[6] and the chromatograms of a commercial pomegranate seed oil analyzed in our laboratory both under the same conditions reported by Sassano et al.[6] and the GC conditions described above for the present study. Fatty acids were expressed as g/100g of total fatty acids. The biohydrogenation indexes (%) for C18:1 *c*9, C18:2 *c*9 *c*12, α-LnA, and PA, and the biohydrogenation completeness (%) in rumen digesta were estimated as reported by Alves et al.[27]

Statistical Analysis. All data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC), with the dietary treatment as the main effect. Data were reported as least-squared means and standard error of means (SEM). Significance was declared at $P \leq 0.05$, while trends toward significance were considered when $0.05 < P \leq 0.10$.

RESULTS

Animals Performance and Intakes. As shown in Table 2, no effect of the dietary treatment was found on the final body and carcass weight, dry matter intake (DMI) and average daily gain (ADG). The intake of NDF was greater for lambs in the WPB group, compared to the control group ($P < 0.001$).

Table 2. Effect of the Dietary Treatment on Lamb Performance and Intakes

	dietary treatment[a]			
	CON	WPB	SEM[b]	*P*-value
no. of animals	8	9		
final body weight, kg	23.6	23.1	0.524	0.637
carcass weight, kg	11.1	10.2	0.330	0.179
DMI[c], g/day	821	882	19.500	0.125
ADG[c], g/day	234	235	7.200	0.921
NDF intake, g/day	191	232	6.650	<0.001
total phenols intake, g/day	2.49	16.7	1.700	<0.001
total tannins intake, g/day	1.16	15.0	1.660	<0.001
vitamin E[d] intake, g/day	6.42	14.8	1.300	<0.001
total FA[c] intake, g/day	10.8	13.6	0.424	<0.001
C18:0 intake, g/day	0.26	0.29	0.007	0.019
C18:1 *c*9 intake, g/day	1.90	1.71	0.044	0.024
C18:2 *c*9 *c*12 intake, g/day	4.56	4.43	0.095	0.547
C18:3 *c*9 *c*12 *c*15 intake, g/day	0.55	0.69	0.022	<0.001
C18:3 *c*9 *t*11 *c*13 intake, g/day	0	1.52	0.181	-

[a]CON: control barley-corn based concentrate diet. WPB: diet including 20% of whole pomegranate byproduct. [b]SEM, standard error of the mean. [c]DMI, dry matter intake; ADG, average daily gain; FA, fatty acids. [d]α-tocopherol.

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Moreover, WPB-fed lambs ingested a greater quantity of total phenols, total tannins and Vitamin E as compared to CON lambs ($P < 0.001$). The daily intake of total FA and stearic acid (C18:0) was higher ($P < 0.001$ and $P = 0.019$, respectively) in WPB group than CON group. The intake of oleic acid (C18:1 *c*9) was greater ($P = 0.024$) in the control group, whereas a comparable intake of linoleic acid (LA; C18:2 *c*9 *c*12) was observed between groups ($P = 0.547$). The intake of α-linolenic acid (α-LnA; C18:3 *c*9 *c*12 *c*15) was greater ($P < 0.001$) for animals in the WPB group compared to those in the CON group. Moreover, a remarkable amount of punicic acid (PA; C18:3 *c*9 *t*11 *c*13) was ingested only by lambs fed the WPB diet (1.52 g/day).

Fatty Acid Composition of Rumen Digesta. The FA composition of the rumen digesta and the BH indexes are presented in Table 3. Feeding the WPB diet tended to increase the sum of saturated fatty acids (SFA; $P = 0.085$) and to decrease total (MUFA; $P = 0.059$). Among the SFA, the proportion of C14:0 tended to be greater in the rumen content from CON group ($P = 0.093$), while stearic acid (C18:0) tended to be greater in the WBP treatment compared to CON group ($P = 0.073$). The proportion of C24:0 was greater in the CON treatment ($P = 0.007$). Within the MUFA, C16:1 *c*9, C18:1 *c*11, C18:1 *t*6 + *t*7 + *t*8 and C22:1 *c*13 were found at greater proportion in the rumen digesta from the CON lambs compared to the WPB group ($P < 0.05$). Vaccenic acid (VA; C18:1 *t*11) tended to be greater in the rumen digesta of WPB lambs as compared to CON ($P = 0.093$), whereas C18:1 *t*10 was reduced ($P < 0.001$) by feeding lambs the WPB diet. The dietary treatment did not affect the total PUFA in the rumen content ($P > 0.05$), while the inclusion of WPB in the diet increased the RA (C18:2 *c*9 *t*11) by three times in the rumen of WPB in comparison with the control group ($P = 0.050$). The pomegranate CLnA (PA, α-ESO, CA and β-ESO) were detected only in the rumen of lambs receiving the WPB diet. In relation to the odd- and branched-chain fatty acids (OBCFA), the inclusion of WPB in the diet decreased their total concentration in the rumen digesta ($P < 0.001$). In particular, C15:0, C15:0 *iso*, C15:0 *anteiso*, C17:0, and C17:0 *anteiso* were significantly higher in the rumen content of CON group compared with the WPB group ($P < 0.05$). No significant difference was found for the index of BH completeness or for the BH index of individual unsaturated C18:1 fatty acids, with the only exception of α-LnA, which tended to be greater in the WPG group ($P = 0.072$)

Fatty Acid Composition of Liver. Table 4 reports the effect of dietary treatment on the fatty acid composition of liver. Total hepatic fat was not affected by the dietary treatment ($P = 0.173$). The sum of SFA was comparable between the treatments ($P = 0.384$), while the proportion of C14:0 and C16:0 was greater in the liver of lambs fed the control diet compared to the WPB lambs ($P = 0.025$ and $P = 0.015$, respectively), and the stearic acid (C18:0) was detected at greater concentration in liver from the animals fed the WPB diet compared with animals in the CON group ($P = 0.033$). Dietary WPB decreased the concentration of total MUFA in liver compared with the CON treatment ($P = 0.002$). Regarding the individual MUFA, most were found in a greater proportion in the liver from the CON-fed lambs, except for C18:1 *c*6, C18:1 *c*14, C18:1 *t*9, and VA, which were increased by feeding the WPB diet ($P < 0.05$).

The sum of PUFA was greater when lambs received the WPB diet compared to the control diet ($P < 0.001$). Within

Table 3. Effect of the Dietary Treatment on pH and Fatty Acid Composition of Rumen Digesta (g/100 g of total FA)

	dietary treatment[a]			
	CON	WPB	SEM[b]	*P*-value
pH	5.85	6.02	0.093	0.375
Fatty Acids				
C12:0	0.34	0.29	0.023	0.255
C13:0	0.10	0.08	0.009	0.186
C13:0 *iso*	0.03	0.03	0.002	0.199
C14:0	0.95	0.68	0.078	0.093
C14:0 *iso*	0.21	0.28	0.026	0.237
C15:0	1.02	0.60	0.059	<0.001
C15:0 *iso*	0.39	0.29	0.024	0.041
C15:0 *anteiso*	1.98	1.00	0.170	0.002
C16:0	16.24	15.94	0.732	0.851
C16:0 *iso*	0.45	0.32	0.048	0.202
C16:1 *c*9	0.18	0.10	0.016	0.007
C17:0	0.54	0.33	0.028	<0.001
C17:0 *iso*	0.16	0.09	0.019	0.086
C17:0 *anteiso*	0.60	0.19	0.068	0.001
C18:0 (SA)	25.35	34.17	2.410	0.073
C18:1 *c*9	9.37	7.80	0.470	0.105
C18:1 *c*11	1.21	0.60	0.099	0.001
C18:1 *c*12	1.03	0.70	0.171	0.369
C18:1 *c*13	0.06	0.07	0.005	0.145
C18:1 *t*5	0.08	0.05	0.007	0.090
C18:1 *t*6 + *t*7 + *t*8	1.35	0.50	0.163	0.006
C18:1 *t*9	0.60	0.37	0.079	0.167
C18:1 *t*10	5.47	0.83	0.745	<0.001
C18:1 *t*11 (VA)	4.35	6.51	0.626	0.093
C18:2 *c*9 *c*12	12.30	9.68	0.781	0.103
C18:2 *c*9 *t*11 (RA)	0.60	2.07	0.376	0.050
C18:2 *t*9 *c*12	0.28	0.29	0.017	0.889
C18:3 *c*9 *c*12 *c*15 (α-LnA)	1.35	1.18	0.082	0.334
C18:3 *c*9 *t*11 *c*13 (PA)	n.d.	1.20	0.165	-
C18:3 *c*9 *t*11 *t*13 (α-ESO)	n.d.	0.51	0.069	-
C18:3 *t*9 *t*11 *c*13 (CA)	n.d.	0.99	0.131	-
C18:3 *t*9 *t*11 *t*13 (β-ESO)	n.d.	0.91	0.107	-
C20:0	0.58	0.63	0.024	0.368
C20:1 *c*11	0.32	0.24	0.020	0.057
C20:4 *n*-6	0.07	0.06	0.002	0.276
C21:0	0.11	0.11	0.010	0.813
C22:0	0.39	0.38	0.009	0.544
C22:1 *c*13	0.16	0.07	0.013	<0.001
C23:0	0.14	0.13	0.004	0.062
C24:0	0.42	0.35	0.013	0.007
SFA	44.27	52.44	2.310	0.085
MUFA	27.27	20.34	1.810	0.059
OBCFA	5.82	3.58	0.301	<0.001
PUFA	15.21	17.19	0.777	0.225
Biohydrogenation Indexes (%)				
C18:1 *c*9	44.98	41.35	3.033	0.575
C18:2 *c*9 *c*12	70.00	72.41	1.874	0.547
C18:3 *c*9 *c*12 *c*15 (α-LnA)	72.86	78.49	1.531	0.072
C18:3 *c*9 *t*11 *c*13 (PA)	n.d.	90.02	10.78	-
completeness (%)	64.72	69.09	1.784	0.242
SA/(SA+VA)	0.81	0.84	0.024	0.489

[a]CON: control barley-corn based concentrate diet. WPB: diet including 20% of whole pomegranate byproduct. [b]SEM, standard error of the mean.

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Table 4. Effect of the Dietary Treatment on Total Hepatic Fat (g/100 g of Liver) and Fatty Acid Composition of Liver (g/100 g of Total FA)

	dietary treatment[a]			
	CON	WPB	SEM[b]	*P*-value
total hepatic fat	3.67	3.85	0.064	0.173
C12:0	0.02	0.02	0.004	0.648
C14:0	0.53	0.42	0.025	0.024
C14:0 *iso*	0.03	0.03	0.003	0.619
C14:1 *c9*	0.02	<0.001	0.002	0.018
C15:0	0.63	0.37	0.047	0.003
C15:0 *iso*	0.12	0.07	0.010	0.024
C15:0 *anteiso*	0.23	0.11	0.022	0.005
C16:0	13.59	12.37	0.256	0.015
C16:0 *iso*	0.22	0.19	0.016	0.317
C16:1 *c7*	0.40	0.32	0.013	0.001
C16:1 *c9*	0.93	0.46	0.070	<0.001
C17:0	2.33	1.36	0.153	<0.001
C17:0 *iso*	0.54	0.40	0.039	0.079
C17:0 *anteiso*	0.94	0.56	0.061	<0.001
C17:1 *c9*	0.79	0.32	0.063	<0.001
C18:0 (SA)	21.08	23.16	0.486	0.033
C18:1 *c6*	0.79	0.98	0.041	0.022
C18:1 *c9*	14.82	12.95	0.422	0.026
C18:1 *c11*	1.53	0.95	0.080	<0.001
C18:1 *c12*	0.65	0.60	0.055	0.697
C18:1 *c13*	0.11	0.09	0.005	0.100
C18:1 *c14*	0.30	0.41	0.017	0.001
C18:1 *t5*	0.06	0.04	0.004	0.021
C18:1 *t6*	0.32	0.18	0.035	0.062
C18:1 *t9*	0.46	0.62	0.033	0.015
C18:1 *t10*	1.79	0.46	0.240	0.003
C18:1 *t11* (VA)	0.85	1.85	0.204	0.012
C18:2 *c9 c12*	11.28	12.59	0.274	0.015
C18:2 *c9 t11* (RA)	0.50	2.22	0.224	<0.001
C18:2 *c10 t12*	0.05	0.06	0.008	<0.001
C18:2 *t8 c13*	0.19	0.20	0.010	0.565
C18:2 *t9 c12*	0.13	0.11	0.006	0.225
C18:2 *t9 c13*	0.19	0.10	0.016	0.002
C18:3 *c6 c9 c12*	0.32	0.24	0.017	0.018
C18:3 *c9 c12 c15* (*α*-LnA)	0.50	0.66	0.027	0.002
C18:3 *c9 t11 c13* (PA)	n.d.	0.53	0.071	-
C18:3 *c9 t11 t13* (*α*-ESO)	n.d.	0.12	0.015	-
C18:3 *t9 t11 c13* (CA)	n.d.	0.02	0.003	-
C18:3 *t9 t11 t13* (*β*-ESO)	n.d.	0.02	0.003	-
C20:0	0.09	0.10	0.003	0.430
C20:1 *c11*	0.18	0.16	0.005	0.025
C20:1 *t11*	0.02	0.02	0.002	0.284
C20:2 *n-6*	0.21	0.26	0.014	0.166
C20:3 *n-3*	0.03	0.03	0.004	0.735
C20:3 *n-6*	1.12	1.32	0.042	0.014
C20:4 *n-6*	8.96	9.33	0.202	0.384
C20:5 *n-3*	0.91	0.89	0.031	0.754
C21:0	0.13	0.07	0.009	<0.001
C22:0	0.04	0.03	0.003	0.573
C22:1 *c13*	0.01	0.00	0.002	0.003
C22:2 *n-6*	0.04	0.09	0.008	<0.001
C22:4 *n-6*	1.82	1.54	0.071	0.052
C22:5 *n-3*	2.44	2.79	0.084	0.033
C22:5 *n-6*	0.62	0.64	0.030	0.756
C22:6 *n-3*	1.35	1.49	0.067	0.339
SFA	35.40	36.15	0.407	0.384
MUFA	23.26	20.22	0.525	0.002
PUFA	31.73	36.59	0.669	<0.001
OBCFA	6.00	3.51	0.376	<0.001

[a]CON: control barley-corn based concentrate diet. WPB: diet including 20% of whole pomegranate byproduct. [b]SEM, standard error of the mean.

the PUFA, the WPB increased the accumulation of RA, LA, C18:2 *c10 t12*, and *α*-LnA in comparison with the CON diet, while the concentration of C18:2 *t9 c13* and C18:3 *c6 c9 c12* was lower in liver from the WPB group compared to CON ($P < 0.05$). The accumulation of some long-chain fatty acids (C20:3 *n*-6, C22:2 *n*-6 and C22:5 *n*-3) in the liver was favored when the animals were fed with the WPB diet ($P < 0.05$). The CLnA isomers (PA, *α*-ESO, CA, and *β*-ESO) were detected only in the liver of lambs receiving the WPB diet. The WPB diet reduced the concentration of total OBCFA in the liver compared to the CON treatment ($P < 0.001$). In particular, the concentration of C15:0, C15:0 *iso*, C15:0 *anteiso*, C17:0, C17:0 *anteiso*, and C21:0 was greater than that found in the liver from lambs fed the CON diet ($P < 0.05$).

Fatty Acid Composition of Intramuscular Fat. Table 5 reports the effect of the dietary treatment on the individual FA in the intramuscular fat. The dietary treatment did not affect the intramuscular fat (IMF). Similarly, the sum of SFA was not different between groups, whereas the concentration of total OBCFA and MUFA was greater in the intramuscular fat of CON lambs as compared to the WPB group ($P = 0.040$ and $P < 0.001$, respectively). Regarding individual OBCFA, the proportion of C15:0, C15:0 *iso*, C15:0 *anteiso*, C17:0, and C17:0 *anteiso* was lowered by feeding WPB compared to the CON diet ($P < 0.05$). Within the MUFA, the proportion of C16:1 *c7*, C17:1 *c9*, C18:1 *t5*, and C18:1 *t10* was higher in the muscle of lambs fed the control diet as compared with WPB ($P < 0.05$), while the intramuscular concentration of C18:1 *c6*, C18:1 *c14*, C18:1 *t9*, and C18:1 *t11* was increased by feeding WPB ($P < 0.05$). The inclusion of WPB in the diet affected the sum of PUFA ($P = 0.017$), whose percentage was greater in the muscle from the WPB lambs in comparison with CON. The proportion of RA was significantly higher in the WPB group as compared to CON ($P < 0.001$), and the same occurred for LA, *α*-LnA (C18:3 *c9 c12 c15*) and C20:2 *n*-6 ($P < 0.05$). Similarly to that reported for the rumen and the liver, CLnA isomers (PA, *α*-ESO, CA and *β*-ESO) were detected only in the muscle from lambs receiving WPB diet.

DISCUSSION

The inclusion of locally available agro-industrial byproducts in the animal diet may contribute to improve the sustainability of the food industry.[28] In particular, the agroindustry could reduce the cost linked to the disposal of waste biomasses, while the farmer could attenuate the cost of animal feeding. Also, replacing conventional feedstuffs, such as cereal grains, with non-human-edible byproducts may lead to a lower feed-to-food competition in livestock production. Finally, bioactive compounds could be transferred from the byproducts, as such or after modification, to the animal products improving their

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Table 5. Effects of the Dietary Treatment on Total Intramuscular Fat (g/100 g of Fresh Muscle) and Fatty Acid Composition of Muscle (g/100 g of Total FA)

	dietary treatment[a]			
	CON	WPB	SEM[b]	*P*-value
intramuscular fat (IMF)	1.88	2.01	0.156	0.690
C10:0	0.15	0.17	0.008	0.425
C12:0	0.11	0.10	0.010	0.714
C14:0	2.17	2.02	0.114	0.530
C14:0 *iso*	0.02	0.02	0.002	0.946
C14:1 *c9*	0.06	0.06	0.006	0.838
C15:0	0.40	0.24	0.025	0.001
C15:0 *iso*	0.07	0.05	0.004	0.012
C15:0 *anteiso*	0.10	0.07	0.006	0.006
C16:0	22.68	22.11	0.497	0.588
C16:0 *iso*	0.13	0.13	0.005	0.768
C16:1 *c7*	0.26	0.23	0.005	0.002
C16:1 *c9*	1.34	1.20	0.058	0.264
C17:0	1.71	0.95	0.127	0.001
C17:0 *iso*	0.34	0.31	0.011	0.216
C17:0 *anteiso*	0.56	0.34	0.033	<0.001
C17:1 *c9*	0.92	0.47	0.072	<0.001
C18:0 (SA)	16.16	16.69	0.553	0.657
C18:1 *c6*	0.35	0.46	0.022	0.005
C18:1 *c9*	36.59	34.75	0.498	0.069
C18:1 *c11*	1.12	0.94	0.050	0.086
C18:1 *c12*	0.45	0.45	0.038	0.976
C18:1 *c13*	0.10	0.11	0.004	0.439
C18:1 *c14*	0.14	0.21	0.011	<0.001
C18:1 *t5*	0.05	0.03	0.006	0.018
C18:1 *t6*	0.16	0.12	0.011	0.124
C18:1 *t9*	0.30	0.35	0.011	0.024
C18:1 *t10*	1.16	0.42	0.113	<0.001
C18:1 *t11* (VA)	0.73	1.38	0.133	0.012
C18:2 *c9 c12*	5.37	7.09	0.409	0.035
C18:2 *c9 t11* (RA)	0.35	0.94	0.084	<0.001
C18:2 *c10 t12*	0.02	0.01	0.004	0.779
C18:3 *c6 c9 c12*	0.06	0.08	0.005	0.188
C18:3 *c9 c12 c15* (α-LnA)	0.37	0.51	0.022	<0.001
C18:3 *c9 t11 c13* (PA)	n.d.	0.42	0.055	-
C18:3 *c9 t11 t13* (α-ESO)	n.d.	0.06	0.008	-
C18:3 *t9 t11 c13* (CA)	n.d.	0.03	0.004	-
C18:3 *t9 t11 t13* (β-ESO)	n.d.	0.01	0.002	-
C20:0	0.12	0.13	0.005	0.635
C20:1 *c11*	0.13	0.14	0.004	0.944
C20:2 *n-6*	0.06	0.08	0.005	0.034
C20:3 *n-3*	0.01	0.01	0.003	0.847
C20:3 *n-6*	0.15	0.24	0.023	0.051
C20:4 *n-6*	1.42	1.92	0.216	0.279
C20:5 *n-3*	0.13	0.20	0.021	0.086
C21:0	0.06	0.05	0.004	0.143
C22:0	0.02	0.03	0.002	0.073
C22:1 *c13*	0.02	0.01	0.003	0.110
C22:2 *n-6*	0.00	0.01	0.003	0.007
C22:4 *n-6*	0.16	0.18	0.018	0.593
C22:5 *n-3*	0.26	0.36	0.036	0.182
C22:5 *n-6*	0.05	0.06	0.007	0.516
C22:6 *n-3*	0.09	0.11	0.016	0.618
SFA	41.43	41.25	0.769	0.912
MUFA	43.01	40.97	0.494	0.040
PUFA	8.82	12.88	0.868	0.017
OBCFA	4.48	2.79	0.261	<0.001
PUFA *n-3*	0.86	1.19	0.087	0.062
PUFA *n-6*	7.29	9.67	0.665	0.080
PUFA *n-6*/*n-3*	8.53	8.20	0.138	0.247
PUFA/SFA	0.22	0.32	0.025	0.051

[a]CON: control barley-corn based concentrate diet. WPB: diet including 20% of whole pomegranate byproduct. [b]SEM, standard error of the mean.

nutritional and technological properties. In the present study, we evaluated the effect of feeding lambs with a diet containing pomegranate pomace for 36 days on the growth performances and on the fatty acid composition of rumen digesta, liver, and muscle. Such short experimental feeding is coherent with the lamb meat production system typical of some Mediterranean regions, where the farms oriented to dairy production represent the major source of sheep meat. With the aim of exploiting the presence of good quality native pasture for milk production in early autumn and to maximize the profit from the meat selling, lambing is concentrated during late summer. Then, the animals are weaned soon and fattened until December, when the markets make requests, and consequently, the demand for lamb meat is high.[29]

Animal Performance. As effect of the inclusion of pomegranate byproducts, the WPB diet contained 17 g of tannins (in TA equivalents)/kg. For a long time, tannins in feeds have been considered as antinutritive and/or toxic compounds because of their potentially detrimental effect on feed palatability, intake, digestion, and animal growth performance.[30] However, the effect of tannins on animal performances is dependent on a number of factors, among which the dosage, the botanical origin and the chemical class of tannins (i.e., hydrolyzable and condensed), as well as the inherent characteristics of the basal diet.[30] Shabtay et al.[31] reported that the fresh pomegranate peel (containing 3.35 and 34.4 g/kg DM of hydrolyzable and condensed tannins, respectively) is very palatable for beef calves, resulting in a greater feed intake and improved growth performances as compared to a control treatment. On the contrary, Oliveira et al.[32] observed a reduction of grain intake and body weight gain when preweaned calves ingested 5 or 10 g/day pomegranate extracts rich in polyphenols (16.9% gallic acid equivalent). Similarly, Hatami et al.[33] reported that feeding a diet including 160 g/kg DM pomegranate marc (containing 3.36% of total tannin in the diet, expressed as TA equivalents) reduced the DMI and ADG of lambs. In the present experiment, despite that the tannin concentration was 12-fold higher in the WPB diet compared with the control diet, no differences were found for voluntary DMI, final body weight, carcass weight, and ADG between treatments, confirming that effects of tannins on animal performance are still controversial. Our findings are in agreement with those reported by Kotsampasi et al.,[18] who incorporated up to 240 g/kg DM of ensiled WPB in growing lamb diet, and confirm that the dietary inclusion of high level of WPB in lamb diet could be a strategy to reduce feeding costs and improve environmental sustainability.

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Fatty Acid Profiles. It is commonly recognized that ruminant products are rich in saturated fatty acids because of the biohydrogenation of dietary unsaturated FA occurring in the rumen.[34] Therefore, in the last decades, several feeding strategies have been proposed with the aim of improving the fatty acid composition of meat and milk, through enhancing the content of those fatty acids (FA) considered beneficial for human health, such as total PUFA, PUFA *n*-3 and rumenic acid (RA; C18:2 *c*9 *t*11). Also, studies have focused on increasing the content of vaccenic acid (VA; C18:1 *t*11), because this fatty acid is extensively converted to RA in mammal tissues by the Δ^9 desaturase.[35] Feeding fresh forages, such as in pasture-based systems, has proved to effectively increase the content of desirable PUFA in meat.[36] However, in the Mediterranean areas, where pasture availability is strongly limited and erratic, another possibility could be represented by the supplementation of diets with sources of PUFA such as oils or oilseeds. However, this latter strategy does not always reach the desired goals because of the high efficiency of the microbial biohydrogenation of PUFA in the rumen.[36] Tannins have been proposed as modulators of PUFA biohydrogenation in the rumen thanks to their direct and indirect action on the bacterial and protozoa community involved in the BH process. Although the effect of tannins is still controversial, it has been generally reported an inhibitory effect on the rumen population, which has been positively associated with the ruminal outflow of PUFA and other desirable fatty acids originating form the biohydrogenation, such as RA and VA.[34]

In the light of the above, the present experiment aimed at improving the FA composition of muscle by feeding lambs with WPB, which contains both PUFA and tannins. In order to better understand the effect of WPB we have investigated the fatty acids in ruminal digesta, liver, and muscle, which represent three important sites involved in ruminant lipid metabolism. Our results clearly show that the fatty acid composition of muscle was improved by the inclusion of WPB in the diet, with the concentration of PUFA, RA, and VA being greater in the muscle of WPB-lambs. The magnitude of this effect was particularly evident for VA and RA, which were found at double and triple concentration in the muscle of WPB-lambs. Interestingly, pomegranate CLnA isomers were detected only in the muscle of lambs of the WPB group, which suggests that some of these molecules were preserved from the complete ruminal BH. In particular, punicic acid was found at the concentration of 0.42 g/100 g of muscle FA. Similar results were found by Emami et al.[14] by feeding lambs with pomegranate seed pulp (100 and 150 g/kg diet DM). To the best of our knowledge, this is the first time that the occurrence of the α-ESO, CA, and β-ESO is reported in ruminant products. For the other PUFA in muscle, we found that the dietary inclusion of WPB increased the proportion of α-LnA and tended to increase the PUFA/SFA ratio. Differing from Emami et al.,[14] we observed that the PUFA *n*-6 to PUFA *n*-3 ratio in muscle was not affected by feeding WPB, which can be attributed to the concurrent increase of PUFA *n*-6 and PUFA *n*-3 in muscle from WPB-fed lambs.

Regarding MUFA, the VA (C18:1 *t*11) is the most abundant among the *trans* octadecenoic acids in ruminant meat and milk fat,[37] and it mainly originates from the principal pathway of rumen BH. However, in animals fed a diet characterized by a high starch-to-forage ratio (e.g., concentrate), the BH pathway may be altered causing an accumulation of C18:1 *t*10 at the expense of C18:1 *t*11 in the rumen, known as "*t*-10 shift", which is then reflected in the ruminant products. The *t*10 isomer has been proposed as a risk factor for human health because of its possible involvement in cardiovascular disease, although this issue is still under debate.[38,39] The occurrence of the "*t*-10 shift" can be evaluated by measuring the C18:1 *t*10/*t*11 ratio, whereby the value of 1 has been proposed as the threshold above which the *t*10-shift can be established.[40] In the present experiment, lambs in both treatments were fed with a concentrate-based diet. Therefore, it was interesting to find that the C18:1 *t*10/*t*11 ratio was strongly reduced in the muscle of WPB lambs in comparison with the control group (0.33 and 1.83 for WPB and CON group, respectively; Figure 1). The same result was found in the liver and rumen digesta,

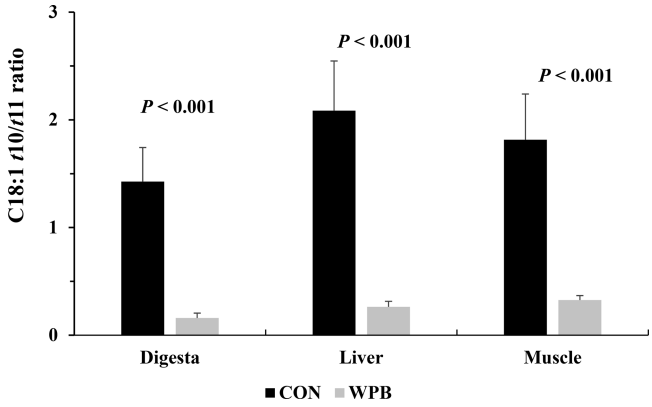

Figure 1. Effect of dietary inclusion of WPB on C18:1 t*10*/*t*11 ratio in rumen digesta, liver, and muscle. CON: control barley-corn-based concentrate diet. WPB: diet including 20% of whole pomegranate byproduct. Values presented are the least-squares means with standard error bars.

suggesting that the WPB diet prevented the *t*10-shift. On the one hand, the greater ingestion of fiber (i.e., NDF) in the WPB group might contribute to explain this observation. Indeed, it is well-known that a low-fiber diet promotes the *t*10-shift[41] mainly by lowering the ruminal pH, with the consequent alteration of the ruminal bacterial species composition.[42] However, in the present study, ruminal pH did not differ between the groups. Other possible mechanisms could explain the lower *t*10-*t*11 C18:1 ratio found in the rumen from the WPB-fed lambs. Among these, the BH of CLnA present in WPB might have contributed to increase the formation of VA. Indeed, although the ruminal BH pathways pomegranate CLnA isomers have not been described so far, these fatty acids are likely to be converted to RA and VA in the rumen,[43] mainly after the saturation of one (Δ^{13}) or two (Δ^9 and Δ^{13}) double bonds, respectively. In the present study, we estimated the BH rate of PA as reported by Alves et al.,[27] and we found that approximately 90% of punicic acid underwent BH. Therefore, it might be supposed that the greater amount of VA produced in the rumen of WPB lambs originated from the BH of punicic acid, with a consequent increase of VA and RA in the liver and muscle. It may also be speculated that the greater intake of vitamin E by lambs in the WPB group contributed to reduce the ruminal production of C18:1 *t*10. Indeed, it has been reported that vitamin E may be involved in preventing the *t*10-shift in the rumen BH, thus reducing the formation and accumulation of C18 *t*10.[44,45] It is still uncertain how vitamin E may affect BH. However, changes involving the rumen population or dynamics could be supposed.[46] Lastly,

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pomegranate tannins could have contributed to modulate the ruminal BH. Indeed, on the one hand, it has been reported that different bacterial strains are responsible for the formation of C18:1 *t*10 and *t*11 isomers and, on the other hand, that tannins can promote shifts in the microbial population of rumen.[47] Therefore, it could be supposed that WPB tannins affected the BH by favoring the production of C18:1 *t*11 instead of *t*10. However, despite total tannins accounted for approximately 90% of total phenols in WPB diet, the effect of non-tannin polyphenols cannot be excluded. Further studies are needed to confirm and to understand how dietary pomegranate by-products may prevent the *t*10-shift in ruminants fed with concentrate-based diets.

Ruminal microroganisms are responsible for the occurrence of odd- and branched-chain fatty acids (OBCFA) in ruminant products.[48] Indeed, rumen bacterial membranes contain high proportions of OBCFA; thus, the measurement of concentration and composition of these fatty acids could be useful to monitor the rumen functionality.[48] In the present study, OBCFA followed a similar trend in the three investigated organ/tissue sites and were strongly reduced by feeding WPB. This is the first study in which fatty acids were analyzed in the rumen digesta from animals fed pomegranate byproducts; therefore, a comparison with the literature is not possible. However, it could be hypothesized that the reduction of OBFCA in the three districts could be due to an inhibitory effect of dietary WPB on rumen microbial population. This effect likely depends on the tannins contained in the WPB diet, which is consistent with inhibitory effect of tannins on rumen bacteria reported both in vitro and in vivo.[49,50]

Nevertheless, the above hypothesis seems to partially contrast with the results obtained on those ruminal fatty acids involved in BH. Indeed, a depressing effect of the rumen BH is one of the most known effects of dietary tannins, which can determine a general depression of the process or the inhibition of specific steps, such as the conversion of VA to SA (last step of BH).[49,51−53] As a result, an increase of BH precursors and intermediates and/or a decrease in SA could have been expected in the rumen of WPB lambs. However, our results suggest that the specific limitation of the terminal step of BH does not seem to have occurred, as demonstrated by the similar SA/(SA+VA) ratio in the ruminal digesta between the treatments. Moreover, despite the greater ingestion of PUFA by WPB lambs, the concentration of PUFA in the rumen digesta was not affected by the dietary treatment, which suggests that feeding WPB did not exert a general depression of the BH. This is further supported by the completeness index of biohydrogenation, which was not different between groups. The greater intake of NDF for lambs fed WPB might partially explain these findings. Indeed, it is well-known that the extent of rumen BH is positively correlated with the NDF level of the diet, because of a longer retention time of feedstuffs in rumen[54] and a favorable environment for bacteria population.[55] Another explanation could be linked to the toxicity of PUFA to rumen bacteria, which increases with increasing degree of unsaturation.[56] Therefore, the BH rate is promoted by feeding high levels of highly unsaturated fatty acids. This could explain why, in spite of the greater intake of highly unsaturated FA (i.e., CLnA) by lambs fed WPB, the proportion of total PUFA in the rumen was not different between the groups. It may be supposed that, in lambs fed the WPB diet, the rumen microorganisms preferentially hydrogenated the highly unsaturated FA from WPB than fatty acids with one or two double bonds. In line with this, no differences between groups were observed for the BH indexes of C18:1 *c*9 and C18:2 *c*9*c*12, while α-LnA acid was saturated at a greater extent in the WPB group and more than 90% of dietary PA underwent BH.

In the present study, the fatty acid composition of the liver generally reflected the intramuscular fatty acid profile. However, some fatty acids in liver followed a different trend compared to muscle, probably caused by a different enzymatic activity. Indeed, we found that many of the Δ^9 desaturase enzyme products (i.e., C14:1 *c*9, C16:1 *c*9, and C18:1 *c*9) were higher in the liver of the control group, suggesting that a higher activity occurred in the liver from animals in this treatment. The C17:1 *c*9 is exclusively synthesized endogenously by the Δ^9 desaturase enzyme, as it is almost absent in the feedstuffs and in the rumen bacteria. Thus, the C17:1 *c*9/(C17:0 + C17:1 *c*9) ratio has been proposed as one of the possible indexes for the indirect estimation of the Δ^9 desaturase enzyme activity.[40] In the present study, this desaturation index (C17Di) was different between the groups in the liver but not in muscle (Figure 2), and a similar result was obtained

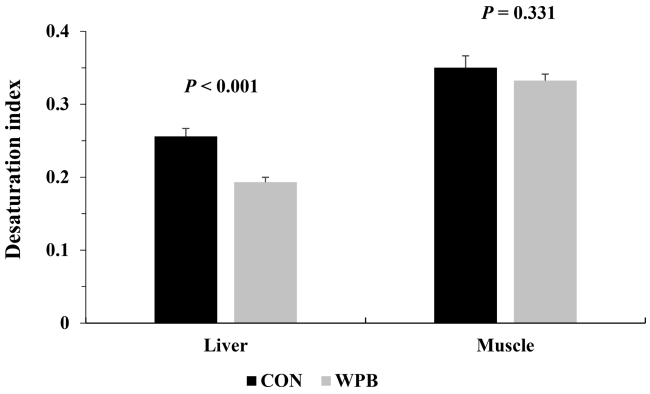

Figure 2. Effect of dietary inclusion of WPB on desaturase index [C17:1 *c*9/(C17:0 + C17:1 *c*9)] in liver and muscle. CON: control barley-corn based concentrate diet. WPB: diet including 20% of whole pomegranate byproduct. Values presented are the least-squares means with standard error bars.

even when the desaturation index was calculated for other fatty acids (C14 to C18; data not shown). The greater percentage of PUFA found in the liver of WPB group could contribute to explain this results. Indeed, PUFA have been described as powerful inhibitors of the Δ^9 desaturase activity in liver or adipocytes through a decreased mRNA expression or stability.[57] Also, Arao et al.[58] reported that the punicic acid has a suppressive effect on the Δ^9 desaturase enzyme activity in rat. Consistently, in the present study we found a negative correlation in liver between the PUFA and the PA with C17Di ($r = -0.861$, $P < 0.001$; $r = -0.740$; $P = 0.009$; for PUFA and PA, respectively). However, a similar result was not observed in muscle suggesting that the influence of PUFA on Δ^9 desaturase enzyme was stronger in the liver than in muscle.

In conclusion, the inclusion of whole pomegranate by-product into a concentrate-based diet can be a profitable strategy to improve the fatty acid composition of lamb muscle, without negatively affecting the animal performances. In particular, the proportion of PUFA, RA and VA in muscle was increased by feeding lambs with WPB, and the health-promoting CLnA of pomegranate was deposited in the intramuscular fat. Moreover, the dietary administration of

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WPB could be proposed as a strategy to prevent the *t*10-shift in intensive concentrate-based feeding systems. Taken together, the results obtained in the ruminal digesta, liver, and muscle suggest that the concurrence of both tannins and polyunsaturated fatty acid in WPB would have favored the deposition of desirable fatty acids in meat. Further specific studies are needed to better understand the mechanisms and the contribution of each WPB bioactive compound and to investigate potential synergistic effects.

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Notes

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ABBREVIATIONS USED

ADF,acid detergent fiber; ADL,acid detergent lignin; BH,biohydrogenation; CA,catalpic acid; CLA,conjugated linoleic acids; CLnA,conjugated linolenic acids; CON,control treatment; DM,dry matter; FA,fatty acid; FAME,fatty acid methyl ester; ME,metabolizable energy; MUFA,monounsaturated fatty acid; NDF,neutral detergent fiber; OBCFA,odd- and branched-chain fatty acid; PA,punicic acid; PUFA,polyunsaturated fatty acid; RA,rumenic acid; SEM,standard error of the means; SFA,saturated fatty acid; VA,vaccenic acid; WPB,whole pomegranate byproduct; α-ESO,α-eleostearic acid; α-LnA,α-linoleic acid; β-ESO,β-eleostearic acid.

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