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PAPER

The effect of dietary supplementation of ensiled pomegranate by-products on growth performance, nutrient digestibility, haematology parameters and meat characteristics of fat-tail lambs

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ABSTRACT

The dietary effects of ensiled pomegranate by-products (EPB) with different levels of polyethylene glycol (PEG) on growth performance, nutrient digestibility, haematological parameters, carcase traits, and meat quality of fat-tail lambs were investigated *in vivo*. Thirty-two lambs were allocated to four experimental diets: containing: (1) diet without EPB as control, (2) diet containing EPB (EPB), (3) diet containing EPB with 5% PEG (EPB5%PEG) and (4) diet containing EPB with 10% PEG (EPB10%PEG). The nutrient digestibility, growth performance, internal organs, carcase traits, and chemical composition of *Longissimus thoracis et lumborum* (LTL) were unchanged between treatments ($p > .05$). However, a significant increase in fatty-acid profile of LTL (vaccenic acid, $p = .04$; C18:2 cis-9 cis12, $p = .05$; rumenic acid, $p = .03$; C18:2 trans-10 cis12, $p = .02$; alpha-linolenic acid, $p = .01$; punicic acid, $p = .03$ and total PUFA, $p < .0001$) was found in EPB-fed animals. The highest total antioxidant capacity ($p = .001$) and the lowest serum malondialdehyde concentration ($p = .05$) were observed in lambs fed EPB. The results indicated that dietary incorporation of EPB without PEG and its partial replacement with grains can improve animal health and meat fatty-acid profile without deleterious effects on growth performance.

HIGHLIGHTS

- The fat-tail lambs were fed with ensiled pomegranate by-products (EPB).
- EPB improved the fatty-acid profile of *Longissimus thoracis et lumborum* and total antioxidant capacity of serum.
- EPB can be partially replaced with cereals without deleterious effect on performance.

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Introduction

Since meat is one of the most widely used sources of animal protein in human nutrition, changing its fatty-acid (FA) profile and chemical composition has a crucial role in meat quality and consumer health. Animal nutritionists can change the FA profile of meat through diet manipulation (Marino et al. 2019). It has been also reported that diets rich in flavonoids (Liu et al. 2019) and natural antioxidants (Velasco and Williams 2011) can improve the meat quality (such as colour, taste, texture, and odour) of lambs. Agricultural by-products are a desirable source of nutrients and bioactive compounds that can be introduced into the diets of ruminants in replacement with traditional and expensive feedstuffs (Grasser et al. 1995; Ajila et al. 2012; Kotsampasi et al. 2021). Pomegranate (*Punica granatum*) is a fruit from the *Punicaceae* family which is extensively produced in Iran (Emami, Ganjkhanlou et al. 2015). Iran is one of the world's largest pomegranate fruit producers with approximately 1.1 million tons in 2019 (Ministry of Agriculture 2019). Some of the compounds such as tannins, alkaloids, glycosides, flavonoids, and phenolic compositions in pomegranate juice, peel, pulp, and seed have been reported in different studies (Noda et al. 2002; Guo et al. 2003; Seeram et al. 2005; Hajimahmoodi et al. 2013). Pomegranate pulp can be included at a high percentage in ruminant's diets because of its suitable content of fibre, crude protein (CP), and fat (Valenti et al. 2019). Punicic acid (PUFA, 18:3 n-5) is classified as a conjugated linolenic acid which is found mainly in the seeds of pomegranate fruit has many biological

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properties such as antidiabetic, antiobesity, antiproliferative and anticarcinogenic activity against different types of cancer (Aruna et al. 2016). Supplementation of diets of small ruminants with pomegranate by-products (PBs) has recently been shown to enrich meat with potentially health-promoting FA such as punicic and rumenic acids (Emami, Fathi Nasri, Ganjkhanlou, Rashidi et al. 2015 ; Natalello et al. 2019). Kotsampasi et al. (2014) reported that supplementation with ensiled pomegranate by-products (EPB) improved the antioxidative capacity and nutritional and functional qualities of lamb's meat. PBs are produced in large quantities worldwide, and they have recently attracted attention in livestock feeding due to their beneficial aspects. Although PBs can also be fed freshly to ruminants, due to its rapid spoiling, it is usually preserved as a dried or ensiled product. Many researchers focussed on the use of pomegranate extracts (Shabtay et al. 2012; Poli et al. 2021; Jami et al. 2020), seeds (Kotsampasi et al. 2021), fresh peels (Shabtay et al. 2008), and dry whole by-products (Natalello et al. 2020a) in livestock feeding, but nutritional aspects of EPB have been less scientifically investigated. Pomegranate seed pulp in replacement with barley and corn grain improved the colour and lipid stability of kid meat (Emami, Fathi Nasri, Ganjkhanlou, Zali et al. 2015). It has been reported that pomegranate peel have relatively high levels of phenolic compounds and total tannins (Hatami et al. 2015) and excessive consumption of tannins can affect the performance of ruminants (Makkar 2003a). It has been also reported that the incorporation of polyethylene glycol (PEG) into diets with high levels of tannins can mitigate its deleterious effects (Makkar 2003a). Therefore, the purposes of this study were to determine whether EPB can be substituted part of the grains and supplementation of PEG affect nutrient digestibility, growth performance, blood metabolites, FA profile, and meat quality of fat-tail lambs.

Material and methods

Preparation of pomegranate by-products silage

PBs containing peels, seed, pith, carpellary membrane, and a small percentage of arils were obtained after juice extracting from a local factory (Narin Noosh Kashmar Co, Kashmar, Iran). After slicing the fresh PB (2–3 cm), three levels (0, 5 and 10% of DM PB) of PEG (Merck, Germany) were added to fresh PB and ensiled in two layers of nylon bags. All prepared silos were kept in a roofed room near the sheep husbandry farm for 60 days until the beginning of the experiment.

Animals, treatments and management

Pomegranate by-product silage (PBS) was used in an experiment with fat-tail lambs, at an industrial sheep husbandry farm (Iran, Khorasan Razavi, Kashmar; 35°14′ N and 58° 27′ E with 206 mm average rainfall, and an average temperature of 17.7 °C). Following a 14-day adaptation period, thirty-two Baluchi male lambs (initial body weight 29.79 ± 2.04, 6-month-old) were randomly allocated to four experimental diets containing: (1) diet without EPB as control, (2) diet containing EPB (EPB), (3) diet containing EPB with 5% PEG (EPB5%PEG) and (4) diet containing EPB with 10% PEG (EPB10%PEG) in a completely randomised design for 90 days. The ingredients and chemical composition of the experimental diets fed to finishing fat-tail lambs are shown in Table 1. Animals were kept based on the guidelines of the Iranian Council of Animal Care (Iranian Council of Animal Care 1995, AEC approval no. 19293). The feed prepared according to NRC (2007) fed to the animals in two equal meals at 7 and 19 h in the form of a total mixed ration (TMR) *ad libitum*. Each lamb was kept in a 2 m × 2 m concrete individual pen and was freely accessed to clean water throughout the experimental period. All experimental diets were isonitrogenous and isoenergetic. Fresh EPB was daily added to the ration of lambs. The offered feed and refusal were recorded daily for each lamb throughout the experimental period. The animals were weighed at the beginning of the experiment and the end of the experiment in order to calculate the average daily gain (ADG). A mean of a 7-day period was considered for the digestion trial (82rd day of the experiment). Total faecal was gathered into the faecal bags. These bags were emptied and weighed twice a day. Before the starting of the digestion trial, animals were adapted in three consecutive days to faecal bags. The gathered samples of total faecal, feed, and refusal were preserved at −20 °C and then were used for DM and chemical analysis. Blood samples were gathered 3 h after morning feeding via jugular vein for two consecutive days (88 and 89) and the mean of these days was considered for final statistical analysis. The gathered blood samples were divided into two tubes, one containing EDTA as an anticoagulant agent for haematology assessment and the other was anticoagulant free for biochemical assay. These samples were preserved at −20 °C for further analysis. At the end of the experimental period, all 8 animals of each treatment were fasted for 18 h (water was allowed), weighed, and slaughtered according to Iranian welfare guidelines at a commercial slaughterhouse. The hot carcase, liver, fat-tail, skin, heart, testicles, kidneys,

lungs, and head were weighted immediately after slaughter. The weight of the cold carcase (with fat-tail) was determined after 24 h chilling at 4 °C. About 250 g of *Longissimus thoracis et lumborum* (LTL) muscle was sampled from the left side of the carcase between the 12th and 13th ribs. The sample was immediately frozen in liquid nitrogen and stored at −20 °C for chemical compounds and FA profile determination.

Laboratory analysis and measurements

For dry matter (DM) determination of feeds and faecal samples, the fresh samples were dried in an air-forced oven (Behdad Co., Iran) (AOAC, 2005) for 48 h. The methods recommended by AOAC (2005) were used for the determination of ash, ether extract (EE) and CP (Kjeldahl, N × 6.25) contents of feed samples, residuals, and EPB (with or without PEG). The concentrations of acid detergent fibre (ADF, for EPW types) and neutral detergent fibre (NDF) in feed samples, residuals, and EPB were determined by procedures of Ankom Technology (2006a, 2006b) with reagents described by Van Soest et al. (1991). The concentrations of total phenolic and total tannins of experimental diets and EPB (with four replicates for each sample) were determined according to the protocol of Makkar (2003b). Total phenols were determined with the Folin–Ciocâlteu reagent using tannic acid (Merck GmbH, Darmstadt, Germany) as a standard, and results were expressed as tannic acid equivalent. Total tannins were calculated as the difference between total phenols and non-tannin phenolic. The serum parameters including albumin, triglyceride, cholesterol, blood urea nitrogen (BUN), glucose, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured using an auto-analyser (A15, Biosystem, Spain) after centrifugation (Eppendorf AG, Hamburg, Germany) at 3000 × g for 10 min. The concentration of haemoglobin (Hb), red blood cells (RBC) count, white blood cells (WBC) count and packed cell volume (PCV) were determined using an automated haematology analyser (CellTac α, MEK-6450, Nihon Kohden, Japan). The malondialdehyde (MDA) concentration was determined as thiobarbituric acid-reactive substances according to Placer et al. (1966). The colorimetric antioxidant assay kit (Cayman Chemical Company, USA) was used for total antioxidant capacity (TAC) determination at the absorbance of 405 nm with a microplate reader. The pH of LTL was measured 45 min and 24 h after slaughter using a Mettler Toledo pH metre with a combination puncture electrode (Mettler-Toledo GmbH Process, Switzerland). The method described by Folch et al. (1957) was employed for the extraction of intramuscular FA in LTL and extraction of FA in EPB with or without PEG. Briefly, a sample of 5 g of LTL or EPB was homogenised with chloroform/methanol (2:1, v/v) solution, filtered, placed in a separating funnel, and then blended with saline solution (0.88% potassium chloride). After differentiation into two phases, the aqueous methanol fraction was thrown away and the chloroform lipid fraction was washed with distilled water/methanol (1:1, v/v). After further filtration and evaporation using a rotary evaporator, lipid extracts were moved to the test tubes. The extracted lipids were methylated using 0.05 mL of 2 N methanolic KOH and 1 mL of hexane (I.U.P.A.C. 1987). The profile of methyl esters of FA was analysed using a gas-chromatograph (Agilent Technologies, 7890 A GC System, Santa Clara, CA, USA) equipped with a flame ionisation detector and fused-silica BPX-70 capillary column (120 m × 0.25 mm i.d., 0.25 μm film thickness). The nonadecanoic acid (Sigma-Aldrich, USA) was used as an internal standard. Helium was used as the carrier gas with a flow rate of 1.0 mL/min. The temperatures of the injector and detector were 220 °C and 280 °C, respectively. A volume of 1.0 μL of the sample with a split ratio of 1:50 was injected into the apparatus. The FAME identification was validated based on electron impact ionisation spectra of FAME and compared with authentic FAME standards (Sigma, St. Louis, MO, USA) and NIST 2007 reference mass spectra library (National Institute of Standard and Technology, Gaithersburg, MD, USA). For cooking loss (%) measurement, the samples of LTL were firstly weighed, transferred into plastic bags, and cooked at a temperature of 75 °C for 60 min in a water bath. After that, bags containing the cooked meats were removed from the water bath and cooled in an ice slurry (Honikel 1998). Then bags were emptied, their contents were dried with paper towels, and reweighed. The weight loss associated with cooking was expressed as a percentage of the fresh weight and reported as the cooking loss percentage. The samples of LTL muscle were grounded and used to determine chemical composition including DM, EE, CP, and ash contents based on AOAC (2005).

Statistical analysis

Thirty-two lambs were analysed in a completely randomised design with four treatments (control, EPB, EPB5%PEG, and EPB10%PEG) and eight replications each, according to the model: $Y_{ij} = \mu + T_i + e_{ij}$, where Y_{ij} = the value of each observation, μ = total mean, T_i

Table 2. Chemical composition and fatty-acid profile of ensiled pomegranate by-products with different levels of polyethylene glycol.

	Silage type				
	EPB	EPB5%PEG	EPB10%PEG	SEM	*p*-value
Chemical composition (% of DM)					
DM (% of as fed weight)	36.37	38.01	37.85	0.38	.14
CP	10.50	10.01	9.80	0.17	.25
NDF	24.43	24.02	23.85	0.28	.73
ADF	16.48	15.20	15.05	0.31	.11
Ash	4.11	4.09	3.90	0.05	.17
EE	4.68	4.60	4.58	0.04	.52
TP	12.55[a]	10.4[b]	9.7[b]	0.44	.0002
TT	11.28[a]	6.80[b]	6.0[b]	0.85	.0005
Fatty-acid profile (% of total fatty acids)					
Myristic acid	0.20	0.18	0.17	0.008	.46
Palmitic acid	3.70	3.65	3.66	0.02	.65
Stearic acid	2.85	2.80	2.82	0.05	.93
Oleic acid	6.37	6.22	6.36	0.07	.67
Vaccenic acid	0.32	0.39	0.29	0.03	.29
Linoleic acid	7.58	7.51	7.51	0.09	.96
Linolelaidic acid	0.32	0.29	0.30	0.03	.93
α-Linolenic acid	3.41	3.15	3.09	0.20	.83
Punicic acid	68.32	67.07	67.16	1.28	.93
α-eleostearic acid	2.85	2.73	2.65	0.19	.93
Catalpic acid	2.67	2.77	2.72	0.14	.97
β-eleostearic acid	0.56	0.58	0.59	0.04	.85
Arachidic acid	0.15	0.10	0.12	0.01	.19

EPB: ensiled pomegranate by-products; EPB5%PEG: ensiled pomegranate by-products with 5% polyethylene glycol (DM basis); EPB10%PEG: ensiled pomegranate by-products with 10% polyethylene glycol (DM basis); DM: dry matter; CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre; TP: total phenolic compounds; TT: total tannins.

= treatment effect and e_{ij} = experimental error. All data were analysed using the MIXED procedure of SAS (SAS Institute Inc. 2002), with the dietary treatment as the main effect. The effect of animal was regarded as random. Data related to each parameter in Tables 1 and 2 with four replicate were also analysed in a completely randomised design. The initial and final weights were considered for ADG calculation. Statistical differences between treatments were determined using Duncan's multiple range test when $p \leq .05$ was detected. Trends were considered when $p < .10$. The data were analysed for normality by the Shapiro–Wilk test.

Results and discussion

Chemical composition and fatty-acid profile of pomegranate by-products

The chemical composition and FA profile of EPB with different levels of PEG are presented in Table 2. With the exception of TP and TT, other parameters including chemical composition and FA profile of EPB were not affected by PEG addition. Similar to our study, the concentrations of TT and TP in ensiled pomegranate peel decreased with increasing PEG (Hatami et al. 2015). To modify the negative effects of dietary tannins, addition of PEG and ensiling have been suggested (Makkar 2003a; Hatami et al. 2015). In this study, to ameliorate the negative effects of tannins, PEG was added to PB at two levels of 5 and 10% DM before ensiling. In our study, the content of DM (36.37 vs. 29.20% fresh weight) was higher, while the concentrations of CP (10.50 vs. 12% DM) and fat (4.68 vs. 6.9% DM) for EPB were lower than those reported by Kotsampasi et al. (2014), however, ADF and ash contents were relatively similar (16.48 vs. 16.9% DM, and 4.11 vs. 4.10%, respectively). The contents of ADF and ash were relatively similar (16.48 vs. 16.9% DM, and 4.11 vs. 4.10%, respectively) to the reports of Kotsampasi et al. (2014). The differences in chemical composition of EPB may be due to various varieties, processing methods, as well as different growing conditions. Similar to the other reports (Khoddami et al. 2014; Laghari et al. 2018), punicic acid (77.80%) was the dominant FA in PB. In contrast with the report of Natalello et al. (2020a), myristic acid was observed in EPB herein. The concentrations of total phenolics and total tannin in whole PB were reported about 17 and 17% of DM, respectively (Natalello et al. 2020a), which were lower (12.55 and 11.28%, respectively) than those reported for EPB in the present study. In some studies, PB has been used in ruminant feeding (Kotsampasi et al. 2014; Natalello et al. 2020a; 2020b) and it contains a high concentration of tannins (Hatami et al. 2015). Due to the lower level of DM in

Table 1. Ingredients and chemical composition of the experimental diets fed to finishing fat-tail lambs.

	Diet			
	Control	EPB	EPB5%PEG	EPB10%PEG
Ingredients (% of DM)				
Alfalfa hay	17.88	17.88	17.88	17.88
Corn silage	21.73	21.73	21.73	21.73
Wheat bran	7.3	7.3	7.3	7.3
Corn grain, ground	16.4	7.13	7.13	7.13
Barley grain, ground	16.4	7.13	7.13	7.13
Sugar beet pulp	3.73	3.73	3.73	3.73
Cotton seed meal	4.1	4.1	4.1	4.1
Soybean meal	8.2	10.33	10.33	10.33
Pomegranate by-products silage	0	16.41	16.41	16.41
Mineral and vitamins supplementa				
Sodium bicarbonate	0.82	0.82	0.82	0.82
Calcium carbonate	0.82	0.82	0.82	0.82
Salt	1	1	1	1
Chemical composition (% of DM)				
DM (% of as fed weight)	57.44	49.92	50.41	50.37
CP	16.45	16.50	16.42	16.38
NDF	37.11	37.91	37.84	37.81
Ash	10.51	10.83	10.83	10.79
EE	2.59	2.78	2.75	2.74
Ca	0.76	0.79	0.79	0.78
P	0.43	0.38	0.38	0.37
ME (Mcal/kg DM)	2.25	2.26	2.26	2.26
TP	0.28	2.62	2.26	1.66
TT	0.16	2.18	1.90	1.32

Control: a basal diet without pomegranate by-product silage; EPB: a diet containing ensiled pomegranate by-products; EPB5%PEG: a diet containing ensiled pomegranate by-products with 5% polyethylene glycol (DM basis); EPB10%PEG: a diet containing ensiled pomegranate by-products with 10% polyethylene glycol (DM basis); DM: dry matter; CP: crude protein; NDF: neutral detergent fibre; EE: ether extract; Ca: calcium; P: phosphorus; ME: metabolism energy; TP: total phenolic compounds; TT: total tannins.
[a]Each kg of supplement contained 100 mg vitamin E, 400000 IU vitamin A, 100000 IU vitamin D, 10 mg vitamin B1, 20 mg vitamin B2, 30 g Calcium, 12 g Phosphorus, 40 g Na, 2000 mg Manganese, 2000 mg Zn, 3000 mg Fe, 1000 mg Cu, 60 mg I, 60 mg Co, and 11 g Mg.

EPB, diets containing EPB (with or without PEG) had a lower percentage of DM compared to the control group (Table 1).

Dry matter intake, nutrient digestibility and growth performance

The effects of diets containing ensiled pomegranate wastes on DM intake, growth performance, and nutrient digestibility in fat-tail lambs are presented in Table 3. We found no significant effect on DM intake, growth performance, and nutrient digestibility when animals were fed different PB silages ($p > .05$). The supplementation of animal's diets with locally available agro-industrial products can be beneficial both for animal husbandries and food industries. The beneficial effects of PB for ruminants have been reviewed by some researchers (Kotsampasi et al. 2014; Natalello et al. 2020a; 2020b). Similar to Kotsampasi et al. (2014), the growth performance and DMI of fattening lambs were not affected by incorporation of EPB into the diet. It is reported that tannins have both detrimental and beneficial impacts on ruminants feeding (Makkar 2003a). Reed (1995) reported that high levels of tannins in diet can reduce DMI, nutrient digestibility (commonly protein and carbohydrates), and animal performance through their negative effect on digestibility and palatability. Although moderate levels of tannins in the ration (3–4% of DM tannins) can have beneficial effects on the availability of protein for ruminants (Min and Solaiman 2018), protein digestibility in EPB-fed animals was not affected by tannins or phenolic compounds available in PB compared to the control group. In the present study, although PEG reduced the level of tannin and phenolic compounds in PB after ensiling, this reduction in the experimental diets did not affect the performance of fat-tailed lambs.

Haematological parameters

The effects of diets containing EPB on haematological parameters of fat-tail lambs are presented in Table 4. Some haematological parameters including triglyceride, cholesterol, Glucose, TAC, and MDA were significantly different between treatments. Lambs fed on

Table 3. Effects of diets containing ensiled pomegranate by-products on dry matter intake, growth performance and nutrient digestibility in fat-tail lambs.

	Diet					
Item	Control	EPB	EPB5%PEG	EPB10%PEG	SEM	*p*-value
DMI (kg/day)	1.341	1.337	1.327	1.357	0.01	.77
Initial BW (kg)	29.79	29.92	29.97	29.48	0.36	.97
Final BW (kg)	45.80	46.09	46.26	46.00	0.33	.96
ADG (g/day)	177.89	179.68	180.97	183.53	1.49	.61
Nutrient digestibility (%)						
DM	65.82	65.94	66.08	66.22	0.23	.94
OM	67.96	68.17	68.13	68.03	0.18	.98
CP	73.62	73.91	74.02	74.29	0.36	.94
NDF	43.68	42.24	43.03	44.11	0.26	.47
EE	49.16	52.11	50.01	50.26	0.50	.20

Control: a basal diet without pomegranate by-product silage; EPB: a diet containing ensiled pomegranate by-products; EPB5%PEG: a diet containing ensiled pomegranate by-products with 5% polyethylene glycol (DM basis); EPB10%PEG: a diet containing ensiled pomegranate by-products with 10% polyethylene glycol (DM basis); DMI: dry matter intake; BW: body weight; ADG: average daily gain; DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fibre; EE: ether extract; SEM: standard error of the mean.

Table 4. Effects of diets containing ensiled pomegranate by-products on haematological parameters of fat-tail lambs.

	Treatment					
Item	Control	EPB	EPB5%PEG	EPB10%PEG	SEM	*p*-value
Serum parameters						
Albumin (g/dL)	3.125	3.541	3.019	3.009	0.13	.45
Triglyceride (mg/dL)	40.64^{a}	35.83^{b}	36.45^{b}	38.51^{ab}	0.59	.01
Cholesterol (mg/dL)	70.05^{a}	66.10^{b}	66.79^{b}	68.66^{ab}	0.58	.05
BUN (mg/dL)	21.92	21.75	21.42	21.22	0.22	.69
Glucose (mg/dL)	69.23^{a}	65.19^{c}	66.52^{bc}	67.54^{ab}	0.43	.004
AST (U/L)	123.56	116.19	120.125	118.87	1.31	.26
ALT (U/L)	27.40	25.99	27.03	26.06	0.36	.42
TAC (mmol/L)	0.219^{c}	0.301^{a}	0.271^{ab}	0.240^{bc}	0.008	.001
MDA (nmol/mL)	2.837^{a}	2.275^{b}	2.368^{b}	2.504^{ab}	0.08	.05
Haematology						
WBC (Cell/μl)	7250.0	7451.6	7469.4	7461.1	52.23	.40
RBC ($\times 10^{6}$/μl)	10.53	10.54	10.58	10.46	0.06	.95
PCV (%)	28.71	29.32	28.22	28.15	0.26	.36
Haemoglobin (g/dL)	10.07	10.14	9.95	10.03	0.06	.73

Different letters along the same row are significantly different according to *p*-value indicated.
Control: a basal diet without pomegranate by-product silage; EPB: a diet containing ensiled pomegranate by-products; EPB5%PEG: a diet containing ensiled pomegranate by-products with 5% polyethylene glycol (DM basis); EPB10%PEG: a diet containing ensiled pomegranate by-products with 10% polyethylene glycol (DM basis); BUN: blood urea nitrogen; AST: aspartate aminotransferase; ALT: alanine aminotransferase; TAC: total antioxidant capacity; MDA: malondialdehyde; WBC: white blood cells; RBC: red blood cells; PCV: packed cell volume; SEM: standard error of the mean.

EPB or EPB5%PEG diets had the lowest triglyceride concentration compared to the control group ($p = .01$). Similar to other studies (Liu et al. 2015; Yang et al. 2018) and in contrast with the results of Safari et al. (2018), the concentrations of triglyceride ($p = .01$) and cholesterol ($p = .05$, Table 4) in lambs fed EPB and EPB5%PEG were significantly decreased compared to the control group. Recently, it has been reported that pomegranate extract with suitable active compounds has a regulatory effect on dysregulation of lipid metabolism and finally alleviates related disorders (Al-Moraie et al. 2013; Yang et al. 2018). The serum glucose concentration in lambs fed the control diet was significantly higher than those fed EPB-diets ($p = .004$). It is reported that PEG can bind dietary tannins (Mkhize et al. 2018). The blood sugar-reducing effect of condensed tannins (CT) was also reported for other CT-containing forages fed to sheep (Wang et al. 1996; Mahgoub et al. 2008). The mechanism by which CT decreases blood glucose concentration is not clear. However, in the study of Peng et al (2016), it was found that purple prairie clover containing CT decreased the molar proportion of propionate which is the only gluconeogenic volatile FAs, which may partly account for the lower serum glucose concentration. Although CT concentration of diets was not measured in this study, it seems that part of the decrease in glucose concentration of the EPB-diets is related to the higher CT percentage compared to control or EPB10%PEG-diets. We found a significant

Table 5. Effects of diets containing ensiled pomegranate by-products on carcase and internal organs weights in fat-tail lambs.

	Treatment					
Item	Control	EPB	EPB5%PEG	EPB10%PEG	SEM	*p*-value
Slaughter BW (kg)	45.27	45.56	45.72	45.46	0.33	.97
Cold carcase (kg)	22.16	22.52	22.40	22.26	0.17	.90
Hot carcase (kg)	22.70	22.04	22.93	22.81	0.17	.92
Cold carcase yield (%)	48.94	49.42	49.00	48.96	0.08	.11
Hot carcase yield (%)	50.13	50.55	50.15	50.17	0.08	.19
Liver weight (kg)	0.734	0.743	0.722	0.733	0.01	.95
Lungs weight (kg)	0.567	0.566	0.567	0.569	0.007	.99
Testicles weight (kg)	0.323	0.331	0.339	0.340	0.004	.55
Spleen weight (kg)	0.108	0.104	0.110	0.103	0.002	.71
Kidneys weight (kg)	0.156	0.155	0.155	0.153	0.002	.94
Fat-tail weight (kg)	3.850	3.914	3.949	3.890	0.07	.97
Heart weight (kg)	0.158	0.160	0.156	0.157	0.002	.80
Skin (kg)	4.887	5.116	5.001	5.026	0.07	.73
Head (kg)	2.469	2.596	2.675	2.562	0.05	.56

Control: a basal diet without pomegranate by-product silage; EPB: a diet containing ensiled pomegranate by-products; EPB5%PEG: a diet containing ensiled pomegranate by-products with 5% polyethylene glycol (DM basis); EPB10%PEG: a diet containing ensiled pomegranate by-products with 10% polyethylene glycol (DM basis); SEM: standard error of the mean.

Table 6. Effects of diets containing ensiled pomegranate by-products on fatty-acid profile (% of total fatty acids) of *Longissimus thoracis et lumborum* in fat-tail lambs.

	Treatment					
Item	Control	EPB	EPB5%PEG	EPB10%PEG	SEM	*p*-value
C10:0	0.47	0.45	0.47	0.49	0.007	.40
C12:0	0.36	0.35	0.36	0.35	0.007	.77
C14:0	3.30	3.19	3.26	3.28	0.02	.30
C16:0	22.63	21.56	22.39	22.67	0.17	.09
C16:1 cis-9	1.71	1.58	1.66	1.70	0.03	.34
C17:0	1.88	1.83	1.87	1.89	0.02	.72
C18:0	16.44	16.14	16.19	16.11	0.14	.84
C18:1 cis-9	37.34	37.25	36.36	36.15	0.23	.17
C18:1 cis-11	0.78	0.82	0.81	0.81	0.02	.90
C18:1 cis-12	0.47	0.51	0.49	0.46	0.02	.76
C18:1 cis-13	0.15	0.17	0.16	0.17	0.007	.63
C18:1 cis-14	0.15	0.16	0.17	0.15	0.005	.37
C18:1 cis-15	0.15	0.14	0.17	0.17	0.006	.22
C18:1 trans-9	0.54	0.42	0.44	0.49	0.02	.23
C18:1 trans-11 (VA)	2.04^{b}	2.56^{a}	2.49^{a}	2.53^{a}	0.07	.04
C18:2 cis-9 cis-12	4.63^{b}	5.06^{a}	4.98^{a}	5.00^{a}	0.06	.05
C18:2 cis-9 trans-11 (RA)	0.79^{b}	0.94^{a}	0.92^{a}	0.91^{a}	0.02	.03
C18:2 trans-10 cis-12	0.11^{b}	0.20^{a}	0.19^{a}	0.18^{a}	0.01	.02
C18:3 cis-9 cis-12 cis-15 (ALA)	0.97^{b}	1.29^{a}	1.26^{a}	1.20^{a}	0.04	.01
C18:3 cis-9 trans-11 cis-13 (PA)	0.67^{b}	0.90^{a}	0.88^{a}	0.89^{a}	0.03	.03
C20:4 n-6	2.56	2.58	2.57	2.56	0.01	.98
C20:5 n-3	1.18	1.21	1.21	1.19	0.01	.70
C22:6 n-3	0.65	0.66	0.68	0.66	0.007	.57
Total SFA	45.10	43.52	44.55	44.79	0.23	.08
Total MUFA	43.32	43.63	42.74	42.63	0.21	.29
Total PUFA	11.58^{b}	12.85^{a}	12.71^{a}	12.58^{a}	0.12	<.0001

Different letters along the same row are significantly different according to *p*-value indicated.
Control: a basal diet without pomegranate by-product silage; EPB: a diet containing ensiled pomegranate by-products; EPB5%PEG: a diet containing ensiled pomegranate by-products with 5% polyethylene glycol (DM basis); EPB10%PEG: a diet containing ensiled pomegranate by-products with 10% polyethylene glycol (DM basis); VA: vaccenic acid; RA: rumenic acid; ALA: alpha-linolenic acid; PA: punicic acid; SFA: short-chain fatty acids; MUFA: mono unsaturated fatty acids; PUFA: polyunsaturated fatty acids; SEM: standard error of the mean.

difference in TAC and MDA among treatments. In line with the reports of Azoz and Basyony (2012), TAC concentration of serum increased significantly when EPB or EPB5%PEG were supplemented to the diets of fat-tail lambs ($p = .001$). Pomegranate is a rich source of natural antioxidant compounds (Tzulker et al. 2007). A greater antioxidant capacity for PB has been reported than its juice (Singh et al. 2002; Tzulker et al. 2007). Pomegranate peel is rich in ellagitannins and also gallic acid, ellagic acid, punicalagin, punicalin, and glycosides of ellagic acid (Gil et al. 2000; Cerdá et al. 2003; Lu and Yuan 2008). Punicalagin has been introduced

Table 7. Effects of diets containing ensiled pomegranate by-products on chemical composition, pH, and cooking loss of *Longissimus thoracis et lumborum* in fat-tail lambs.

	Treatment					
Item	Control	EPB	EPB5%PEG	EPB10%PEG	SEM	*p*-value
DM (% of fresh meat)	23.9	24.6	24.3	24.4	0.13	.30
Ash (%)	1.05	1.07	1.07	1.05	0.02	.94
CP (%)	21.4	22.0	21.8	21.5	0.12	.27
EE (%)	2.99	2.82	2.87	2.95	0.05	.72
pH, 45 min after slaughter	6.61	6.64	6.71	6.73	0.03	.46
pH, 24 h after slaughter	5.87	5.90	5.94	5.94	0.02	.77
Cooking loss (%)	21.71	23.0	22.59	22.01	0.19	.15

Control: a basal diet without pomegranate by-product silage; EPB: a diet containing ensiled pomegranate by-products; EPB5%PEG: a diet containing ensiled pomegranate by-products with 5% polyethylene glycol (DM basis); EPB10%PEG: a diet containing ensiled pomegranate by-products with 10% polyethylene glycol (DM basis); DM: dry matter; CP: crude protein; EE: ether extract; SEM: standard error of the mean.

as a major antioxidant concerning health in human beings (Lin et al. 2001; Tzulker et al. 2007). Therefore, increasing in TAC of serum in EPB fed lambs can be attributed to antioxidant characteristics of PB. Similarly (Ibrahim et al. 2017), the concentration of MDA in growing rabbits fed on PB decreased significantly compared to the control group ($p = .05$). In this regard, the presence of phenolic compounds in PB may explain their activity as antioxidants and radical scavenging agents.

Carcase yield and internal organs weight

The effects of diets containing EPB on carcase and internal organs weight in fat-tail lambs are shown in Table 5. Despite having relatively appropriate concentrations of phenolic compounds and tannins in EPB diets, the parameters related to the carcase and internal organs weight were not affected by the experimental diets ($p > .05$). It has been reported that incorporation of plants containing tannins into the diets of ruminants can improve carcase quality (Priolo and Vasta 2007; Vasta et al. 2007). Similar to the present study, internal organs weight and carcase yield of growing lambs were not affected by the EPB-diets (Kotsampasi et al. 2014). It has been reported that pomegranate peel is rich in tannins and other polyphenolics compounds (Ben Nasr et al. 1996). Similarly, Emami, Ganjkhanlou et al. (2015) did not find any significant effect on carcase characteristics of kids when they were fed pomegranate seed pulp. It has been reported phenolics and polyphenols in PB possess antioxidant, antimitotic, anti-inflammatory, immunomodulatory, and antimicrobial properties both *in vivo* and *in vitro* (Adams et al. 2006; Jayaprakasha et al. 2006; Rosenblat and Aviram 2006).

Fatty-acid profile of Longissimus thoracis et lumborum

The effects of diets containing EPB on FA profile of LTL in fat-tail lambs are presented in Table 6. We found a significant difference in FA profiles between the experimental diets. The sum of MUFA was not different between the experimental groups, whereas the concentration of PUFA was greater in the LTL fat of EPB lambs (0, 5, and 10% PEG) as compared to the control group ($p < .001$). The concentration of SFA tended to decrease in EPB-fed animals (0, 5 and 10% PEG) compared to the control group ($p = .08$). Regarding individual PUFA, the concentrations of C18:2 cis-9 cis-12 ($p = .05$), C18:2 cis-9 trans-11 ($p = .03$), C18:2 trans-10 cis-12 ($p = .02$), alpha-linolenic acid ($p = .01$), and punicic acid ($p = .03$) were increased by feeding EPB (0, 5, and 10% PEG) compared to the control diet. The vaccenic acid (C18:1 t11) content of LTL in EPB-fed lambs was also higher than in the control group ($p = .04$). The FA profile of meat has a great effect on its quality, sensorial characteristics, consumer satisfaction, and mankind's safety (Wood et al. 2008). It is mostly explained that ruminant's products are rich in saturated fatty acids (SFA) due to the biohydrogenation of dietary unsaturated FA that occurs in the rumen (Vasta and Bessa 2012). Therefore, several feeding strategies have been presented to improve the FA profile of meat through enhancing the content of those FA considered advantages for human health recently (Natalello et al. 2020b). Similar to the other reports (Kotsampasi et al. 2014; Natalello et al. 2020b), the results of the present study indicated that EPB has been able to change the profile of FA. Among the FA profile detected, the concentrations of C18:1 cis-9, C16:0, and C18:0 were predominant which is similar to Natalello et al (2019) and Kotsampasi et al (2021). The higher concentration of vaccenic acid in EPB-fed lambs

is useful, because this FA is reported to extensively convert to rumenic acid (C18:2 cis-9 trans-11) in mammal tissues by the Δ^9 desaturase (Palmquist et al. 2004). Although EPB had a high concentration of punicic acid, it is reported that approximately 90% of punicic acid is biohydrogenated in the rumen and it is likely to be converted to rumenic and vaccenic acids, mainly after the saturation of one (Δ^{13}) or two (Δ^9 and Δ^{13}) double bonds, respectively (Natalello et al. 2019). The increase in vaccenic and rumenic acids of EPB-fed lambs (0, 5 and 10% PEG) is supported by the results of Natalello et al. (2019) who reported that the greater amount of vaccenic acid was produced in the rumen of lambs fed whole PB originated from the biohydrogenation of punicic acid, and consequently increased vaccenic and rumenic acids in the liver and muscle. It is also reported that pomegranate tannins can contribute to modulate the ruminal biohydrogenation. Indeed, different bacterial strains are responsible for the formation of some FA isomers (vaccenic acid) and tannins can promote shifts in the microbial population of the rumen (Buccioni et al. 2012). Although PEG bound tannins and phenolic compounds in EPB, we found no significant differences for some FA among the EPB diets (0, 5, and 10% PEG). It might be contributed to the lower use of PEG or lower inclusion of pomegranate in the diet. A higher concentration of total PUFA in LTL of lambs fed EPB could be due to higher concentrations of C18:2 cis-9 cis-12, C18:2 cis-9 trans-11, C18:2 trans-10 cis-12, alpha-linolenic acid, and punicic acid. Contrary to the present study, Kotsampasi et al. (2014) did not find any changes in the proportion of PUFA when EPB was incorporated into the diets of lambs. In line with our results, addition of whole PB (Kotsampasi et al. 2014) into the diet increased PUFA of meat fat in lambs. Although linoleic and alpha-linolenic acids are two important components of animal and plant cell membranes (Simopoulos 1999), their increase in meat (Table 6) can be beneficial for human health (Williams 2000). A trend to decrease in the C16:0 of LTL muscle in EPB-fed lambs can be beneficial for consumers because palmitic acid is effective in the reduction of human serum cholesterol (Gilmore et al. 2011).

Chemical composition, pH and cooking loss of Longissimus thoracis et lumborum

The cooking loss and chemical composition containing DM, ash, CP, and EE of LTL in fat-tail lambs were not affected by the experimental diets ($p > .05$, Table 7). There is no comprehensive data about the quality of meat of fat-tail lambs when they are fed with EPB. Similar to the present study, cooking loss and the meat pH (45 min and 24 h after slaughter) of kids were not affected by the incorporation of PB into the diet (Emami, Fathi Nasri, Ganjkhanlou, Rashidi et al. 2015). The values of pH (Table 7) were also within the normal range reported for sheep meat (Oliveira et al. 2021). Regarding meat quality, the analysis of pH, cooking losses, chemical composition, FA composition as well as other parameters can be effective on the selection power of consumers (Lima Júnior et al. 2016). Similar to the present study, supplementation of the diet of finishing lambs with EPB did not affect the chemical composition (moisture, CP, EE, and ash) of the meat (Kotsampasi et al. 2014). Unlike the current study, feeding sheep with EPB increased the fat content of the LTL muscle (Kotsampasi et al. 2014).

Conclusion

It was confirmed that PEG could decrease the total tannins and phenolic contents of EPB. The punicic acid was the abundant FA detected in EPB. The FA profile of LTL muscle and antioxidant capacity of serum improved when EPB was incorporated into the diet of fat-tail lambs. Due to the economical point of view and the lack of effect of PEG on measured parameters, it would be better to emphasise that EPB can be used in fat-tail lambs without PEG addition. Generally, replacing part of the diet grains with EPB can be effective in reducing feed costs and improving meat FA profile, and animal health.

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Ethical approval

The Animal Ethics Committee at the University of Torbat-e Jam approved all the animal protocols used in the present experiment.

Disclosure statement

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Data availability statement

The data that support the findings of this study are available on reasonable request from the corresponding author.

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