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Feeding pomegranate pulp to Ghezel lambs for enhanced productivity and meat quality

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ABSTRACT

Agrifood by-products contain nutrients and bioactive compounds that can be used in the diets of livestock – thereby value-adding to an otherwise waste product of environmental and economic significance. This study investigated the effect of dietary pomegranate pulp in the total mixed ration of Ghezel lambs, evaluating its effect on growth performance, blood parameters, carcass traits, as well as meat quality and shelf life. 3-month-old Ghezel lambs (individually housed, $n = 8$) were randomly assigned to be either non-supplemented (control) or supplemented with 100 g/kg DM of sun-dried pomegranate pulp for 28 days, post-adjustment. Results showed that supplementation of lamb diets with pomegranate pulp significantly increased liveweight and average daily gains, while not significantly affecting dry matter intake. Lamb serum urea and alkaline phosphatase concentrations and hot carcass weight were increased with pomegranate pulp supplementation. Compared to control lambs, the meat from lambs fed the supplemented diet had higher concentrations of intramuscular fat, mono- and polyunsaturated fatty acid, total unsaturated fatty acid, and meat phenolic compounds. Pomegranate pulp supplemented lambs also had a higher ratio of polyunsaturated to saturated fatty acids; and produced liver tissue with less fat and ash contents. Meat oxidative status (thiobarbituric acid reactive substance) and quality (water holding capacity, colour, and pH) were improved when lambs were supplemented with pomegranate pulp. These findings demonstrate that using pomegranate pulp as a feed for Ghezel lambs has advantageous effects on animal performance and meat quality, offering valorisation of an agrifood by-product.

1. Introduction

The (semi-)intensive production of lamb meat often relies on cereal and grain-based feeds and their being of high quality, low cost, and ready availability. Competition, climate, and geopolitics have had significant impact on the reliability of cereal and grain-based feeds for these production systems (OECD-FAO, 2021; Ponnampalam & Holman, 2023; Sandström et al., 2022), resulting in many prime lamb producers seeking alternative and comparable feedstuffs. In this application, agrifood and industrial by-products may be viable candidate feedstuff – being the organic residues of horticultural and agronomic production as well as from food processing, biofuel and oil production, and forestry sectors (Salami et al., 2019; Yang et al., 2021). There are several advantages to using agro-industrial by-products within the total mixed rations (TMR) for prime lambs, including their low cost, little competition from alternative uses (food vs. feed) or industries, redirection from potentially detrimental waste-streams, and sources for bioactive compounds and dietary nutrients (Vasta & Bessa, 2012; Yang et al., 2021). The effect of agrifood by-products on lamb production as well as carcass and meat quality must be validated on a case-by-case basis. These checks must, therefore, address the increased interest in using pomegranate by-product within the TMR for prime lambs.

The fruit of pomegranate (*Punica granatum* L.) trees is a fleshy berry that is widely consumed and cultivated in Mediterranean, Asian, and

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other countries with comparable climates. The whole fruit is half exocarp (peel) with the other half being edible by humans (40 % arils and 10 % seed), yet both contain phytonutrients such as hydrolysable tannins, condensed tannins, flavanols, anthocyanins, phenolic and organic acids, etc. (Kandylis & Kokkinomagoulos, 2020). When processed for juice, the remaining pomegranate pulp (PP) contains approximately 91 % DM, 7 % CP, 31 % NDF, 23 % ADF, 3 % EE and 4 % ash (Valenti et al., 2019). Combined with its phytonutrient contents, these nutritional factors support PP as a feed ingredient in the TMR for ruminants.

Recent studies have reported advantages to feeding ruminants with PP – with improvements to animal performance as well as milk and meat products qualities observed (Chan et al., 2018; Eliyahu et al., 2015; Kotsampasi et al., 2014; Obeidat, 2023; Safari et al., 2018; Valenti et al., 2019). Of particular note were the findings of Natalello et al. (2020), who found colour, polyunsaturated fatty acid concentration, and the antioxidant capacity of meat was improved when Comisana male lambs were fed pellets containing PP. Natalello et al. (2023) further observed differences in the organic volatile compounds released when the meat of PP fed lambs was grilled; albeit consumer sensory panels could not detect any difference in lamb flavour. The act of pelletising can impact on nutrient bioavailability and the concentration of reactive phytonutrients in feedstuff (Karimizadeh et al., 2017). There is, therefore, a need to confirm these results when non-pelletised and sundried PP is included into the TMR. In addition, a variety of breeds were investigated by these previous studies of PP effects on lamb performance, carcasses and meat quality, including Comisana (Natalello et al., 2023), Baluchi (Kazemi & Valizadeh, 2021), Pelagnoia (Kotsampasi et al., 2021), Awassi (Obeidat, 2023), and Mehraban (Ghoreishi et al., 2021). These studies and the literature have not described the dietary effects of non-pelletised PP in the TMR of Ghezel lambs, a major breed used in the prime lamb production systems of Iran and neighbouring countries (Barzegari et al., 2010).

This study aimed to quantify the effect of non-pelletised and sundried PP, fed at 10 % of the TMR, on the growth rate, liveweight, carcass and meat quality of Ghezel lambs. This included the analysis of measures for performance, serological status, carcass and carcass component yields, tissue chemical composition, water-holding capacity, colour stability, fatty acid composition, and oxidative stability. The null hypothesis was assumed.

2. Materials and methods

The study was conducted at the research facilities of the University of Tabriz (IRN). All protocols and procedures were approved by the Biomedical Ethics Committee of the University of Tabriz.

2.1. Animals and study design

A total of 8 Ghezel lambs (initial liveweight: 35 ± 2 kg) of 3 months age were randomly allocated to be fed with (PP, 100 g/kg DM) or without (control, 0 g/kg DM) PP included in their TMR. The lambs were held within well ventilated, indoor individual metabolic crates (area: 1.5 × 0.75 m^2) for the 14 day adjustment period and 28 day experimental period (total: 42 days). During the adjustment period and when allocated, alfalfa hay and barley straw in the TMR was partially replaced with PP. PP was sourced weekly from a commercial juice manufacturer, immediately after the fruit had been mechanically squeezed (Tabriz, IRN). The fresh PP was sundried for 7 days and then chopped into 1–2 cm pieces, which were included into the TMR (unpelletized). The TMR was formulated according to the lamb requirements for maintenance and 200 g/day live weight gains (National Research Council, 2007). Table 1 includes the proportions of alfalfa hay, barley straw, wheat bran, barley grain, wheat grain, calcium carbonate, vitamin and mineral premix, sodium bicarbonate, and mineral salt used to formulate the TMR. Representative samples of the PP and TMR were analysed and dry matter (DM), crude protein (CP), ether extract (EE), neutral digestible fibre (NDF), and ash content determined as per Taghizadeh and Zabihollah (2008) (Table 1). Throughout the study, lambs had ad libitum access to drinking water and a mineral salt lick. Feed refusals were recorded each day and used to calculate dry matter intake (DMI). Lambs were weighed each week of the study using an electronic walk-over scales and the change in live weight was recorded. DMI and body weight gain (BWG) were used to calculate feed conversion ratio (FCR).

Table 1
Ingredients and chemical composition of the dietary treatments.

	Dietary treatment		Pomegranate pulp
	Control	Supplemented	
Ingredient (%)			
Alfalfa hay	49.24	41.32	–
Barley straw	2.11	0.00	–
Wheat bran	14.06	14.08	–
Barley grain, ground	17.59	17.60	–
Wheat grain	14.07	14.08	–
Pomegranate pulp	0.00	10.00	–
Calcium carbonate	1.04	1.04	–
Minerals and vitamins supplement[1]	1.04	1.04	–
Sodium bicarbonate	0.53	0.53	–
Common salt	0.32	0.32	–
Chemical composition			
ME, Mcal/kg of DM	2.22	2.29	–
DM,%fresh weight	64.93	63.14	89.44
CP,%DM	13.94	13.13	9.63
Ether extract,%DM	2.22	2.51	12.16
NDF,%DM	38.63	36.25	49.60
Ash,%DM	8.98	9.10	4.74
Total phenols, g GAE/100 g	–	–	3.06

[1] Premix supplement provided the following per kilogram of diet: vitamin A 250,000 IU/kg, vitamin D 50,000 IU/kg and vitamin E 1500 IU/kg, manganese 2.25 g/kg, calcium 120 g/kg, zinc 7.7 g/kg, phosphorus 20 g/kg, magnesium 20.5 g/kg, sodium 186 g/kg, iron 1.25 g/kg, sulphur 3 g/kg, copper 1.25 g/kg, cobalt 14 mg/kg, iodine 56 mg/kg and selenium 10 mg/kg. Abbreviations include dry matter (DM).

2.2. Blood collection and analysis

On Day 42 of the feeding study, and following a 14 h period of fasting, 10 mL blood samples (Lithium heparin) were collected using venepuncture from the jugular vein of each lamb. Samples were held on ice and within 2 h of collection they were centrifuged at 1300 × *g* for 15 min to separate the serum from the plasma, the former frozen at −20 °C until analysis. Serum concentrations of urea (using enzymatic Urease-GLDH method; mg/dL), triglycerides (Catalogue number: 117,500; mg/dL), total cholesterol (Catalogue number: 128,500; mg/dL), low density lipoprotein cholesterol (using enzymatic methods; mg/dL), high density lipoprotein cholesterol (mg/dL), aspartate amino transferase (Catalogue number: 118,400; U/L), alanine amino transferase (U/L), alkaline phosphatase (Biochrom WPA Biowave S2100 Diode Array Spectrophotometer; U/L), and lactate dehydrogenase were quantified as per the manufacturer's instructions of the listed laboratory kits (Pars Azmoon Co., IRN) and a benchtop autoanalyzer (ALCYON-300, Abbott, USA) (Nemati et al., 2023).

2.3. Slaughter and carcass assessment

On Day 42 of the feeding study and following a 14 h period of fasting, final curfew live weights were recorded. All the lambs were transported and slaughtered as a single group at a commercial Iranian abattoir – following head stunning and Halal practices. The carcasses were eviscerated and dressed. Individual organ and carcass components were weighed, and liver tissue samples were collected at this same time. Hot carcass weights were recorded ~ 45 min post-slaughter and prior to

entry into the chiller (3–4 °C). Cold carcass weights were recorded ~ 24 h post-slaughter and used with final curfew liveweight to calculate dressing percentages. At this time, both *longissimus lumborum* (LL) muscles were removed. The left LL were frozen at −20 °C and analysed for chemical composition, total phenolic content, and fatty acid composition. The right LL was divided into 4 equal portions that were vacuum-packaged and allocated to one of four ageing periods (1, 4, 8, or 12 days; 3–4 °C). These aged samples were analysed for colour, cooking loss, drip loss, pH, thiobarbituric acid reactive substances (TBARS), and water-holding capacity (WHC).

2.4. Liver and muscle chemical composition

Liver and LL sample chemical compositions were determined as per AOAC (2006). Dry matter (DM) and ash contents were determined by gravimetric methods and using a muffle furnace respectively set a 102–105 °C for 45 min and 550 °C for 24 h, when a constant weight was achieved. Ether extract (EE) were determined using a Soxhlet extractor unit (liquid-solid extraction) set at 85 °C for 90 min. Crude protein (CP) was determined using the Kjeldahl method and the $N \times 6.25$ correction factor. of 10 g were homogenised with 100 mL distilled water (IKA, T50 Ultra-Turrax, GER), allowed to equilibrate to room temperature, and assessed using a pH meter (Hanna, Methrom, CHE). The pH meter was calibrated using room temperature standard buffer solutions of pH 4 and pH 7 (Nemati et al., 2021a).

2.5. Cooking loss

Cooking loss was measured according to Mortensen et al. (2006). Meat samples of ~ 20 g were placed into polyethylene containers and incubated in a 75 °C water bath for 10 min. The samples were then removed, cooled to room temperature, and weighed. The percentage change in sample weight before and after cooking was reported as the cooking loss.

2.6. Total phenolic content

Sample total phenolic content was determined using the Folin-Ciocalteu method described by Liu et al. (2009). In brief, 50 g samples were combined with 100 mL of distilled water, homogenised (IKA, T50 Ultra-Turrax, GER) for 60 s at 15,000 × *g*, and then allowed to stand for 20 min at room temperature. The homogenate was filtered, cooled, and transferred into a 50 mL glass tube with 5 mL of Na_2CO_3 solution and 2.5 mL of Folin-Ciocalteau reagent. This was vortexed and left for 60 min under darkness. Samples were then measured using a benchtop spectrophotometer (model 3210, Hitachi Ltd., JAP) set to measure absorbance at 700 nm. Data were compared to a linear standard curve prepared using a serial dilution of gallic acid (Liu et al., 2009).

2.7. Fatty acid composition

Fresh samples of 5 g were first extracted as per Folch et al. (1957) and the lipid fraction analysed for fatty acid composition as per Nemati et al. (2021a). The fatty acid methyl esters (FAME) were separated using a gas chromatograph fitted with an Agilent capillary column (30 $m \times 0.25$ mm i.d., CPS Analitica, ITA) and the temperature settings for the 86 min total run time are described by Damirchi et al. (2005). Helium was used as the carrier gas and nitrogen as the makeup gas, at a flow rate of 30 mL/min. The FAMEs were quantified by injecting 1 μL of the sample into GC–MS. Tridecanoic acid (C13:0) methyl ester was used as an internal standard. FAME data converted so that the concentration of fatty acids was reported as mg per 100 g of fresh sample.

2.8. Water-holding capacity

Water-holding capacity (WHC) was determined using the gravimetric method, whereby samples of weight 10.0 ± 0.1 g were centrifuged at 200 × *g* (Universal 320R, Hettich, GER) at room temperature and the percentage change in sample weight recorded as WHC.

2.9. Thiobarbituric acid reactive substances

The TBARS concentrations of aged samples were determined as per the colorimetric method of Nemati et al. (2021b) and using a benchtop spectrophotometer (model 6405, Jenway, UK) set to measure absorbance at 532 nm. The results were calculated using a 0–10 ppm standard curve of 1,1,3,3-tetraethoxypropane and expressed as mg of malondialdehyde (MDA) per kg of fresh meat (mgMDA/kg).

2.10. Colour parameters

Sample surfaces were measuring using a simple digital imaging system and the method of León et al. (2006). First, a digital camera (Sx620 HS PowerShot, Canon Ltd., JAP) with a resolution of 20 mp was placed 30 cm vertical to the sample, on which muscle fibre orientation was perpendicular to the cut surface. An image was recorded with light sources positioned at 45° to the lens axis. Photoshop (Adobe) was used to analyse the images, with three locations selected, at random albeit with care to avoid connective tissue and fatty deposits, and the mean L^*, a^*, and b^* CIE colour coordinates calculated. Hue angle and chroma values were calculated *post-hoc* (AMSA, 2012).

2.11. Statistical analysis

Data were analysed using SAS statistical software (Version 9.2., SAS Institute Inc., USA) using base linear regression models (PROC REG) fitted with the fixed effect of dietary treatment and the random effect of animal. The additional fixed effects of ageing period and its interaction with dietary treatment were fitted for the analysis of colour parameters, cooking loss, drip loss, pH, TBARS, and WHC data. Means were compared using Tukey multiple comparison tests with the level of significance set at 5 %.

3. Results

The chemical composition and phenolic compounds of PP are presented in Table 1.

3.1. Growth and performance

BWG (8.6 v. 6.1 kg; $P = 0.049$) and ADG (246.4 v. 175.0 g/day; $P = 0.049$) were both higher for lambs fed PP than observed for control lambs (Table 2). Lamb DMI, FBW and FCR were not affected by dietary treatment ($P > 0.05$).

3.2. Blood biomarkers

Serum urea (29.0 v. 23.8 mg/dL; $P = 0.012$) and alkaline phosphatase (636.7 v. 501.0 U/L; $P = 0.009$) concentrations were both higher for lambs fed PP than was observed for control lambs (Table 2). No other blood biomarker was affected by dietary treatment ($P > 0.05$).

3.3. Carcass parameters

The spleen weight for lambs fed PP was higher than was observed for control lambs (126.7 v. 89.7 g; $P < 0.001$; Table 3). The large intestine (220.0 v. 155.0 g; $P = 0.003$) and full abomasum (231.6 v. 267.4 g; $P = 0.031$) weights for control lambs was higher than was observed for lambs fed PP (Table 3). No other carcass parameter was affected by dietary treatment ($P > 0.05$).

Table 2
Mean and standard error (SEM) growth performance and blood parameters for the Ghezel lambs fed total mixed rations with or without pomegranate pulp.[1].

	Dietary treatment		SEM	*P*-value
	Control	Supplemented		
Growth performance				
Dry matter intake, kg/day	2.18	2.17	0.01	0.766
Initial live weight, kg	34.8	34.4	1.7	0.879
Final live weight, kg	40.9	43.0	1.4	0.308
Body weight gain, kg	6.13[b]	8.63[a]	0.72	0.049
Average daily gain, g/day	175.0[b]	246.4[a]	20.5	0.049
Feed conversion ratio	13.3	8.9	1.5	0.090
Blood parameters[2]				
Urea, mg/dL	23.75[b]	29.00[a]	1.06	0.012
Total cholesterol, mg/dL	58.50	63.00	6.17	0.624
Triglycerides, mg/dL	10.50	12.75	1.55	0.344
High density lipoprotein cholesterol, mg/dL	26.00	27.00	2.78	0.808
Low density lipoprotein cholesterol, mg/dL	30.25	36.00	3.87	0.333
Aspartate amino transferase, U/L	103.5	109.7	11.1	0.703
Alanine amino transferase, U/L	20.50	19.75	3.07	0.868
Alkaline phosphatase, U/L	501.0[b]	636.7[a]	25.5	0.009
Lactate dehydrogenase, U/L	456.2	457.0	21.3	0.981

[1] Means within the row with differing letter superscripts are different ($P < 0.05$).

Table 3
Mean and standard error (SEM) carcass parameters for the Ghezel lambs fed total mixed rations with or without pomegranate pulp.[1].

	Dietary treatment		SEM	*P*-value
	Control	Supplemented		
Carcass efficiency,%	48.7	47.6	1.1	0.500
Hot carcass weight, kg	20.0	20.1	0.5	0.453
Cold carcass weight, kg	19.6	19.1	0.7	0.643
Dressing percent,%	51.3	52.4	1.1	0.500
Organ weights				
Heart, g	182.6	165.1	5.5	0.084
Liver, g	590.0	625.0	16.5	0.207
Lungs, g	470.0	515.0	42.1	0.491
Kidney, g	105.1	125.9	12.0	0.286
Spleen, g	89.7[b]	126.7[a]	1.6	< 0.001
Large intestine, g	155.0[b]	220.0[a]	8.9	0.003
Small intestine, g	735.0	895.0	60.9	0.136
Full rumen, g	640.0	725.0	53.6	0.324
Full omasum, g	132.8	140.4	7.2	0.496
Full abomasum, g	267.4[a]	231.6[b]	7.8	0.031
Full reticulum, g	156.9	116.57	17.2	0.172
Head, g	2290	2385	24.9	0.410
Testis, g	211.8	267.1	27.8	0.231

[1] Means within the row with differing letter superscripts are different ($P < 0.05$).

3.4. Meat quality and oxidative status

The percentage fat of the meat from lambs fed PP were higher than was observed for control lambs (1.7 v. 1.3%; $P < 0.001$; Table 4). The percentage ash of the meat (2.4 v. 2.3%; $P = 0.023$) and percentage fat of the liver (1.5 v. 1.1%, $P = 0.020$) were observed to be highest for control lambs, compared to those fed PP. No other proximate component of the meat or liver tissue were affected by dietary treatment ($P < 0.05$). The pH at 24 h post-slaughter of meat from lambs fed PP was higher than was observed for the control lambs (6.55 v. 5.99; $P = 0.002$; Table 4). The pH of meat from control lambs was higher than was observed for the lamb's fed PP at Day 12, with increases to pH observed with increased ageing period (6.9 v. 6.5; $P < 0.05$; Fig. 1). The WHC of meat from lambs fed PP was lower than was observed for control lambs at Day 4 and thereafter (Fig. 1; $P < 0.05$). The meat of lambs fed PP had lower cooking loss than was observed for control lambs ($P < 0.05$). No significant dietary

Table 4
Mean and standard error (SEM) physiochemical parameters for the meat and liver from Ghezel lambs fed total mixed rations with or without pomegranate pulp.[1].

	Dietary treatment		SEM	*P*-value
	Control	Supplemented		
pH at 24 h *post-mortem*	5.99[b]	6.55[a]	0.06	0.002
Moisture,%DM	74.46	75.14	0.29	0.167
Ash,%DM	2.35[a]	2.28[b]	0.01	0.023
Crude protein,%DM	22.48	21.87	0.30	0.220
Intramuscular fat,%DM	1.32[b]	1.69[a]	0.03	< 0.001
Liver fat,%DM	1.48[a]	1.14[b]	0.07	0.020
Total phenols, g GAE/100 g	9.37[b]	14.52[a]	0.01	< 0.001

[1] Means within the row with differing letter superscripts are different ($P < 0.05$). Abbreviations included dry matter (DM).

treatment by ageing period interaction was observed for meat cooking loss in the present study. ($P > 0.05$). The cooking loss value of meat increased as ageing period increased ($P < 0.01$), being lowest at Day 1 (30.3 %) and highest at Day 12 (41.0 %) (Table 6).

The total phenolic content of meat from lambs fed PP was higher than was observed for control lambs (14.5 v. 9.4 g GAE/100 g; $P < 0.001$; Table 4). From Day 4 onwards, the TBARS content of meat from control lambs was higher than was observed for the lambs fed PP, the difference being greatest at Day 12 (Fig. 1).

3.5. Fatty acid composition and indices

The meat of lambs fed PP had higher concentrations of C15:0 (15.3 v. 5.9 mg/100 g; $P = 0.012$), C16:1n-6 (36.0 v. 17.0 mg/100 g; $P = 0.049$), C17:0 (38.5 v. 17.0 mg/100 g; $P = 0.003$); C18:0 (271.7 v. 186.9; $P = 0.019$), C18:1n-9 (474.5 v. 298.3 mg/100 g; $P = 0.009$), C18:2n-6 (162.3 v. 73.6 mg/100 g; $P = 0.002$), and C20:3n-3 (22.3 v. 5.8 mg/100 g; $P = 0.021$) than was observed for control lambs (Table 5). The meat of control lambs had a higher concentration of C22:0 (122.3 v. 11.8 mg/100 g; $P = 0.001$) than was observed for lambs fed PP. The meat of lambs fed PP had higher concentrations of ∑MUFA, ∑PUFA, and ∑TUFA as well as a higher PUFA:SFA value than was observed for control lambs ($P < 0.05$; Table 5). Peroxidability index was not affected by dietary treatment ($P > 0.05$).

3.6. Colour parameters

The b^* value of meat from lambs fed PP was lower than was observed for the control lambs ($P = 0.039$; Table 6). The L^* value of meat declined as ageing period increased ($P < 0.001$), being highest at Day 1 (26.2) and lowest at Day 12 (19.0). The b^* value of meat increased as ageing period increased ($P = 0.009$), being lowest at Day 1 (10.0) and highest at Day 12 (13.7) (Table 6). Fig. 2 shows the significant dietary treatment by ageing period interaction effects on a^*, hue angle, and chroma values. The a^* value of meat from lambs fed PP was higher that was observed for the control lambs at Day 12 ($P < 0.05$). The hue angle value of meat from lambs fed PP was higher for control lambs than was observed for lambs fed PP at Day 12 ($P < 0.05$). There was a significant decline in chroma values between Day 4 and Day 8 observed only for the meat from lambs fed PP ($P < 0.05$).

4. Discussion

The PP had comparatively higher concentration of CP, NDF, and ether extract to the PP used in previous research – for example, the PP used by Natalello et al. (2020) was 90.0 % DM, 6.3 % CP, 28.8 % NDF, 20.7 % ADF, and 3.5 % Ash. The total phenolic content of the PP was also lower than those previously reported (Kazemi & Valizadeh, 2021; Natalello et al., 2020). These differences in total phenolic content and proximate composition are likely due to the pomegranate variety used,

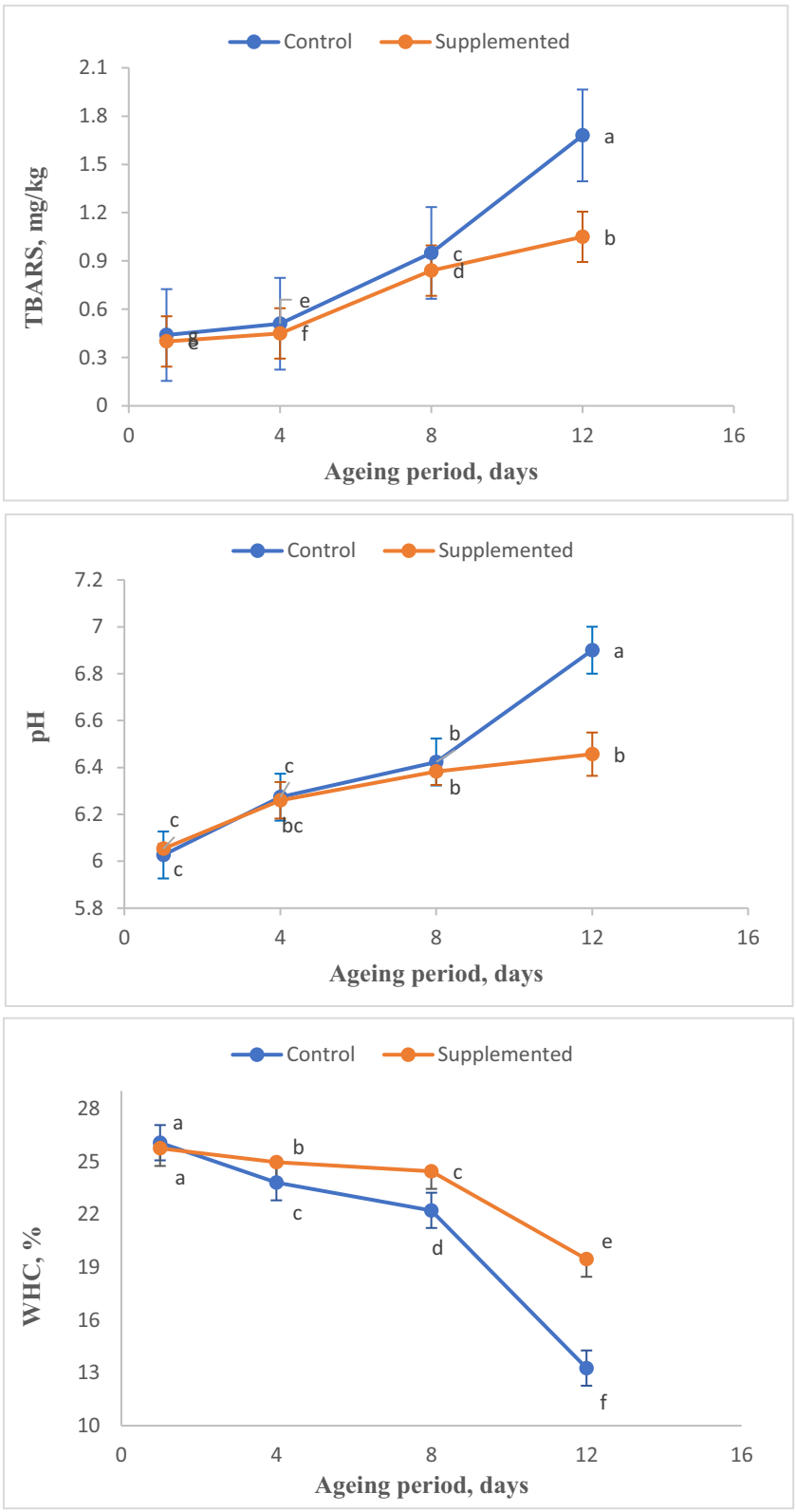

Fig. 1. The effect of dietary supplement by ageing period interactions on thiobarbituric reactive substance (TBARS) concentrations, pH, and the water holding capacity (WHC) of meat from Ghezel lambs. Different letters indicates significant differences between mean values ($P < 0.05$).

the processing methods from which PP by-product was sourced, and the pre-processing of the PP before it was fed to the lambs within a TMR.

The physiochemical properties of lamb meat are dynamic across an ageing period, resulting in changes that can affect consumer acceptability and health (Bekhit et al., 2021; Coombs et al., 2017; Fernández et al., 1997). PP is rich source of nutrients and bioactive compounds which are valuable to meat shelf-life and animal health (Ponnampalam et al., 2022), including phenolic compounds (flavonoids, anthocyanins, phenolic acid and mainly hydrolysable tannin), minerals and polysaccharides, while proteins, fibres, vitamins, minerals, pectin, sugars,

Table 5
Mean and standard error (SEM) fatty acid (mg/100 g) concentrations for the meat from Ghezel lambs fed total mixed rations with or without pomegranate pulp.[1].

	Dietary treatment		SEM	*P*-value
	Control	Supplemented		
C10:0	8.72	8.95	2.99	0.959
C12:0	5.62^{b}	11.99^{a}	1.80	0.066
C14:0	27.65	32.87	10.38	0.740
C15:0	5.94^{b}	15.29^{a}	1.55	0.012
C16:0	228.2	303.5	27.8	0.128
C16:1n-6	19.80^{b}	35.99^{a}	4.12	0.049
C17:0	16.96^{b}	38.53^{a}	2.40	0.003
C18:0	186.9^{b}	271.7^{a}	15.8	0.019
C18:1n-9	298.3^{b}	474.5^{a}	26.5	0.009
C18:2n-6	73.59^{b}	162.3^{a}	8.74	0.002
C18:3n-3	18.08	21.29	4.14	0.612
C20:0	37.16	70.90	14.68	0.179
C20:3n-3	5.80^{b}	22.30^{a}	3.19	0.021
C20:4n-3	83.95	78.92	5.36	0.543
C22:0	122.3^{a}	11.83^{b}	2.84	0.001
C24:0	51.74	0.51	17.19	0.102
C20:5n-3	6.60	18.67	8.08	0.350
C22:6n-3	16.17	15.32	6.95	0.331
MUFA	318.1^{b}	510.5^{a}	30.6	0.011
PUFA	204.2^{b}	318.9^{a}	11.3	0.002
TUFA	522.3^{b}	829.3^{a}	28.3	0.001
SFA	691.2^{b}	766.1^{a}	10.83	0.008
PUFA:SFA	0.29^{b}	0.41^{a}	0.01	0.002
PI	905.8	908.8	3.3	0.554

[1] Means within the row with differing letter superscripts are different ($P < 0.05$). Abbreviations included monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), total unsaturated fatty acids (TUFA), saturated fatty acids (SFA), the ratio of polyunsaturated to saturated fatty acids (PUFA: SFA), and peroxidability index (PI).

polyphenols, isoflavones, and fatty acids. Of the latter, lipid extracted from pomegranate seeds contains high concentration (12–20 %) of PUFA (including linolenic and linoleic acids, as well as other lipids such as punicic acid, oleic acid, stearic acid, and palmitic acid). Ellagitannins, a member of hydrolysable tannin, is present in the pericarp and seeds of pomegranate, which are converted to urolithins by the intestinal microflora (Kandylis & Kokkinomagoulos, 2020) and thereby increase the production of short chain fatty acids. Further, Akhtar et al. (2015) reported the antioxidant potential of pomegranate peel is higher than was observed for pomegranate arils and seeds. The applied PP at the present study contained 3.1 g GAE/100 g total phenolic compounds; so, the benefits of dietary PP supplementation in lambs' performance and meat shelf life were attributed to their bioactive compounds.

Recent study of 2 month old lambs (average body weight of 14.8 ± 2.0 kg) and a pelletised concentrate diet inclusive of 200 g/kg DM of whole pomegranate by-product did not find any significant difference between the control and supplemented group on liveweight, carcass weight, DMI, ADG or FCR (Natalello et al., 2020). In other research of beef calves, feeding with pomegranate peels in fresh form was found to significantly increase DMI (Shabtay et al., 2008). In contrast, as reported by Shaani et al. (2016), inclusion of 200 g/kg DM pomegranate pulp in lactating cows significantly decreased DMI. Kotsampasi et al. (2014) fed weaned male lambs (18.8 ± 2.28 kg average weight; 65 ± 5 days of age) with 120 and 240 g/kg DM pomegranate by-product silage for 9 week and reported no change in liveweight, BWG, DMI, and FCR attributable to dietary treatment (Kotsampasi et al., 2014). It is possible that numerical NDF differences between the control and supplemented dietary treatments contributed to the ADG and BWT outcomes – an effect noted by the non-significant trend in FCR data. The variance in these data must be considered, however, it was confirmed that dietary PP supplementation supports gains to Ghezel lamb live weight.

The cold carcass weight and carcass yield as well as other carcass traits were similar among dietary treatments of this previous study, with increasing level of pomegranate by-product silage (Kotsampasi et al., 2014). Using goats, Emami et al. (2015) reported that kid carcass characteristics were observed not to be affected by incorporation of PP into the diet. In agreement with the results of current study, Kotsampasi et al. (2014) reported that the feeding of pomegranate by-product silage was not affected the weight of liver, heart, lungs, kidneys, and small intestine in growing lambs; however, in contrast with the present study the increase of spleen weight causing with PP feeding was not significant (Kotsampasi et al., 2014). Also, Eliyahu et al. (2015) reported that the lambs received 51 % PP silage TMR have higher daily DMI than control group. These and the current study demonstrate that PP can be fed to ruminants, specifically Ghezel lambs, and deliver a TMR that supports the nutritional requirements of growing ruminants.

PP is rich of tannins which due to their low palatability and low digestion of protein and carbohydrates have negative effect on diet consumption and growth performance (Ponnampalam et al., 2015a; Reed, 1995). Therefore, it seems that using higher amounts of PP in animal diets reduce the positive effects through reducing feed consumption (Eliyahu et al., 2015). As described by Min et al. (2003), low and moderate levels of dietary condense tannin have beneficial effect in ruminant efficiency production with no effect on intake; a result that aligns with the result of the current study, in which the administration of moderate level of 100 g/kg DM PP in growing lambs statistically improved BWG. These results confirm those of Rajabi et al. (2017), who reported that the feeding of pomegranate peel extract to lambs had no effect on DMI, even though ADG was improved; a result attributed to the greater supply of microbial protein.

In agreement with the results of the present study, feeding of pomegranate seed (2.2 and 2.9 % DM in prepartum and postpartum, respectively) or pomegranate seed pulp (seed + peels; 12 and 16 % DM in prepartum and postpartum, respectively) to dairy cows did not have significant effect on serum total cholesterol, total glucose, and aspartate amino transferase (Safari et al., 2018). In contrast with the result of the present study, Hussein and Shugaa (2013) reported reduced concentrations of urea in the serum of Awassi lambs fed with increasing levels of pomegranate peel extract. Other than a potential breed effect, the discrepancy between the results of these studies was unknown.

Following slaughter, changes on the permeability of cell membrane, lead to low WHC of the meat (Huff-Lonergan & Lonergan, 2005). This change is mainly related to the anaerobic glycolysis reaction and lactic acid accumulation in the muscles as well as the stopping blood flow, which induces cellular hypoxia and reduce the pH of the meat to an ultimate pH of 5.4–5.8 (Lambert et al., 2001). The pH affects the physiochemical properties of meat including, colour, WHC, texture,

Table 6
The effect of dietary treatment and ageing period on the cooking loss, L^* (lightness), and b^* (yellowness) of meat from Ghezel lambs fed total mixed rations with or without pomegranate pulp.[1].

	Dietary treatment		SEM	Ageing period, days				SEM	P-value		
	Control	Supplemented		1	4	8	12		Diet	Ageing	Interaction
Cooking loss,%	38.0^{y}	34.3^{x}	0.5	30.3^{a}	35.5^{b}	37.8^{b}	41.0^{c}	0.631	0.004	< 0.001	0.666
L^*	22.6	23.7	0.5	26.2^{a}	24.3^{ab}	23.0^{b}	19.0^{c}	0.745	0.213	< 0.001	0.301
b^*	13.0^{y}	10.6^{x}	0.6	10.0^{c}	10.7^{bc}	12.8^{ab}	13.7^{a}	0.680	0.039	0.009	0.741

[1] Means within the row and fixed effect with differing letter superscripts are different ($P < 0.05$). Abbreviations included standard error (SEM).

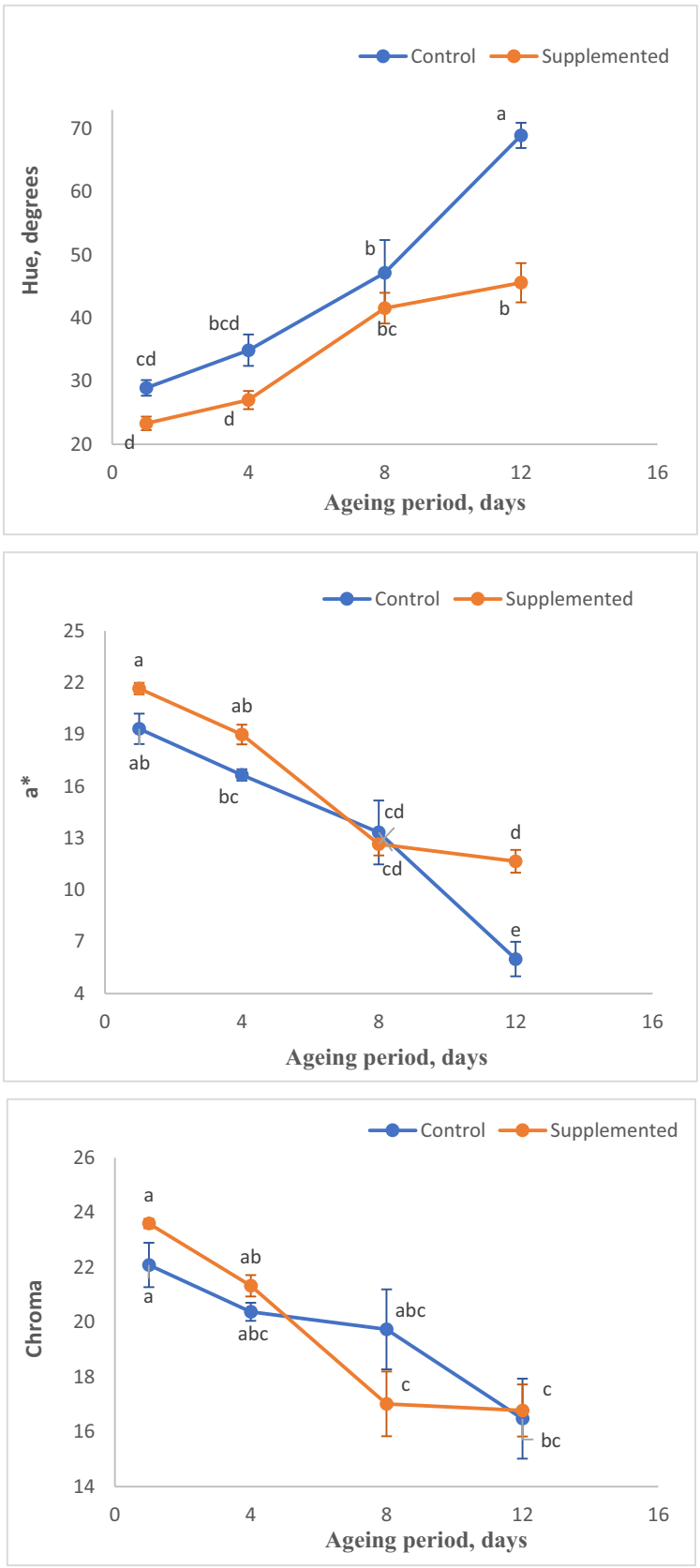

Fig. 2. The effect of dietary supplement by ageing period interactions on chroma, *a**, and hue values for meat from Ghezel lambs. Different letters indicates significant differences between mean values ($P < 0.05$).

sensory, cooking loss and safety (Shang et al., 2014; Zhang et al., 2021). The observed difference in pH between control lambs and those fed PP could, therefore, provide a basis for the WHC differences observed in the study. Alternatively, the antagonistic relationship between protein oxidation and degradation may account for the variation in WHC, the former inhibited by antioxidant compounds (e.g., those found in PP) and the latter associated with reductions to WHC (Estévez, 2011).

Kotsampasi et al. (2014) reported that dietary pomegranate by-product silage supplementation did not differ in moisture and protein and ash content in lamb muscle samples among treatments. Instead, the meat from Ghezel lambs fed PP had the higher moisture content than control. These results confirm with those reported by Ahmed et al. (2015), who showed that the moisture content of breast meat of broilers fed diet supplemented with the pomegranate (*Punica granatum* L.) by-products had significantly higher moisture content than in the 0 % pomegranate by-products supplemented group. This increase in moisture content might be related to the high phenolic content of meat samples fed diet supplemented with PP.

The results of the current study are in agreement with the findings of Kotsampasi et al. (2014), who evaluated the effects of dietary pomegranate by-product silage supplementation on meat quality of growing lambs. These authors showed that 240 g/kg pomegranate by-product in diet increased total fat content from 3.4 to 4.3 % but total fat contents were lower than those found by Kotsampasi et al. (2014) and Shabtay et al. (2008). In contrast, Ahmed et al. (2015) reported that the total fat contents were higher in thigh meat of broilers fed diets supplemented with 0.5, 1.0, or 2.0 % pomegranate by-product silage compared to control. These variations in total fat content of meat can be related to pomegranate peel chemical composition as well as different varieties and processing methods.

The pH results of current study are in agreement with Akhtar et al. (2015). These authors evaluated the effect of pomegranate peel powder meal dietary supplementation on quality of breast meat in broilers and indicated that treated samples with pomegranate peel displayed lower pH values compared with control. Ahmed et al. (2015) reported a similar finding, with the meat from broilers supplemented with pomegranate (1 %) found to have a significantly lower pH values to control broilers on Days 14, 21, and 28. These authors reported the pH value of control meat to be ~ 6.1, which was significantly higher than treated samples with pomegranate (5.86) (Ahmed et al., 2015).

Results of current study show that by including PP to the diet of lamb, the fatty acid composition of the meat can be manipulated – a finding similar to that of other recent studies (Kotsampasi et al., 2014; Natalello et al., 2020). Our results showed that concentrations of three fatty acids including oleic acid, plasmatic acid, stearic acid and linoleic acid were the predominant among the fatty acids detected in lamb meat which is similar to which what has been reported by Kotsampasi et al. (2014); Natalello et al. (2019). The concentration of PUFA, MUFA, and TUFA, and PUFA:SFA values for meat from lamb fed PP was higher compared to the control group by 51.2 %, 42.6 %, 45.8 % and 41.0 % respectively. It has been mostly reported that the fatty acid composition of red meat are rich in SFA form due to biohydrogenation of unsaturated fatty acids in the rumen (Ponnampalam et al., 2015b; Vasta & Bessa, 2012). Recently, improvements to fatty acid profile of meat have been targeted, using different feeding strategies to improve lamb meat 'healthiness' within a human diet (Natalello et al., 2020; Ponnampalam et al., 2021). Generally, PUFA:SFA is a vital factor in determining the nutrient value of fats and lower value (< 0.4) is considered a negative factor (Santos-Silva et al., 2002). In the present study, the PUFA:SFA value for control lambs (0.29) was lower than the stated value whereas the PUFA:SFA value for lambs fed PP (0.41) was higher than both control lambs and the stated limit. The higher concentration of TUFA observed in the meat of lambs fed diet PP is probably due to the PUFA and MUFA content and bioactive components present in pomegranate (tannins). Bioactive substances of pomegranate by products could change the composition of meat fatty acids by changing the biohydrogenation rate of unsaturated fat (protected fats) in the rumen (Natalello et al., 2019).

The presence of PP in Ghezel lamb diet significantly reduced the oxidation of lipids, as indicated by the lower TBARS value of meat across the 12 day ageing period. In present study PP was found to have substantial high levels of phenolic compounds. In the research of Shabtay et al. (2008), higher total polyphenols content of ensiled PP was found. Kazemi and Valizadeh (2021) also found that pomegranate peels and seeds have significant free radical scavenging activity (antioxidant activity) and high amounts of phenolic compounds. Therefore, their presence, in high amounts, into pomegranate by-product assures its considerable nutritional value. A higher concentration of PUFA was observed in meat of lambs fed PP, therefore, its higher susceptibility to lipid oxidation can be expected because of their susceptibility for autoxidation (Cosgrove et al., 1987). Nevertheless, in this study, there was no reduction in meat shelf life (in terms of TBARS, colour, and WHC) compared to the control group. These results confirm that feeding lambs with PP improves the antioxidant capacity of the meat (Natalello et al., 2020).

In the current study, the presence of PP in diet improved redness (a^*) of lamb meat and thereby presented similar findings to those observed for the meat from pomegranate supplemented goat kids (Emami et al., 2015). A consumer threshold value for lamb meat has been proposed as $a^* \geq 14.5$ (Khliji et al., 2010), meaning that the meat from lambs fed PP are acceptable to consumers even after a 12 day ageing period, unlike meat from the control lambs. In the current study, after 12 days of ageing, the a^* values where higher and hue angle values lower for meat from lambs fed PP compared with control lambs. This finding confirms that of Emami et al. (2015), who reported that feeding pomegranate by-product to kids caused increase in meat a^* value and decrease hue angle value with *post-mortem* ageing (storage time). Improved colour stability of meat from lambs fed PP is probably a result of the observed increases in oxidative stability and corresponding reduction to myoglobin oxidation and discolouration of lamb meat (Luciano et al., 2011; Ponnampalam et al., 2022).

5. Conclusions

This study demonstrated that non-pelletised PP can be included into the TMR of Ghezel lamb lambs and support lamb growth rates to deliver carcasses of high quality. The inclusion of PP at 100 g/kg DM has antioxidant effects in the meat of lambs, offering enhancement to PUFA and MUFA concentrations, colour stability, and shelf life. The identified alternative use for PP, being a by-product from pomegranate processing, could redress associated social and environmental issues such as food security, proper waste disposal and environment pollution in a world with limited resources. While sufficiently replicated to identify variance between dietary treatments, the inclusion of additional experimental units (lambs) would have enhanced the statistical power of the current study. The effects of alternative levels of PP inclusion into the diets of Ghezel lambs must also be understood. Further studies should focus on these and determine the variability and bioavailability of nutrients in pomegranate by-products as well as its application in semi-intensive production systems that use alternative basal feeds.

Ethical statement

All protocols and procedures were approved by the Biomedical Ethics Committee of the University of Tabriz.

CRediT authorship contribution statement

Zabihollah Nemati: Writing – review & editing, Writing – original draft, Supervision, Software, Methodology, Data curation, Conceptualization. **Saeid Amirdahri:** Writing – original draft, Data curation. **Ardashir Asgari:** Software, Methodology, Conceptualization. **Akbar**

Taghizadeh: Validation, Software. **Shahida Anusha Siddiqui:** Writing – review & editing. **Magsoud Besharati:** Visualization, Investigation. **Kazem Alirezalu:** Visualization, Investigation. **Benjamin W.B. Holman:** Writing – review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

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