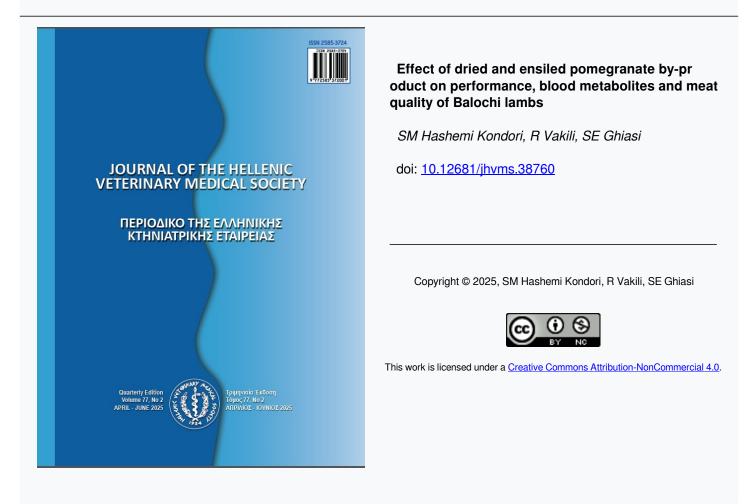




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Effect of dried and ensiled pomegranate by-product on performance, blood metabolites and meat quality of Balochi lambs

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ABSTRACT: This study aimed to investigate the addition of dried and ensiled pomegranate by-products on the performance, rumen parameters, blood metabolites and meat quality of Balochi ram lambs. A total of 18 lambs at 90 d age with similar body weight (BW, 28.0 ± 3.5 kg) with 6 replicates per treatment were used. The experimental treatments including 1) Control, 2) 20% of dried pomegranate by-product, 3) 20% of ensiled pomegranate by-product. According to the results, it was found that the use of pomegranate by-products does not have a significant effect on daily weight gain, feed consumption and feed conversion ratio (P>0.05). Except the constant rate of gas production, other rumen parameters were significantly affected by the experimental treatments (P<0.05). Furthermore, the use of pomegranate by-products had a significant effect on blood cholesterol (P<0.05). Moreover, the dietary treatments have no significant effect on meat traits and its components (P>0.05). The overall results showed that ensiling pomegranate by-products could reduce the amount of tannin in them to a greater extent.

Keyword: Balochi lamb; Tannin; Pomegranate; Silage; Meat quality.

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INTRODUCTION

C urrently, there are many political and social pressures to reduce pollution caused by industrial activities. Almost all developed and underdeveloped countries are trying to adapt their processes to waste recycling. One of the strategies of management of waste and by-products includes the effectual development of nutritious low-cost sustainable animal feed. In the animal industries, by-products could improve animal performance and simultaneously reduce the number of by-products and environmental pollution (Kotsampasi et al., 2014). It should be noted, agricultural by-products have long been used as an effective strategy to reduce feed costs in ruminant nutrition (Salami et al., 2019).

Pomegranate (Punica granatum L.) is an important source of vital compounds and has been used in the past as a medicine to prevent and treat many diseases (El-Hamamsy and El-Khamissi, 2020). Recently, pomegranate by-products, are attracting significant attention for their potential applications in the agri-feed sector. Pomegranate were the leading fruit in the Iran based on production quantity, with some 1.1 million tons produced in 2019. Pomegranate is widely grown in many regions and is an important source of antioxidant, anticancer, antimicrobial compounds and contains a significant number of phenolic compounds (El-Hamamsy and El-Khamissi, 2020). Pomegranate are among the most processed fruits in the world that generate huge quantities of processing waste biomasses. Moreover, the use of other orchard by-products such as dried citrus pulp in ruminant diets has also been studied, showing promising results in improving animal performance (Ahooei et al., 2011).

Several active compounds such as tannins, sterols and terpenoids are present in seeds, bark and leaves of Pomegranate. These bioactive compounds include specific conjugated fatty acids, phenolic compounds, and vitamins that have demonstrated antioxidant, antimicrobial, anti-inflammatory, anti-tumor, and immune system-modulating properties *in vivo* (Carulla et al., 2005; Miguel et al., 2010; Johanningsmeier and Harris, 2011) For this reason, pomegranate waste can be considered as an excellent alternative feed supplementation to improve growth performance, digestibility of nutrients and immunity of animals (Ibrahim et al., 2017). Pomegranate by-products have high nutritional value as feed for ruminants and can be effectively used and may replace grains in the animal diet. However, these products can be used as fresh, they are usually stored dry or ensiled due to their high potential for rapid (Kotsampasi et al., 2014).

In previous studies, the use of fresh pomegranate peel in the diet of beef calves increased feed intake and alpha-tocopherol concentration in plasma (Shabtay et al., 2008). Evidence of the beneficial effects of pomegranate seeds on the antioxidant status of goats has been reported (Emami et al., 2015b). Few studies have investigated the meat shelf life of ruminants fed pomegranate by-products. The researchers reported that, goats were fed with 150 g/ kg (based on dry matter) of pomegranate pulp, more stability in fat and meat color was observed (Emami et al., 2015a). It has been reported that feeding fresh pomegranate peels caused a significant increase in feed intake, along with a positive tendency to gain body weight in male calves (Shabtay et al., 2008). In addition, the use of pomegranate peel at different levels (0, 1, 2 or 4%) in the diet of Kurdish lambs improved their growth performance (Sadq et al., 2016). It has been reported that diets rich in flavonoids (Liu et al., 2018) and natural antioxidants (Velasco and Williams, 2011) can improve meat quality (such as color, taste, texture, and smell) of lambs. Recently, supplementation of small ruminant diets with pomegranate by-products has been shown to enrich meat with potentially health-promoting fatty acids such as punicic acids and rumen fatty acids (Natalello et al., 2019). In other studies, it was found that the use of ensiled pomegranate by-products (EPB) improved the antioxidant capacity, nutritional quality and performance of lamb meat (Kotsampasi et al., 2014).

Therefore, the objective of this study was to investigate the effects of dietary supplementation of dried and ensiled pomegranate by-products on growth performance, blood parameter, and meat quality of Blachi lambs.

MATERIALS AND METHODS

localization and ethical manifestation

The study was performed at the livestock farm located in Zindajan village of Kashmar city (35°14' N and 58° 27' E), from April 1401 and for 90 days. All experimental procedures with the sheep were performed according to regulations established by the Animal Protection Law enacted by the Kashmar State of Iran (Iranian Council of Animal Care AEC approval no. 19293). **Preparation of experimental animals and diets** At the beginning of the breeding period, all male lambs were injected with 2 mL of vitamin B-complex and 3 mL of vitamin AD3E. The albendazole was consumed as anti-parasitic drugs.

Each lamb was maintained in a $2 \text{ m} \times 2 \text{ m}$ concrete individual pen and was freely accessed to clean water throughout the experimental period. The total mixed ration (TMR) was prepared, and given to the lambs twice a day (7:00 in the morning and 5 in the evening). The ration was adjusted according to the Small Ruminant Nutrient System (SRNS) (Tedes-

chi et al., 2010). The components and composition of experimental diets are reported in Table 1 and Table 2. The analysis of chemical compounds of feed including dry matter, organic matter and ash, crude protein, crude fat and Neutral detergent fiber (NDF) was done according to the AOAC (Chemists and Chemists, 1931).

Following a 2 weeks adaptation period, eighteen Baluchi male lambs (initial body weight of 28.0 ± 3.5 kg, 6-month-old) were randomly allocated to three experimental diets with 6 replicates for 90 days including: Treatment 1 or control: diet without pome-

	Experimental diets					
Diet components	Control	Diet containing dried pomegranate by- product	Diet containing pomegranate by-product silage			
Alfalfa	1.32	0.21	0.12			
Corn	9.59	9.40	3.54			
Bran	0.7	8.5	4.5			
Dried Pomegranate by-product	-	4.31	-			

Table 1. Diet components and chemical composition (based on dry matter percentage)

Alfalfa	1.32	0.21	0.12
Corn	9.59	9.40	3.54
Bran	0.7	8.5	4.5
Dried Pomegranate by-product	-	4.31	-
Pomegranate by-product silage	-	-	2.27
Mineral and vitamin supplement	0.1	0.1	0.1
Chemical composition (percentage)			
Dry matter	89	93	64
Crude protein	12	12	12
Neutral detergent fiber (NDF)	38	64	45
Ash	0.6	5.5	0.5
Crude fat	00.3	75.1	75.1
Calcium	63.0	47.0	46.0
Phosphorus	52.0	41.0	36.0
Metabolizable energy (Mcal/kg)	67.2	68.2	60.2

Table 2. Chemical composition of diet components (percentage in dry matter)

items	Dry matter (DM)	Crude protein (CP)	Neutral detergent fiber (NDF)	Ether extract (EE)	Ash	рН	Metabolizable energy (ME) (MCal/kg)
Alfalfa	0.94	0.18	0.30	1.2	-	-	0.2
corn	0.87	0.9	0.12	5.2	8.1	-	0.3
Dried Pomegranate dry by-product	0.95	0.12	0.49	5.11	0.2	-	3.2
Pomegranate by- product silage	0.34	0.17	0.36	5.6	0.2	3.4	1.2

granate by-products in which the lambs received the basic diet. Treatment 2: containing 20% dried pomegranate seed (50%) and peel pulp (50%). Treatment 3: containing 20% pomegranate silage containing seed (50%) and peel pulp (50%).

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). At the beginning of each day before feeding, the feed was collected and weighed and the feed intake (FI) of each lamb was calculated according to the feed. The percentage of feed conversion ratio (FCR) was eventually calculated.

Measurement of gas production, pH and ammonia

Gas production was measured in 96 hours and the resulting parameter was calculated according to the standard method of Khe (1988) (Kh, 1988). After 24 hours, the pH of the contents of the vials was recorded using a digital pH meter. To measure ammonia concentration, four ml of rumen fermentation liquid was mixed with four ml of 0.4 normal hydrochloric acid and kept at -20 ^{°C until} the time of measurement. After that, the samples were centrifuged at 3000 g for 20 minutes. The upper liquid was used to measure ammonia nitrogen by the phenol-hypochlorite method with a spectrophotometer (CE 1021.1000 SE-RIES, CECIL instruments, Cambridge, England) at a wavelength of 625 nm (Broderick and Kang, 1980).

Analysis of blood biochemical indices and meat properties

Blood sampling was measured by kit (*Pars Azmoon*, Iran), before morning feeding from the jugular vein of each lamb (Alhidary and Abdelrahman, 2016). The measured blood parameters included albumin, total protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol, triglyceride and glucose.

To investigate meat traits, three of each treatment

were selected. At the end of the period, the day before the slaughter, the lambs were deprived of water and feed 12 hours before the slaughter. After the slaughter, the weight of the carcasses was recorded and stored at 4 ° C for 24 hours. The weight of the carcass was also recorded. The left -handed muscle tissue was sampled and covered with liquid nitrogen in aluminum foil after being frozen and kept at -80 ° C until measuring. Meat physicochemical characteristics, including cooking loss, shear force, juiciness, general acceptability, pH, flavour intensity, and drip loss were investigated. Additionally, moisture content, total protein, ether extract and meat ash were analyzed according to the method of AOAC (Chemists and Chemists, 1931).

Statistical analyses

This experiment was conducted as a completely randomized design. Data was analyzed were conducted using SAS version 9.4 (version 9.4, SAS Institute Inc., Cary, NC, USA). Statistical differences between experimental groups were determined using Tukey's test when $p \le 0.05$ was detected. The model used in this project is as follows:

- $Yij = \mu + Ti + eij$
- Yij= the value of each observation in the experiment
- μ = population mean
- Ti =Diet effect
- eij= experimental error

RESULT

As indicated by the results in Table 3, the results showed that although the use of pomegranate by-products does not have a significant effect on ADG, FI and FCR of lambs (P<0.05); the highest amount of average weight gain was related to the treatment containing dried pomegranate by-product in the diet (Table 3).

According to the Table 4, Except the constant rate of gas production, other rumen parameters were

Table 3. The effect of using dried and ensiled pomegranate by-products on the performance of Balochi ram lambs

		Experimental trea				
	Control	Dried Pomegranate by-product	Pomegranate by- product silage	SEM	P-Value	
Weight gain (grams)	81.204	00.205	80.201	78.27	9958.0	
Feed intake	54.1410	45.1377	37.1370	32.43	7843.0	
Feed conversion ratio	056.7	992.7	377.7	08.1	8263.0	

significantly affected by the experimental treatments (P<0.05). The amount of gas production, digestibility of organic matter and rumen metabolizable energy in the control treatment (without pomegranate by-products) increased significantly compared to other experimental treatments (P<0.05). The use of dried pomegranate by-product and pomegranate by-product silage caused a significant decrease in rumen volatile fatty acids, ammonia nitrogen and pH compared to the control group (P<0.05). The amount of ammonia nitrogen in this study showed a significant decrease in treatments containing pomegranate products compared to the control, and the lowest amount was related to dried pomegranate by-product.

As shown in Table 5, the results showed that although the use of pomegranate by-products has a significant effect on blood cholesterol (P<0.05), other biochemical indicators including albumin, to-tal protein, creatinine, triglyceride, glucose, alanine aminotransferase, aspartate aminotransferase were not affected by the experimental treatments (P>0.05).

As shown in Table 6, the use of pomegranate by-products did not have a significant effect on the meat characteristics of the lambs (P < 0.05).

Table 4. The effect of using dried and ensiled pomegranate by-products on the rumen metabolites of Balochi male lambs

Rumen metabolites	Experimental treatment					
	ControlPomegranate dry by-productPomegranate by- product silage		0 1	- SEM	P-Value	
Ammonia nitrogen	^a 64.11	^b 85.8	^{ab} 88.10	63.0	0199.0	
pH	^a 55.6	^{ab} 96.5	^b 73.5	17.0	0094.0	
Gas produced in 24 hours	^a 03.118	ь78.101	° 15.89	90.2	0004.0	
Gas produced in 48 hours	^a 14.143	^b 25.120	^b 78.103	48.5	0024.0	
Gas produced in 96 hours	^a 61.162	^b 97.135	^b 01.116	98.6	0034.0	
Rate constant of gas production (c)	027.0	021.0	021.0	003.0	4506.0	
Organic matter digestibility (%) OMD	^a 92.35	^b 02.33	° 79.30	510.0	0004.0	
Metabolizable energy (Mcal/kg)	^a 49.12	ь 88.11	^b 88.11	061.0	002.0	
Volatile fatty acids	^a 51 .0	^b 44.0	° 39.0	012.0	0004.0	

^{abc} Different letters indicate statistically significant differences (P≤0.05).

Table 5. The effect of using dried and ensiled pomegranate by-products on blood biochemical indices of Baluchi ram lambs

Pland his shaming a new motors		Experimental trea				
Blood biochemical parameters	ControlDried Pomegranate by-productPomegranate by- product silage		0 1	SEM	P-Value	
Albumin (mg/dl)	89.3	62.3	75.3	34.0	8586.0	
total protein (mg/dl)	05.6	29.6	48.6	23.0	4630.0	
Creatinine (mg/dl)	14.1	12.1	18.1	09.0	9141.0	
Triglycerides (mmol/dl)	21.38	58.36	59.35	53.1	4916.0	
Cholesterol (g/dl)	^a 38.70	^{ab} 53 .68	^b 13.64	08.1	0030.0	
Glucose (mg.d)	97.65	11.68	46.71	70.1	1024.0	
Alanine aminotransferase (UL)	32.17	57.17	54.17	24.1	9766.0	
aspartate aminotransferase (UL)	85.86	57.84	91.88	49.4	7938.0	

^{abc} Different letters indicate statistically significant differences (P≤0.05).

Characteristics of meat		Experimental trea			
	Control	Dried Pomegranate by-product	Pomegranate by- product silage	SEM	P-Value
cooking loss (percentage)	38.32	29.33	27.33	43.2	9551.0
Shear force (kg)	53.4	89.4	73.4	22.0	5514.0
Juiciness ¹	65.5	57.5	40.5	18.0	7228.0
General acceptability ¹	16.5	33.5	12.5	18.0	6867.0
pН	44.5	57.5	73.5	13.0	3261.0
Flavour intensity ¹	05.6	09.6	21.6	26.0	84476.0
Drip loss	77.3	73.3	9072.3	45.0	9619.0

Table 6. The effect of using dried and ensiled pomegranate by-products on the meat characteristics of Balochi ram lambs

^{abc} Different letters indicate statistically significant differences (P≤0.05).

² Scale 1 to 8: Scale: 1: less, dry and unpleasant and desirable taste, 8: contains highly watered, delicious and desirable meat aroma.

In Table 7, Experimental treatments had no significant effect on moisture, protein, ether extract and meat ash (P<0.05). Although the percentage of ether extract and ash were not significant, they tended to increase and the amount of meat protein was very close to significance.

DISCUSSION

It was also found that pomegranate silage up to 240 g/kg DM could be used in the diet of growing lambs, in complete replacement with hay, without negative effects on the animal's productive traits (Kotsampasi et al., 2014). Similar to the results of the present study, the use of 20% of pomegranate by-products in the diet of lambs had no effect on weight gain (Natalello et al., 2019). In the present study, lamb fed with dried pomegranate by-product showed the highest average weight gain, although it was not statistically significant. This weight gain may be due to the presence of compounds such as fiber, protein, minerals and various vitamins in pomegranate (thiamine, riboflavin, tocopherol, ascorbic acid, etc.) that

have beneficial effects on ruminants (El-Hamamsy and El-Khamissi, 2020). Also, Researchers stated that tannin-containing feed decreases the protein digestion rate, increases microbial protein synthesis in the rumen, and decreases methanogenesis (Mergeduš et al., 2018). because, the tannin presents in pomegranate products (Sadq et al., 2016) make complexes with the proteins and act as a protective material for protein, delays protein degradation in the rumen, and increases its flow to the abomasum and small intestine (place of enzymatic digestion), leads to an increase in the weight of the animal. In addition to, the weight gain may also occur due to the improvement of nitrogen balance in the rumen and reduction of nitrogen loss in the form of ammonia gas, improving the rumen environment and increasing the formation of microbial protein, which leads to an increase in protein synthesis in the body (Abarghuei et al., 2013). Although, the high concentration of tannin reduced the digestibility of carbohydrate and hemicellulose in the rumen, the digestibility increased after the rumen (Mergeduš et

Table 7. The effect of using dried and ensiled pomegranate by-products on the blood components of Balochi male lambs

The percentage of meat		Experimental tre			
components	Control Pomegranate by_		Pomegranate silage by-product	SEM	P-Value
Humidity	32.72	23.71	66.71	95.0	72.0
Protein	26.21	47.20	43.22	51.0	056.0
Ether extract	54.0	73.0	64.0	20.0	46.0
Ash	43.1	49.1	67.1	10.0	2931.0

al., 2018). In addition, tannins reduce gas production from feed starch (Mergeduš et al., 2018). Reduction of methane and ammonia nitrogen causes nitrogenous compounds to be converted into microbial protein (Matthews et al., 2019). In addition, tannins can reduce the rumen biohydrogenation process and increase the flow of unsaturated fatty acids to the duodenum, but this effect is dependent on the tannin dose. As a result, tannins improve nitrogen utilization efficiency by retaining more nitrogen in the body (Besharati et al., 2022).

Similar to the present study and other studies, it was found that when different levels (0, 5.0, 1 and2%) of dried pomegranate by-product were used in the diet of lambs, their feed intake was reduced and feed conversion was improved (Omer et al., 2019). This could be due to the fact that high concentrations of tannins may reduce feed intake, digestibility of protein and carbohydrates, as well as animal performance through their negative effect on palatability and digestibility of feed nutrients (Kotsampasi et al., 2014). It could be a reason for the decrease in feed consumption in the treatments containing pomegranate by-products compared to the control in the present study. On the other hand, low and medium concentrations of condensed tannins (20 to 45 mg/g DM) in the diet may improve production without increasing feed intake by preventing bloat and increasing the flow of non-ammonia nitrogen and essential amino acids from the rumen (Min et al., 2003). In the present study, although the amount of tannin in the treatments containing pomegranate by-products is higher than the control, no significant difference was observed between the experimental treatments on the performance of lambs, which indicates the controversial effect of tannins on the performance of ruminants. Several factors could make the effect of tannin negative, positive or indifferent. This may be due to the chemical composition or different type of tannins, dosage, metabolic status of the animals, species and the diet to which tannins were added (Patra and Saxena, 2011). They were characterized by high chemical structural diversity, with results that cannot be easily generalized from one type of tannin to another (Rodríguez et al., 2013).

The amount of ammonia nitrogen in this study showed a significant decrease in treatments containing pomegranate products compared to the control, and the lowest amount was related to lambs fed with dried pomegranate by-products group. In other studies, where plants containing condensed tannins were used in animal feed, reduction of ammonia nitrogen in the rumen was observed (Carulla et al., 2005). In past studies, a positive correlation between rumen ammonia nitrogen production and crude protein has been reported (Getachew et al., 2004). Due to the formation of a complex between the tannins in pomegranate products and protein, and as a result, creating less accessibility for raw protein and restricting effects of proteolytic enzymes, there is a reason for the reduction of ammonia nitrogen in the experimental treatments compared to the control group (Frutos et al., 2004). On the other hand, it has been considered that, tannins by affecting the rumen microbial population, may cause changes in rumen parameters such as ammonia and volatile fatty acids. The decrease in ammonia nitrogen in the rumen can be due to the decrease in the number of protozoa, which plays an important role in the breakdown of dietary protein (Newbold et al., 1995). Probably, tannins can reduce ammonia nitrogen in the rumen by limiting the growth and activity of certain bacteria such as ammonia-producing bacteria that have deaminase activity (Newbold et al., 2004). It is known that the phenolic group of tannins is a strong hydrogen donor to bond with the carboxyl group of proteins. This characteristic of tannins increases their tendency to form bonds with proteins, which can reduce the amount of ammonia produced in the rumen (Getachew et al., 2004). Also, by increasing the secretion of salivary glycoproteins, tannins decrease the rate of protein decomposition and ammonia production in the rumen (Reed et al., 1990).

In this study, experimental treatments caused a significant decrease in rumen pH. Similar to the results of the present study, other researchers also reported that the rumen pH decreases when using tannin in ruminant diets (Yanez Ruiz et al., 2004). The reason for the decrease in pH in the present study may be due to a change in the growth pattern of rumen bacteria, especially cellulolytic bacteria (Gee et al., 1996).

The gas produced in 24, 48 and 96 hours in the control treatment was significantly higher than the treatments containing pomegranate by-products. Furthermore, the groups used dried pomegranate by-product showed more gas production than the groups used pomegranate by-product silage similar result with Shabtay et al (2008) (Shabtay et al., 2008). It is noteworthy, the use of tannin sources in sheep (Carulla et al., 2005) and dairy cows (Grainger et al., 2009) caused a decrease in the activity of

methanogens, followed by a decrease in gas production; However, in another study, no significant difference was observed between the gas production of dried and ensiled pomegranate products (Taher-Maddah et al., 2012). It has been found that gas production has a negative correlation with the bacteria cell wall (De Boever et al., 2005). In general, the chemical compounds in plants can reduce the production of hydrogen (substrate for bacteria) through a direct effect on bacteria and an indirect effect on the digestion of raw fiber, thus affecting methanogenic bacteria and gas production in the rumen (Tavendale et al., 2005).

In this study, the use of pomegranate by-products in the diet caused a significant decrease in volatile fatty acids and metabolizable energy. In other studies, pomegranate peel silage had less metabolizable energy and short chain fatty acids than its dried form (Shabtay et al., 2008). The reduction of metabolizable energy and fatty acids in ensiled pomegranate by-products may be due to the difference in the chemical composition of pomegranate silage compared to its dried form. Also, in this study, the reason for the observed decrease in volatile fatty acids may be due to the decrease in crude protein digestion.

In line with the results obtained in this study, Shabtay et al., (2008) reported that the digestibility of organic matter for pomegranate silage and pomegranate peel was 63 and 2.76, respectively. Also, these researchers stated that, ensiling reduced the digestibility of organic matter (Shabtay et al., 2008). This reduction may be due to the digestibility of organic matter to the reduction of rumen degradability, the formation of bonds between tannins with dietary protein and carbohydrates, the reduction of the activity of cellulolytic and proteolytic bacteria, and the general fermentation of the rumen (Muhammed et al., 1994).

Studies showed that the use of 0, 400, 800 and 1200 mg of pomegranate peel extract in the diet of dairy cows did not have a significant effect on glucose, albumin, total protein and triglyceride in the blood of cows, but it significantly reduced cholesterol (Abarghuei et al., 2013). It has been well established that saponins in pomegranate peel form insoluble complexes with cholesterol (Nasri and Salem, 2012). It has been said that the main effect of pomegranate saponins on blood cholesterol is through a direct connection between saponin and dietary cholesterol in the intestine, which prevents its absorption from the small intestine. Second, the binding between saponin and bile acids in the intestine may lead to a decrease in the hepatic blood circulation of bile acids and an increase in the excretion of cholesterol in the feces.

The normal range of ALT and AST enzymes in lamb is 5-21.6 and 36-115 IU/L, respectively (Al-Hadithy, 2013). Therefore, in the present study, it was determined that the use of pomegranate by-products did not cause liver damage. Similar to this review, in a study, the use of tannin in the diet of sheep and goats had no effect on the concentration of creatine, ALT, and AST (Salem et al., 2011). In contrast, researcher showed that the use of chestnut hydrolysable tannin to lambs' diet at 08.2% DM, increased the activity of ALT and AST enzymes without effect on blood creatine (Frutos et al., 2004). This difference in the results may be due to the difference in the plant used in the diet and the design of the experiment.

In the present study, the effect of treatments on blood glucose and triglycerides was not dramatically change which corresponds to no significant change in feed consumption. It has been said that the presence of condensed tannin in the diet increases the molar ratio of propionate, which is a glucogenic precursor formed in the rumen, therefore it is expected to increase blood glucose concentration (Abarghuei et al., 2013). In the present study, although the treatments had no significant effect on blood glucose, its value tended to increase. Furthermore, the concentration of total protein and albumin was not affected by the experimental treatments, which is consistent with the results of Salem et al., 2011. It is possible that the decrease in serum albumin level is due to less feed consumption and less protein intake in the present study.

According to the results of the present study, the use of different levels of tannin in the diet had no effect on the loss of cooking, juiciness and sheer force of meat (Dentinho et al., 2020; Pimentel et al., 2021). It has been determined that the two indices of cooking loss and shear force required for meat crispiness are among the influencing factors in meat quality and choice by consumers. These factors are influenced by the amount of fat stored in the meat, the degree of fatness and the final pH of the meat (Priolo et al., 2001). Muscle fat separates the connective tissue inside the muscle and reduces the density of collagen fibers (Koohmaraie, 1996). The acceptable shear force for lamb is considered to be 3 kg, and if this number reaches 6 kg, it is unacceptable (Watanabe et al., 1996). In this study and according to other reviewer, the shear force and meat quality were acceptable (Guerreiro et al., 2020).

It was shown that the use of pomegranate silage up to 240 g/kg DM in the diet of lambs has no effect on moisture, protein and ash of meat, while meat fat increased (Kotsampasi et al., 2014). According to the results of the present study, the inclusion of pomegranate by-products in the diet of lambs may not be enough to change the meat properties, but it will improve the quality of the meat to some extent.

CONCLUSION

In the present study, Although the use of pomegranate by-products had no significant effect on feed consumption, daily weight gain and feed conversion ratio of Baluchi male lambs, best daily weight gain and feed conversion ratio was related to dry pomegranate by-products. Except the constant rate of gas production, other rumen parameters were significantly affected by the experimental treatments. The amount of in vitro gas production, organic matter digestibility and rumen metabolizable energy in the experimental treatments were significantly reduced compared to the control group. The results showed that the use of pomegranate by-products does not have a significant effect on physicochemical properties and components of lamb meat. Additionally, none of the blood biochemical parameters except cholesterol were affected by the experimental treatments. The amount of alanine aminotransferase enzyme was the lowest in the treatment containing dried pomegranate by-products. Finally, the results of this study showed that although dried pomegranate products have nutritional value, pomegranate silage can reduce the amount of tannin in it to a greater extent. As a result, pomegranate silage can be used in animal feed. According to the previous studies, different types and concentrations of tannins have different effects on animal performance, so it is better to investigate the long-term effects of pomegranate products on young animals.

REFERENCES

- Abarghuei MJ, Rouzbehan Y, Salem A, Zamiri MJ (2013) Nutrient digestion, ruminal fermentation and performance of dairy cows fed pomegranate peel extract. Livestock Science 157(2-3):452-461. https://doi.org/https://doi.org/10.1016/j.livsci.2013.09.007.
- Ahooei GR, Foroughi AR., Tahmasbi AM, Shahdadi AR, Vakili R (2011). Effects of Different Levels of Dried Citrus Pulp and Urea on Performance of Fattening Male Calves. Journal of animal and veterinary advances, 10(14), 1811-1816. 10.3923/javaa.2011.1811.1816.
- Al-Hadithy H (2013) Estimation of serum liver enzymes activities in Awassi sheep. The Iraqi Journal of Veterinary Medicine 37(1):115-120.
- Alhidary I, Abdelrahman M (2016) Effects of naringin supplementation on productive performance, antioxidant status and immune response in heat-stressed lambs. Small Ruminant Research 138:31-36. https:// doi.org/https://doi.org/10.1016/j.smallrumres.2016.03.030.
- Besharati M, Maggiolino A, Palangi V, Kaya A, Jabbar M, Eseceli H, De Palo P, Lorenzo JM (2022) Tannin in ruminant nutrition. Molecules. Nov 27;27(23):8273. https://doi.org/10.3390/molecules27238273.
- Broderick G, Kang J (1980) Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. Journal of dairy science 63(1):64-75. https://doi.org/https://doi. org/10.3168/jds.S0022-0302(80)82888-8.
- Carulla J, Kreuzer M, Machmüller A, Hess H (2005) Supplementation of Acacia mearnsii tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. Australian journal of agricultural research 56(9):961-970. https://doi.org/https://doi.org/10.1071/ AR05022.
- Chemists AoOA, Chemists AoOA. (1931). Official methods of analysis of the Association of Official Analytical Chemists (Vol. 3). Association of Official Analytical Chemists.
- De Boever J, Aerts J, Vanacker J, De Brabander D (2005) Evaluation of the nutritive value of maize silages using a gas production technique. Animal Feed Science and Technology 123:255-265. https://doi.org/ https://doi.org/10.1016/j.anifeedsci.2005.04.019.
- Dentinho M, Paulos K, Francisco A, Belo A, Jerónimo E, Almeida J, Bessa R, Santos-Silva J (2020) Effect of soybean meal treatment with Cistus ladanifer condensed tannins in growth performance, carcass and meat quality of lambs. Livestock Science 236:104021. https://doi.org/https://doi.org/10.1016/j.livsci.2020.104021.

- El-Hamamsy S, El-Khamissi H (2020) Phytochemicals, antioxidant activity and identification of phenolic compounds by HPLC of pomegranate (Punica granatum L.) Peel extracts. Journal of Agricultural chemistry and biotechnology 11(4):79-84. https://doi.org/ https://doi.org/10.21608/jacb.2020.95837.
- Emami A, Nasri MF, Ganjkhanlou M, Rashidi L, Zali A (2015a) Dietary pomegranate seed pulp increases conjugated-linoleic and-linolenic acids in muscle and adipose tissues of kid. Animal Feed Science and Technology 209:79-89. https://doi.org/https://doi.org/10.1016/j. anifeedsci.2015.08.009.
- Emami A, Nasri MF, Ganjkhanlou M, Zali A, Rashidi L (2015b) Effects of dietary pomegranate seed pulp on oxidative stability of kid meat. Meat science 104:14-19. https://doi.org/https://doi.org/10.1016/j. meatsci.2015.01.016.
- Frutos P, Mantecón ÁR, Angulo GH, García FJG (2004) Tannins and ruminant nutrition. Spanish journal of agricultural research 2(2):191-202. https://doi.org/https://doi.org/10.5424/sjar/2004022-73.
- Gee J, Wortley G, Johnson I, Price K, Rutten A, Houben G, Penninks A (1996) Effects of saponins and glycoalkaloids on the permeability and viability of mammalian intestinal cells and on the integrity of tissue preparations in vitro. Toxicology in vitro 10(2):117-128. https://doi.org/https://doi.org/10.1016/0887-2333(95)00113-1.
- Getachew G, Robinson P, DePeters E, Taylor S (2004) Relationships between chemical composition, dry matter degradation and in vitro gas production of several ruminant feeds. Animal Feed Science and Technology 111(1-4):57-71. https://doi.org/https://doi.org/10.1016/ S0377-8401(03)00217-7.
- Grainger C, Clarke T, Auldist M, Beauchemin K, McGinn S, Waghorn G, Eckard RJ (2009) Potential use of Acacia mearnsii condensed tannins to reduce methane emissions and nitrogen excretion from grazing dairy cows. Canadian Journal of Animal Science 89(2):241-251. https://doi.org/https://doi.org/10.4141/CJAS08110.
- Guerreiro O, Alves SP, Soldado D, Cachucho L, Almeida JM, Francisco A, Santos-Silva J, Bessa RJ, Jerónimo E (2020) Inclusion of the aerial part and condensed tannin extract from Cistus ladanifer L. in lamb diets–Effects on growth performance, carcass and meat quality and fatty acid composition of intramuscular and subcutaneous fat. Meat science 160:107945. https://doi.org/https://doi.org/10.1016/j. meatsci.2019.107945.

- Ibrahim M, Arafa SA, Hassan FA, Zaki EE (2017) Utilization of pomegranate (Punica granatum L.) by-product powder as a natural growth promoter in growing rabbit diets. Egyptian Journal of Rabbit Science 27(2):197-217. https://doi.org/https://doi.org/10.21608/ ejrs.2017.46568.
- Johanningsmeier SD, Harris GK (2011) Pomegranate as a functional food and nutraceutical source. Annual review of food science and technology 2(1):181-201. https://doi.org/https://doi.org/10.1146/ annurev-food-030810-153709.
- Kh M (1988) Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Anim Res Dev 28:7-55.
- Koohmaraie M (1996) Biochemical factors regulating the toughening and tenderization processes of meat. Meat science 43:193-201. https://doi.org/https://doi.org/10.1016/0309-1740(96)00065-4.
- Kotsampasi B, Christodoulou V, Zotos A, Liakopoulou-Kyriakides M, Goulas P, Petrotos K, Natas P, Bampidis V (2014) Effects of dietary pomegranate byproduct silage supplementation on performance, carcass characteristics and meat quality of growing lambs. Animal Feed Science and Technology 197:92-102. https://doi.org/https:// doi.org/10.1016/j.anifeedsci.2014.09.003.
- Liu W, Ding H, Erdene K, Chen R, Mu Q, Ao C (2018) Effects of flavonoids from Allium mongolicum Regel as a dietary additive on meat quality and composition of fatty acids related to flavor in lambs. Canadian Journal of Animal Science 99(1):15-23. https:// doi.org/https://doi.org/10.1139/cjas-2018-0008.
- Matthews C, Crispie F, Lewis E, Reid M, O'Toole PW, Cotter PD (2019) The rumen microbiome: a crucial consideration when optimising milk and meat production and nitrogen utilisation efficiency. Gut microbes. Mar 4;10(2):115-32. https://doi.org/10.1080/19490976 .2018.1505176.
- Mergeduš A, Pšenková M, Brus M, Janžekovič M (2018) Tannins and their effect on production efficiency of ruminants. Agricultura Scientia.15(1/2):1-1. https://doi.org/10.18690/agricultura.15.1-2.1-11.2018.
- Miguel MG, Neves MA, Antunes MD (2010) Pomegranate (Punica granatum L.): A medicinal plant with myriad biological properties-A short review. Journal of Medicinal Plants Research 4(25):2836-2847.
- Min B, Barry T, Attwood G, McNabb W (2003) The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. Animal Feed Science and Technology 106(1-4):3-19. https://doi.org/https://doi.org/10.1016/S0377-8401(03)00041-5.
- Muhammed S, Stewart C, Acamovic T. (1994). Effects of tannic acid on cellulose degradation, adhesion and enzymic activity of rumen microorganisms. Proceedings of the Society of Nutrition Physiology (Germany).
- Nasri S, Salem HB (2012) Effect of oral administration of Agave americana or Quillaja saponaria extracts on digestion and growth of Barbarine female lamb. Livestock Science 147(1-3):59-65.
- Natalello A, Luciano G, Morbidini L, Valenti B, Pauselli M, Frutos P, Biondi L, Rufino-Moya PJ, Lanza M, Priolo A (2019) Effect of feeding pomegranate byproduct on fatty acid composition of ruminal digesta, liver, and muscle in lambs. Journal of agricultural and food chemistry 67(16):4472-4482. https://doi.org/https://doi. org/10.1021/acs.jafc.9b00307.
- Newbold C, Lassalas B, Jouany J (1995) The importance of methanogens associated with ciliate protozoa in ruminal methane production in vitro. Letters in applied microbiology 21(4):230-234. https://doi. org/https://doi.org/10.1111/j.1472-765X.1995.tb01048.x.
- Newbold C, McIntosh F, Williams P, Losa R, Wallace R (2004) Effects of a specific blend of essential oil compounds on rumen fermentation. Animal Feed Science and Technology 114(1-4):105-112. https://doi. org/https://doi.org/10.1016/j.anifeedsci.2003.12.006.
- Omer HA, Abdel-Magid SS, Awadalla IM (2019) Nutritional and chemical evaluation of dried pomegranate (Punica granatum L.) peels

and studying the impact of level of inclusion in ration formulation on productive performance of growing Ossimi lambs. Bulletin of the National Research Centre 43:1-10. https://doi.org/https://doi. org/10.1186/s42269-019-0245-0.

- Patra AK, Saxena J (2011) Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. Journal of the Science of Food and Agriculture. Jan 15;91(1):24-37. https://doi.org/10.1002/ jsfa.4152.
- Pimentel P, Pellegrini C, Lanna DPD, Brant L, Ribeiro C, Silva T, Barbosa A, da Silva Júnior J, Bezerra L, Oliveira R (2021) Effects of Acacia mearnsii extract as a condensed-tannin source on animal performance, carcass yield and meat quality in goats. Animal Feed Science and Technology 271:114733. https://doi.org/https://doi. org/10.1016/j.anifeedsci.2020.114733.
- Priolo A, Micol D, Agabriel J (2001) Effects of grass feeding systems on ruminant meat colour and flavour. A review. Animal research 50(3):185-200. https://doi.org/https://doi.org/10.1051/animres:2001125.
- Reed J, Soller H, Woodward A (1990) Fodder tree and straw diets for sheep: intake, growth, digestibility and the effects of phenolics on nitrogen utilisation. Animal Feed Science and Technology 30(1-2):39-50. https://doi.org/https://doi.org/10.1016/0377-8401(90)90050-I.
- Rodríguez R, De la Fuente G, Gómez S, Fondevila M (2013) Biological effect of tannins from different vegetal origin on microbial and fermentation traits in vitro. Animal Production Science. Nov 26;54(8):1039-46. https://doi.org/10.1071/AN13045.
- Sadq SM, Ramzi DO, Hamasalim HJ, Ahmed KA (2016) Growth performance and digestibility in Karadi lambs receiving different levels of pomegranate peels. Open Journal of Animal Sciences 6(01):16.
- Salami SA, Luciano G, O'Grady MN, Biondi L, Newbold CJ, Kerry JP, Priolo A (2019) Sustainability of feeding plant by-products: A review of the implications for ruminant meat production. Animal Feed Science and Technology 251:37-55. https://doi.org/https:// doi.org/10.1016/j.anifeedsci.2019.02.006.
- Salem A, López S, Ranilla MJ, González JS. (2011). Influence of quebracho tannins administration on some blood metabolites in sheep and goats. In Challenging strategies to promote the sheep and goat sector in the current blobal context (Vol. 99, pp. 273-278). CI-HEAM, FAO Zaragoza.
- Shabtay A, Eitam H, Tadmor Y, Orlov A, Meir A, Weinberg P, Weinberg ZG, Chen Y, Brosh A, Izhaki I (2008) Nutritive and antioxidative potential of fresh and stored pomegranate industrial byproduct as a novel beef cattle feed. Journal of agricultural and food chemistry 56(21):10063-10070. https://doi.org/https://doi.org/10.1021/ jf8016095.
- Taher-Maddah M, Maheri-Sis N, Salamatdoustnobar R, Ahmadzadeh A (2012) Comparing nutritive value of ensiled and dried pomegranate peels for ruminants using in vitro gas production technique.
- Tavendale MH, Meagher LP, Pacheco D, Walker N, Attwood GT, Sivakumaran S (2005) Methane production from in vitro rumen incubations with Lotus pedunculatus and Medicago sativa, and effects of extractable condensed tannin fractions on methanogenesis. Animal Feed Science and Technology 123:403-419. https://doi.org/https:// doi.org/10.1016/j.anifeedsci.2005.04.037.
- Velasco V, Williams P (2011) Improving meat quality through natural antioxidants. Chilean journal of agricultural research 71(2):313-322.
- Watanabe A, Daly C, Devine C (1996) The effects of the ultimate pH of meat on tenderness changes during ageing. Meat science 42(1):67-78. https://doi.org/https://doi.org/10.1016/0309-1740(95)00012-7.
- Yanez Ruiz D, Moumen A, Martin Garcia A, Molina Alcaide E (2004) Ruminal fermentation and degradation patterns, protozoa population, and urinary purine derivatives excretion in goats and wethers fed diets based on two-stage olive cake: Effect of PEG supply. Journal of animal science 82(7):2023-2032. https://doi.org/https:// doi.org/10.2527/2004.8272023x.