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The effect of tannin extract and n-3 fatty acid source on nutrient digestibility, blood metabolites, enzyme activity, and ruminal parameters of lactating ewes

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Abstract: This study aimed to evaluate the effects of tannin extract and n-3 fatty acid supplementation on nutrient digestibility, ruminal parameters, blood metabolites, and enzyme activity in lactating ewes. Thirty-six lactating ewes were allocated to 6 experimental groups. The dietary treatments were as follows: 1) control, 2) diet supplemented with 2% dry matter (DM) oak leaf tannin extract, 3) diet supplemented with 2% DM grape pomace tannin extract, 4) diet supplemented with 2% DM linseed oil, 5) diet supplemented with 2% DM oak leaf tannin extract and 2% DM linseed oil, and 6) diet supplemented with 2% DM grape pomace tannin extract and 2% DM linseed oil. The results showed the significant effects of treatments on final body weight, digestibility, and some blood metabolites ($P < 0.05$). Therefore, the treatment groups had higher final body weight, better nutrient digestibility, and higher values of blood parameters. In conclusion, our results showed that the dietary tannin extract and the n-3 fatty acid source improved nutrient digestibility and blood metabolites, and they had a trend of improving the ruminal volatile fatty acid profile while not having any adverse effects on the animals' performance.

Key words: Grape pomace, linseed oil, oak leaves, lactating ewe

1. Introduction

Tannins are polyphenolic polymers with a high molecular weight and a high number of hydroxyl groups, which enable them to bind different components and make new complexes [1]. Depending on the type of tannin and its chemical structure, the quantity of feeding, and the species of the animals, these components could be detrimental, harmless, or even beneficial to animals [2]. Tannins have beneficial effects for ruminants, such as enhancing protein utilization, preventing bloat, controlling internal parasites, increasing wool growth, and improving the milk fatty acid profile; therefore, there is a growing interest in using tannin-rich feeds in ruminant nutrition [3,4].

Livestock production systems increasingly use tannin-rich sources such as shrubs, tree foliage, or even agroindustrial byproducts [5] to reduce the feeding costs. *Quercus* species (oak trees) have been reported to contain high levels of both forms of condensed and hydrolyzable tannins [6,7], and grape pomace (*Vitis vinifera* sp.) as a tannin source is produced in different parts of the world [8].

The effects of tannin-containing oak and grape pomace and linseed oil as an n-3 fatty acid source have been studied individually and in different animal species [9–15], but there is no report on the possible interactions between these tannin-rich feeds and the n-3 fatty acid-rich oil. This study was designed to survey the effects of the oak leaf (*Quercus castanifolia*) or grape pomace (*Vitis vinifera*) tannin extracts, individually or mixed, with linseed oil as an n-3 fatty acid source on performance, blood metabolites, hepatic enzyme activity, and ruminal parameters in lactating ewes.

2. Materials and methods

2.1. Materials

Oak leaves were collected from the local habitat of oak trees located south of Ardabil Province, in the northwest of Iran; grape pomace was obtained from a juicer factory located in Urmia Province in the northwest of Iran; and linseed oil was bought from a commercial company in Fars Province, located in the south of Iran. Oak leaves and grape pomace were air-dried for 2 weeks, then milled

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using a mesh size of 1 mm and kept in a dark and cold place until extraction.

2.2. Animals, experimental diets, and management

The study was conducted at the Moghani Sheep Breeding Center, Ardabil Province, Iran (39°26′20.6″N, 48°5′26.4″E). The experimental protocols were reviewed and approved by the Animal Care Committee of the Agriculture Office. Thirty-six monoparous lactating ewes (at 5–7 weeks postpartum, 55 ± 5 kg) were randomly assigned to six treatments in a completely randomized design with six ewes in each treatment. The animals were kept in individual pens.

The basal diet consisted of alfalfa hay and concentrate (forage:concentrate ratio of 40:60). The following experimental diets were formulated individually according to the 2007 nutrient requirements of the National Research Council: (1) control (C), (2) 2% oak leave tannin extract (OE), (3) 2% grape pomace tannin extract (GPE), (4) 2% linseed oil (LO), (5) 2% oak leave tannin extract + 2% linseed oil (OEL), (6) % grape pomace tannin extract + 2% linseed oil (GPL). The experiment lasted 45 days, including 15 days of treatment adaptation and 30 days of data collection.

The animals were fed every day in the morning (08:30) and evening (16:30). Treatments were mixed evenly with the concentrate each morning. The ingredients and chemical compositions of the diets are presented in Table 1.

2.3. Measurements and sampling procedures

2.3.1. Diet sampling, body weight, and feed intake measurement

For each diet, samples of the feed were collected weekly and used for chemical analysis. Ewes' body weights were recorded at the beginning and end of the experimental period. Individual feed intake was measured by collecting the amount of feed and refusals for each animal weekly.

2.3.2. Blood and rumen sampling

At the 1st, 15th, and 30th days of the experimental period, blood samples were taken 4 h after feeding from the jugular vein of each ewe. Blood samples were collected into 10-mL tubes (anticoagulant-free), put on ice, and centrifuged at 3000 × *g* for 15 min to separate the serum. The serum samples were stored at –20 °C until analyses. The blood samples were analyzed to measure glucose, cholesterol, triglyceride, total protein, albumin, urea, and the activities of gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), alkaline phosphatase

Table 1. Ingredients and chemical composition of the experimental diets

Item	Alfalfa hay	Diets					
Ingredient (%)		C	OE	GPE	LO	OEL	GPL
Dehydrated alfalfa hay		40	40	40	40	40	40
Whole barley grain		32	32	32	29	29	29
Whole corn grain		14	13.8	13.8	10/8	10	10
Soybean meal		4	3.5	3.5	4	3.5	3.5
Wheat bran		8.5	7.2	7.2	12.7	12	12
Salt		0/5	0/5	0/5	0/5	0.5	0.5
Vitamin-mineral premix		0/5	0/5	0/5	0/5	0.5	0.5
Sodium bicarbonate		0.5	0.5	0/5	0.5	0.5	0.5
Linseed oil		-	-	-	2	2	2
Oak leave tannin extract		-	2	-	-	2	-
Grape pomace tannin extract		-	-	2	-	-	2
Chemical composition (%)							
OM	91.2	96.61	95.95	95.11	97.17	96.82	96.82
CP	17.92	16.96	16.81	17.76	16.79	15.90	15.90
NDF	34.47	28.5	26.15	25.5	27.25	26.18	26.18
ADF	23.85	11.75	10.56	11.12	11.25	10.25	10.25
Ether extract	5.6	6.3	6.8	7.4	8. 6	9.8	9.8

C: Control, OE: oak leave extract, GPE: grape pomace extract, LO: linseed oil, OEL: oak leave extract + linseed oil, GPL: grape pomace extract + linseed oil.

(ALP), and alanine aminotransferase (ALT) using a UV spectrophotometer (UNICO-Spectrophotometer, S2100-Vis, South Plainfield, NJ, USA); due to the lower spectrum required, the enzyme activity was determined using a UV spectrophotometer (UV-M51, UV/VIS spectrophotometer, BEL Engineering, Monza, Italy) with commercial kits (Pars Azmun Laboratory, Tehran, Iran) according to the instructions of manufacturer. Serum globulin (GBL) concentration was estimated using the difference between total protein and albumin concentrations.

According to Toral et al. [14], on the last day of the experimental period, animals had free access to the rations for 1 h; the feed was then removed and 3 h later the rumen liquors were collected by a stomach tube connected to a manual pump. Immediately after collection, the rumen liquors were strained through four layers of cheesecloth, and the pH of the samples was determined. After that, the samples were prepared (acidified with 10 mL of 50% H_2SO_4) for volatile fatty acid (VFA) analyses and stored at –18 °C. Gas chromatography equipment (Agilent 6890, Mississauga, Canada) was used to analyze the VFA (C2:0, acetic; C3:0, propionic; C4:0, butyric; *iso* C4:0, isobutyric; C5:0, valeric; *iso* C5:0, isovaleric).

2.3.3. Chemical analyses

Samples of feed were analyzed for dry matter (DM), organic matter (OM), ether extract (EE), and crude protein (CP) by the procedures of the AOAC [16]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Van Soest et al. [17].

For digestibility calculations, first the acid insoluble ash (AIA) [18] in feed and fecal samples was measured as an internal marker, and then total tract apparent DM digestibility was calculated using the following formula: 100 – 100 × [(Fecal con. / Feed con.) × (Feed AIA / Fecal AIA)].

2.4. Statistical analyses

The data on body weight, digestibility, and ruminal parameters were analyzed as a completely randomized design using PROC GLM, while periodically recorded items with repeated measures (blood metabolites, enzyme activity, and feed intake) were analyzed using the MIXED procedure of SAS 9.1.3 [19] according to the following model:

$$Y_{ij} = \mu + T_i + P_j + T_i \times P_j + e_{ij},$$

where Yi = dependent variable; μ = overall mean of the population; Ti = treatment; Pj = sampling time; $T_i \times P_j$ = treatment and sampling time interaction; and eij = random error. Comparison of means was performed using the Tukey test. The probability of 0.05 was used to determine significant differences among means.

3. Results

The effects of dietary treatments on the ewes' final body weight is presented in the Figure. The results showed that the final body weights were significantly different between the experimental groups ($P < 0.05$). The GPL, GPE, and OEL treatments had higher final body weights at the end of this study (60.65, 60.38, and 59.41 kg, respectively).

Table 2 shows the effects of tannin extract and linseed oil on the feed intake of the experimental groups. There were significant differences between the treatments and the periods for feed intake values ($P < 0.05$). Additionally, the interaction of treatment and period was significant, too ($P < 0.05$). In the whole period, the GPE, OEL, and GPL groups had the highest feed intake, respectively. At the first sampling, the OEL group had more feed intake, but in the next sampling series, the GPE group had the highest feed intake compared to the other groups ($P < 0.05$).

The results of the apparent digestibility assay are represented in Table 3. Digestibility of DM, OM, ash, CP,

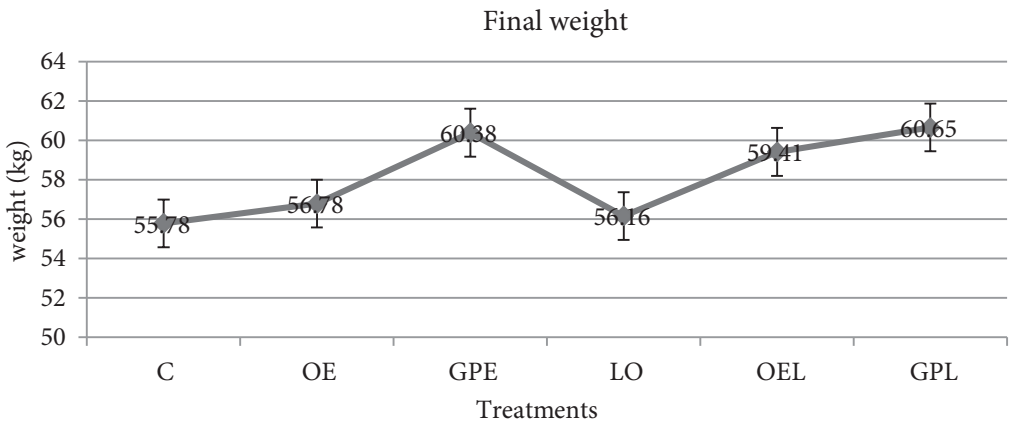

Figure. Effects of tannin extract and linseed oil on final body weight (kg). C: Control, OE: oak leave extract, GPE: grape pomace extract, LO: linseed oil, OEL: oak leave extract + linseed oil, GPL: grape pomace extract + linseed oil.

Table 2. Effects of tannin extract and linseed oil on feed intake.

Item (g)	Period	Treatment						SEM	P-value		
		C	OE	GPE	LO	OEL	GPL		T	P	T × P
Intake	1	1129	1346	1428.6	1452.8	1467.3	1408.8	15.56	0.0016	0.0002	0.0033
	2	1348.6	1411	1490.6	1432.8	1441.3	1462				
	3	1356.4	1377.1	1500	1434	1465.3	1481.8				
	4	1310.4	1386.6	1500	1435.6	1455.5	1472.6				

C: Control, OE: oak leave extract, GPE: grape pomace extract, LO: linseed oil, OEL: oak leave extract + linseed oil, GPL: grape pomace extract + linseed oil. T: Treatment, P: period, T × P: interaction of treatment and period.

Table 3. Effects of dietary treatments on apparent digestibility of nutrients.

Item (%)	Treatment						SEM	P-Value
	C	OE	GPE	LO	OEL	GPL		
DM	74.95	72.16	72.61	68.04	72.66	72.17	1.38	0.0792
OM	66.95	71.96	73.11	63.46	72.12	74.03	1.57	0.0030
Ash	57.58	65.89	64.46	55.97	63.63	58.01	2.04	0.0186
CP	55.15	65.03	65.24	63.54	65.10	67.42	2.34	0.0138
EE	46.29	53.003	52.01	36.77	50.38	58.78	2.003	0.0006
NDF	56.58	62.11	63.42	58.21	61.15	64.30	2.05	0.1288
ADF	51.87	53.72	54.72	52.45	58.83	56.43	1.93	0.1841

C: Control, OE: oak leave extract, GPE: grape pomace extract, LO: linseed oil, OEL: oak leave extract + linseed oil, GPL: grape pomace extract + linseed oil. T: Treatment, P: period, T × P: interaction of treatment and period.

and EE was affected by the dietary treatments ($P < 0.05$). Higher digestibility for OM, CP, EE, and NDF was seen for the GPL group, while the digestibility of ash and ADF was higher in the OE group. In contrast, more DM digestibility was seen in the control group.

The effect of dietary treatments on blood metabolites is shown in Table 4. According to the results, the effect of dietary treatments on glucose, cholesterol, and triglyceride was significant ($P < 0.05$). Moreover, the concentration of glucose, cholesterol, triglyceride, total protein, albumin, and globulin was significantly different between the sampling periods ($P < 0.05$). Significant interaction was also observed between the dietary treatments and the sampling periods for serum glucose, triglyceride, globulin, and urea content ($P < 0.05$). Noticeably, higher concentrations of almost all the serum parameters in the sampling periods were seen in the tannin-including treatments (mostly in OEL and GPL). The exception was higher cholesterol concentration in the linseed oil group.

Table 5 represents the effects of the dietary tannin extracts and the linseed oil on some hepatic enzyme activity in ewes. The effect of treatment was significant on AST concentration ($P < 0.05$). The concentrations of ALP, ALT, and AST changed significantly during the sampling periods ($P < 0.05$). The interaction of treatment and sampling period was not significant. During the sampling periods, the increasing or decreasing trend of the enzymes among treatments was inconsistent; for example, the concentrations of ALP, AST, and GGT generally showed ascending trends among the periods, but this trend was not observed in all treatments, whereas for ALT higher concentrations were observed in periods 2 and 3.

Table 6 shows the effects of the dietary tannin extract and linseed oil on ruminal pH and VFA concentration. The results showed that the dietary treatments did not affect the pH or VFA content of the rumen content.

4. Discussion

In this study, the highest final body weights were recorded for the ewes fed GPE. Moreover, the OEL and GPL groups (including both tannin extract and linseed oil) had higher body weights. Similar results were reported by Titi and

Table 4. Effects of extract and linseed oil on blood metabolites

Item	Period	Treatment						SEM	P-Value		
		C	OE	GPE	LO	OEL	GPL		T	P	T × P
Glucose (mg/dL)	1	46.09	46.35	47.83	41.37	46.35	42.51	2.11	0.0298	<0.0001	0.0420
	2	35.98	33.85	30.11	29.39	37.33	32.76				
	3	25.15	28.40	30.81	25.88	31.59	28.15				
Cholesterol (mg/dL)	1	52.97	82.63	86.70	86.43	112.2	90.08	5.39	0.0027	<0.0001	0.0593
	2	30.98	47.73	52.71	57.28	53.29	53.01				
	3	49.28	66.40	50.72	76.80	70.24	56.28				
Triglyceride (mg/dL)	1	18.15	44.32	51.54	36.27	23.1	29.45	18.83	<.0001	0.0009	<.0001
	2	24.99	18.61	21.34	26.97	29.19	27.99				
	3	54.44	75.53	58.59	55.55	54.36	45.72				
Total Protein (g/dL)	1	5.72	7.42	7.27	5.58	6.73	6.88	0.74	0.236	<0.0001	0.0592
	2	7.36	10.87	8.58	9.59	9.84	10.56				
	3	8.19	6.77	6.81	6.08	7.56	6.45				
Albumin (mg/dL)	1	2.65	2.95	3.17	2.79	2.92	2.07	0.33	0.290	<0.0001	0.3353
	2	4.04	4.39	5.03	4.68	4.82	5.14				
	3	2.91	3.67	3.25	3.67	3.29	3.55				
Globulin (mg/dL)	1	3.08	4.48	4.10	2.79	3.81	4.81	0.73	0.258	0.0197	0.0353
	2	3.31	6.48	3.54	4.91	5.01	5.41				
	3	2.90	3.10	3.56	2.40	4.27	5.28				
Urea (g/dL)	1	41.97	61.71	64.18	78.17	57.60	58.42	7.64	0.137	0.342	0.0015
	2	45.58	68.30	49.89	55.43	60.29	53.53				
	3	37.42	38.80	51.28	54.74	65.13	81.25				

C: Control, OE: oak leave extract, GPE: grape pomace extract, LO: linseed oil, OEL: oak leave extract + linseed oil, GPL: grape pomace extract + linseed oil. T: Treatment, P: period, T × P: interaction of treatment and period.

Al-Fataftah [20], who reported that feeding vegetable oil to Awassi ewes improved the final body weight. They announced that since the animals were under a positive energy balance before the experiment and the energy and protein supplied by the diets were adequate to meet the requirements, the final body weights increased. In a similar study by Bucciuni et al. [21], there was no adverse effect of diets containing tannin extract and oil on animal performance.

Based on the results for dry matter intake (DMI), the GPE, OEL, and GPL groups had higher DMI than the control and other experimental groups. The results concerning the effects of tannin extract and oil supplementation on DMI are inconsistent. In a study by Titi and Al-Fataftaha [20], insignificant effects of vegetable oil on DMI were reported. Toral et al. [22] and Cieslak et al. [5] reported that inclusion of tannin in the diet did not have any adverse effect on DMI. Martin et al. [23] reported that dietary linseed oil (LSO) supplementation reduced DMI through reducing corn silage intake. They noted that the reduction in DMI could not be explained only by the effects of the LSO on ruminal function (like microbial activity in feed digestion); therefore, it seems that the fat supplement has a direct inhibitory effect on feed intake by restricting reticulorumen motility. It was also noted that tannins could reduce DMI by reducing palatability and digestibility [2]. However, it seems that in the present study, the level of tannin supplementation was not enough to affect the palatability. Similar results have been reported by Aboagye et al. [24], who found that the mixture of condensed and hydrolyzable tannins resulted in a higher DMI in Holstein steers. Salem et al. [25] reported that the effects of tannin on DMI were related to the dosage of tannin, so doses lower than 4.5–44.5 g/kg DM were used safely in the studies. In line with our results, Naserian et al. [26] in a study on dairy goats reported increased DMI by increasing pistachio skins (a tannin source) as a replacement for wheat bran. The authors reported that this

Table 5. Effects of tannin extract and linseed oil on hepatic enzyme activity.

Item	Period	Treatment						P-Value			
		C	OE	GPE	LO	OEL	GPL	SEM	T	P	T × P
ALP (U/L)	1	223.91	170.02	226.53	339.48	260.87	304.67	42.54	0.7283	0.0013	0.2368
	2	220.81	249.97	196.24	250.61	211.97	252.56				
	3	327.58	281.37	308.54	317.72	304.85	275.55				
ALT (U/L)	1	23.05	23.27	25.14	25.25	25.58	24.81	1.38	0.0917	0.0283	0.3278
	2	25.80	25.03	28.34	24.59	28.45	27.90				
	3	24.66	26.24	25.91	23.82	24.15	29.55				
AST (U/L)	1	174.22	153.38	117.43	118.53	122.28	123.71	13.32	0.043	0.013	0.324
	2	117.98	117.21	115.11	120.40	116.65	116.21				
	3	171.20	119.52	141.69	138.38	128.12	142.13				
GGT (U/L)	1	50.11	71.92	82.09	72.50	66.13	60.79	13.61	0.8494	0.4009	0.1085
	2	60.79	73.53	76.94	65.49	59.63	110.65				
	3	70.81	74.43	52.24	76.10	66.01	51.98				

C: Control, OE: oak leave extract, GPE: grape pomace extract, LO: linseed oil, OEL: oak leave extract + linseed oil, GPL: grape pomace extract + linseed oil. T: Treatment, P: period, T × P: interaction of treatment and period.

Table 6. Effects of tannin extract and linseed oil on ruminal fermentation parameters

Item	Treatments						SEM	P-value
	C	OE	GPE	LO	OEL	GPL		
pH	6.95	6.80	6.74	6.78	6.36	6.66	0.26	0.7094
Acetic acid (mmol/L)	15.30	16.83	26.45	24.31	30.68	20.51	2.88	0.0537
Propionic acid (mmol/L)	5.27	4.50	5.81	6.05	7.24	6.44	0.97	0.5153
Butyric acid (mmol/L)	5.92	5.03	5.83	5.57	7.10	6.73	0.99	0.7111
Isobutyric acid (mmol/L)	ND	ND	ND	ND	ND	ND	-	-
Valeric acid (mmol/L)	1.63	0.56	0.61	0.67	0.69	ND	0.07	0.4003
Isovaleric acid (mmol/L)	0.76	0.76	0.88	0.80	1.05	ND	0.39	0.4863
Total VFA (mmol/L)	28.72	27.69	39.60	37.41	45.89	33.69	4.02	0.1107

C: Control, OE: oak leave extract, GPE: grape pomace extract, LO: linseed oil, OEL: oak leave extract + linseed oil, GPL: grape pomace extract + linseed oil. ND: Not determined.

result could be caused by the positive effect of secondary metabolites such as tannin on ruminal fermentation and higher nutrient digestibility.

In this study, the digestibility results showed that inclusion of tannin extract and linseed oil in the diet had no adverse effects on nutrient digestibility. Only DM showed higher digestibility in the control group. The effect of tannins on animals depends on their biological activity, plant maturity [6], and the level of intake [27]. Abargouei et al. [11] studied the effect of grape pomace and oak leaf tannin on in vitro organic matter digestibility in sheep. They reported that the grape pomace and oak leaf tannins decreased the in vitro digestibility of OM. These results are different from our findings. Ueda et al. [28] reported a decrease in the ruminal digestibility of OM and fiber following supplementation of the diet with linseed oil. However, they also noted the increase in total tract digestibility of those fractions with a higher forage ratio in the diet. The reason for this increase was the compensatory effect of fiber digestion in the large intestine. They noted

that the forage-to-concentrate ratio is a key factor to determine the effects of lipid supplementation on ruminal digestibility. In diets with lower concentrate ratios (30%–35%), n-3 supplements had a positive effect on NDF and OM digestibility. Ahnert et al. [29] noticed the adverse effects of tannin on OM, DM, NDF, and ADF digestibility with supplementation levels over 4%; moreover, they reported a reduction in fiber digestibility at levels up to 6% supplementation. Barry et al. [30] reported that less than 4% dietary tannin supplement could be beneficial for ruminants. Barry and McNobb [31] reported that lower dosages of secondary plant metabolites (i.e. tannins) could have beneficial effects for animals; they can also reduce protein degradability in the rumen, consequently improving CP digestibility and animal performance. In line with this report, the dosage of tannin extract in the present study was lower than 4% and it improved the nutrient digestibility without any adverse effects on animal performance.

The effects of dietary treatments on blood parameters and hepatic enzymes activity were variable, and the sampling period was a more compelling factor. The lack of effect on blood parameters such as urea and albumin in the present study could be a result of the inability of tannin sources to affect the protein metabolism in the rumen [26]. Although dietary treatments did not have a significant effect on blood parameters, numerically higher levels of blood parameters were seen in ewes receiving experimental treatments. In line with the results of this study, Szczechowiak et al. [32] reported increased levels of glucose by supplementing a tannin extract and oil blend. They noted that this result could be due to the higher propionate concentration in the rumen.

However, these researchers reported reduced blood triglycerides by linoleic acid in linseed and the mixture of tannin extract and oil [32], which is in contrast to our findings and was attributed to the improvement in the lipoprotein lipase activity, which affects the fat oxidation. Consequently, reduction in NEFA levels resulted in lower TG levels. The same result in TG reduction was reported in a study by Titi and Al-Fataftah [20].

Salem et al. [25] reported that supplementing the diet with tannin did not affect the serum cholesterol content, which is different from our results. It was reported that supplementation of fat in the diet could increase the cholesterol synthesis in the small intestine and increase the chance of fat absorption [33]. This conclusion is in agreement with the higher concentration of serum cholesterol in oil treatments of the present study.

The liver has the main fraction of circulating proteins. There are comparable total protein concentrations in the serum of most animal species, but there are variations in the distribution of protein fractions. Aganga et al. [1] reported that proper dosages of tannins have positive effects on protein metabolism, especially the effects on reducing protein degradation in the rumen, which could lead to more amino acid absorption in the intestine. Following the results of this study, Salem et al. [25] reported that the concentrations of total protein, albumin, and globulin were unaffected by tannin supplementation.

Measuring some enzyme activities and their concentrations in the blood is helpful to monitor liver health and function [33]. Aminotransferases are responsible for amino acid metabolism, and their activity is highly related to the organ's energy status [32]. Bucciuni et al. [21] noted that condensed tannins are not absorbed in the intestine, so they could not have adverse effects on the metabolism of organs like the liver. The concentrations of GGT, ALT, and AST enzymes did not increase in their study. They also mentioned that hydrolyzable tannins are capable of being degraded by rumen microbes; therefore, the higher concentration of the enzymes in the blood resulted from the liver's inability to detoxify tannin. The report of these researchers on liver enzyme activities being unaffected by tannin extract is in line with our results, except for AST. In another similar study, Bucciuni et al. [34] studied the effects of tannin extract on blood parameters in grazing ewes fed a diet containing soybean oil. They reported that the treatments could not affect blood parameters, but the concentration of urea, transaminase, total protein, globulin, glucose, and triglycerides was different among the sampling periods. The result of the effects of period on the concentration of glucose, triglycerides, total protein, globulin, urea, and transaminase enzymes (ALT, GGT, and AST) in that study was in agreement with our findings. In contrast, Nudda et al. [12] reported the significant effect of dietary grape byproducts and linseed oil on blood parameters of dairy ewes. Unlike our results, they found significant effects of treatments on blood metabolites. However, the increase in protein and albumin agreed with our findings, but not the reduction trend of ALP. These researchers noted that there were reports on the hepatoprotective role of grape seed extract in laboratory animals. They announced that the variation in most of the blood parameters during the period of sampling could be a result of the metabolic changes related to the progress of lactation. As Mohamed [35] noted, lactation has a significant impact on the intensity of metabolism and metabolic parameters in the blood.

The effects of treatments on ruminal fermentation parameters (pH and VFA concentration) were insignificant in the present study. In a comparable report, Bucciuni et al. [21] found that the tannin extract did not have any significant effects on ruminal pH, but a comparison of the results showed lower pH in treatment groups; this report is in agreement with the results of this study, in which

ruminal pH was unaffected during the experimental period. In line with our findings, Toral et al. [13], in a study using tannin extract in a linoleic acid-containing diet, reported that tannin extract had no effects on ruminal parameters (including pH and molar proportions of VFA). Unlike those insignificant effects of treatments, the results of this study showed higher VFA content in treatment groups, which is in contrast to the results of Bucciuni et al. [21]. They reported the reduction in concentrations of acetic, propionic, valeric, and isovaleric acid affected by quebracho tannin extracts and they announced the increasing effect of chestnut tannin extract on acetic and propionic acid. The same trend was seen in this study. This could be related to the fact that hydrolyzable tannins might be metabolized to gallic and ellagic acids in the rumen, which can convert to acetic and butyric acids [4]. Finally, it was concluded that the rumen microbiota might have been changed to the tannin hydrolyzing population, but the change was minor, so there was not a significant effect on VFA concentration in the present study [24].

In conclusion, the results of this study showed that the dietary tannin extract and linseed oil had no adverse or toxic effects on lactating ewes. Moreover, tannin extract and linseed oil, individually or together, have a positive effect on ewes' final body weight and could change blood metabolites. Our results showed that the effects of the treatments were not significant for VFA molarity, but treatment groups had higher molarity of ruminal VFA.

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