Journal of Agriculture and Food Research 19 (2025) 101658

Contents lists available at ScienceDirect

Journal of Agriculture and Food Research

journal homepage: www.sciencedirect.com/journal/journal-of-agriculture-and-food-research

Feeding walnut leaf and green tea ethanolic extracts enhances performance and improves plasma metabolites in fat-tailed ewes during the transition period

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ARTICLE INFO

Keywords:
Milk composition
Plant extract
Polyphenolic
Plasma metabolites
Milk fatty acid profile
Metabolic health
Transition period

ABSTRACT

The transition period in ewes, characterized by significant physiological changes, plays a critical role in determining the health and productivity of both the ewe and her offspring. This study explored the effects of leaf extracts of walnut (WLE) and green tea (GTE) on dry matter intake, colostrum and milk production, composition, and plasma metabolites in fat-tailed ewes in the transition period. Forty-eight fat-tailed Makui ewes (58 ± 1.2 kg body weight) were randomly assigned to four treatments (12 ewes per group): a control group (CON) receiving a basal diet and three experimental groups supplemented with 100 mg/kg body weight of WLE, GTE, or a combination (WL + GT), and the extracts were administered orally twice daily. Ewes fed the WL + GT combination had higher ($P < 0.05$) dry matter intake, body weight, and body condition score both *pre-* and *post-partum* than the other groups. The WL + GT ewes also exhibited lower plasma non-esterified fatty acids, reduced insulin levels *post-partum*, and lower liver enzyme concentrations (aspartate aminotransferase and alanine aminotransferase) *versus* CON ewes. Additionally, WL + GT supplementation reduced ($P < 0.05$) plasma monounsaturated fatty acids and increased polyunsaturated fatty acids and Omega-6 levels. Milk yield during the first week of lactation was highest in the WL + GT group, which also showed increased ($P < 0.05$) milk fat and lactose content. Furthermore, milk from supplemented ewes showed a higher concentration ($P < 0.05$) of polyunsaturated fatty acids, Omega-6, eicosapentaenoic acid, and docosahexaenoic acid in both WL + GT and WLE ewes than GTE and CON. These findings suggest that the supplementation of WLE and especially the combination of WL + GT positively influences feed intake, body weight, and milk production, while also enhancing the nutritional profile of the milk by increasing beneficial fatty acids such as eicosapentaenoic acid and docosahexaenoic acid. The improvements in metabolic health markers, including reduced ($P < 0.05$) non-esterified fatty acids and liver enzyme concentrations, indicate a potential role of these extracts in optimizing energy metabolism and reducing metabolic stress during the critical transition period, with potential benefits for sustainable dairy production. Further research is needed to fully elucidate the underlying mechanisms of these effects and their long-term implications for ewe and lamb health.

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https://doi.org/10.1016/j.jafr.2025.101658
Received 22 October 2024; Received in revised form 5 January 2025; Accepted 15 January 2025
Available online 17 January 2025

1. Introduction

The transition period in ruminants, spanning from 3 weeks before to 3 weeks after parturition, is a critical phase marked by significant metabolic changes. These changes include reduced feed intake, milk production, and heightened nutrient demands for fetal growth and lactation [1]. This imbalance results in a negative energy balance, mobilization of adipose tissue, and elevated levels of circulating non-esterified fatty acids (NEFA) [2]. Increased NEFA levels lead to triglyceride accumulation in muscle and liver, causing reduced feed intake and metabolic disturbances [3]. To address these issues, plant extracts rich in polyphenols have gained attention for their antimicrobial and antioxidative properties [4]. Flavonoids, in particular, have been shown to enhance nutrient absorption, improve performance, and increase milk production [5]. Green tea is notable for its bioactive components, especially flavonoid polyphenols like catechins, caffeine, and benzimidazole, while walnut leaves contain polyphenols such as juglone and flavonoids, also exhibiting antioxidant and anti-inflammatory properties [6]. Studies on green tea have demonstrated improvements in body weight gain in broilers [7], calves [8], and goats when tea byproducts were included in their diet [9]. Additionally, phenolic compounds in green tea extracts can influence milk fatty acid composition [10]. Both green tea and walnut leaf extracts have shown positive effects on blood glucose and lipid profiles in animals and humans [11,12] Previous research has primarily focused on the general effects of plant extracts in livestock nutrition, including their antioxidant, anti-inflammatory, and growth-promoting properties. However, these studies have largely concentrated on more commonly studied breeds, rather than fat-tailed ewes. For instance, studies on green tea and walnut leaf extracts have often been limited to ruminants like cattle or goats, without addressing the unique physiological characteristics of fat-tailed sheep. Despite the importance of the peripartum period, there is a lack of targeted research on how plant-derived compounds affect animals during this phase. In addition, while caffeine has been studied for its metabolic and antioxidant effects in monogastric animals, its role in ruminants, especially fat-tailed sheep, remains underexplored. Similarly, benzimidazole derivatives have been researched primarily as anthelmintic agents, but their broader physiological effects when consumed as part of plant extracts (e.g., walnut leaves) have not been adequately investigated. Benzimidazole (CAS no. 51-17-2) has shown potential in reducing plasma triacylglycerol and cholesterol and enhancing glucose transport into tissues via adenosine monophosphate-activated protein kinase (AMPK) activation or other mechanisms [13]. AMPK = 5′ AMP-activated protein kinase or 5′ adenosine monophosphate-activated protein kinase is an enzyme (EC 2.7.11.31). Benzimidazole also has antibacterial, antibiotic, anti-inflammatory, and antidiabetic properties [14]. Its derivatives are potent inhibitors of protein glycation and oxidative damage) [15].

While both extracts independently possess significant bioactive compounds with antioxidant, anti-inflammatory, and metabolic effects, we hypothesize that their combination may produce a synergistic effect. This synergy is expected due to the complementary nature of their active components, particularly flavonoids, catechins, caffeine, and benzimidazole derivatives. Combining these extracts may better address the multifaceted metabolic challenges of fat-tailed ewes, including reduced feed intake, elevated non-stratified fatty acid levels, and changes in milk composition. The potential for combined effects on adenosine monophosphate-activated protein kinase activation and fatty acid mobilization underscores the innovation in exploring these extracts together.

By highlighting the expected synergistic effects of walnut and green tea, this study aimed to investigate the metabolic pathways affected by green tea and walnut leaf extracts, focusing on their impact on plasma non-stratified fatty acids, metabolites, and fatty acid profiles. If the results indicate significant improvements in milk production, composition, and metabolic health, these extracts could be considered for inclusion in commercial feed formulations for fat-tailed ewes during the transition period. Such plant-based additives not only offer potential benefits for productivity but also support sustainable agriculture by minimizing dependence on synthetic supplements and promoting animal welfare through natural solutions.

2. Materials and methods

The trial took place at the Urmia University dairy farm in Iran, spanning from October to December 2017. Approval from the Animal Care and Use Committee of Urmia University (IACUC Protocol #IR2018011) was obtained upfront. The rules of the Iranian Council of Animal Care [16] were followed.

2.1. Animals and diets

The research protocol was approved by the university's Animal Care and Use Committee (IACUC Protocol #IR2018011), following the guidelines of the Iranian Council of Animal Care [16]. The ewes' estrous cycles were synchronized using progesterone sponges, and 35 days after mating, they were confirmed pregnant through ultrasonography. Forty-eight fat-tailed Makui ewes (58 ± 1.2 kg body weight) were divided into 4 treatments (n = 12 per each) in a randomized design: a control group receiving no extract (CON), a group receiving 100 mg/kg BW (body weight) of walnut leaf ethanolic extract (WLE), a group receiving 100 mg/kg BW of green tea leaf ethanolic extract (GTE), and a group receiving 100 mg/kg BW of a 1:1 combination of both extracts (WL + GT). The main experiment lasted 60 days, preceded by a two-week adaptation period for the ewes to adjust to their diet and housing. The ewes were housed individually with access to fresh water, salt, and mineral–vitamin licking blocks. They were fed a total mixed ration, formulated to meet their energy and crude protein (CP) needs (Table 1; NRC [17]), and provided *ad libitum* twice daily. The plant extracts were administered orally, dissolved in double distilled water to

Table 1
Ingredients and chemical composition of the basal total mixed rations used as experimental diets during pre- and *post-partum*.

Ingredients, % DM	*Pre-Partum*	*Post-Partum*
Alfalfa hay	34.2	25.6
Corn Silage	34.1	35.2
Barley grain	13.0	15.7
Wheat bran	6.57	9.09
Soybean meal	8.86	10.1
Dicalcium phosphate	0.74	0.50
Mineral-vitamin supplement[a]	0.74	1.00
Fat supplement (calcium salts)[b]	1.80	2.80
Dietary nutrients and energy		
Metabolizable energy, Mcal/kg dry matter (DM)	2.30	2.53
Crude protein, % DM	13.9	15.0
Ether extract, % DM	4.90	5.30
Neutral-detergent fiber, % DM	44.8	43.1
Ash, % DM	7.30	6.50
Calcium, % DM	1.08	0.85
Phosphorus, % DM	0.49	0.48
Fatty acid profiles, % of fatty acids		
C16:0	11.67	13.25
C16:1	1.12	1.34
C18:0	6.31	8.11
C18:1	34.36	37.06
C18:2	29.34	30.36
C18:3	6.42	5.71

[a] Each kg contains: 500,000 IU of vitamin A, 100,000 IU of vitamin D, 0.1 mg of vitamin E, 180 g of calcium, 90 g of phosphorus, 20 g of magnesium, 60 g of sodium, 2 g of manganese, 3 g of iron, 0.3 g of copper, 3 g of zinc, 0.1 g of cobalt, 0.1 g of iodine, 0.001 g of selenium, 3 g of antioxidants.

[b] Calcium salts of fatty acids (PersiaFat®,Iran). Fatty acid profiles as described by the producer: Palmitic acid: 20 %; Palmitoleic acid 2 %; Oleic acid 40 %; Stearic acid 8 %; Linoleic Acid 22 %; Linolenic acid 5 %.

form a stable emulsion, and given in equal doses before morning and evening feedings. The 100 mg/kg BW dose was based on prior studies evaluating effective extract doses on ruminal fermentation [18,19]. Feed intake and nutrient composition were monitored weekly by sampling feed and orts. The chemical analyses followed AOAC [20] guidelines for dry matter (DM), organic matter (OM), CP, and ether extract (EE). For fatty acid analysis, bi-weekly feed samples were collected, lipids were extracted, and fatty acid methyl esters were prepared using mild methanolysis/methylation methods, as described by Ichihara and Fukubayashi [21].

2.2. Preparation of the extracts

In September 2017, walnut leaves (*Juglans regia*) were gathered from the Urmia University research garden (37′N, 44′E), West Azerbaijan province, Iran, and green tea leaves (*Camellia sinensis*) were collected from tea farms in Lahijan, Iran. The collected leaves were air-dried in the shade at ambient room temperature (approximately 22–25 °C) to prevent degradation of sensitive bioactive compounds. After the milling process, a 96 % ethanol solution (containing 4 % water) was used to soak dried walnut and green tea leaves separately for 24 h at room temperature (22–25 °C), and then the solution was filtered using filter paper. After filtration, solid residues were submerged in 75 % ethanol solution (containing 25 % water) under the same conditions (22–25 °C) for another 24 h. The filtration process was then repeated and the filtrates from both of the extraction procedures (96 % ethanol and 75 % ethanol) were combined for each of the leaves. Subsequently, the solvent extraction was carried out using a Heidolph Laborota 4000 rotary device (Heidolph Instruments GmbH and CO. KG, Schwabach, Germany). The solvent was nearly completely evaporated, operating at 50 °C with a rotation speed of 70 rpm, resulting in a non-liquefied form according to Solar et al. [22]. The concentrated solutions were preserved in small containers at 4 °C until subjected to analysis. Based on the analysis of extracts using GC-MS in previous studies [18,19], benzimidazole was identified as the compound with the highest concentration in green tea leaf extract, while caffeine was the predominant component in walnut leaf extract.

2.3. Dry matter intake, body weight and body condition score change

Dry matter intake was monitored daily by subtracting the weights of individual feed offered and the remaining ort. Accordingly, the total mixed ration and refusals were sampled weekly to determine the dry matter content [20]. Body weight (BW) and body condition score (BCS) assessments were conducted weekly in the morning before feeding. A calibrated digital scale with a maximum capacity of 100 kg ± 10 g (1 m length × 0.5 m width, Pand Electronics, Tehran, Iran) was used for weighing, and trained evaluators assigned the BCS on a scale ranging from 1 to 5, following the criteria established by Russel et al. [23]. Additionally, ewes were weighed after lambing. Fat tail characteristics such as volumetric weight, length, width, and diameter were measured by a caliper and recorded weekly as an indirect measure for changes in fat tail reserves [24].

2.4. Blood sampling and measurement of plasma metabolites

Blood samples from 48 ewes were collected at five points: 30 days *pre-partum*, 14 days *pre-partum*, the day of lambing, and 10- and 30-days *post-partum*. Each ewe was sampled using two tubes to collect both serum (for insulin measurement) and plasma (heparinized tubes with sodium fluoride and potassium oxalate). Plasma and serum samples were stored at −20 °C until further analysis. Analyses included glucose, triglycerides, cholesterol, blood urea nitrogen (BUN), albumin, total protein, non-esterified fatty acids (NEFA), liver enzymes (AST, ALT), and alkaline phosphatase (ALP). Serum insulin was measured with an ELISA kit, and plasma lipid profiles were determined through lipid extraction and fatty acid analysis as described previously [25].

2.5. Colostrum, milk yield and composition

The first secretion of the mammary glands after parturition was considered colostrum. Total colostrum was milked from both of the glands, thoroughly mixed, weighted, and sampled to determine the composition (Table 5). Milk yield was recorded daily at 12:00 after milking the ewes with portable milking machines. Milk samples were taken in 7-day intervals from individual ewes on two consecutive days to determine the milk composition. Both glands were milked, and a sample was chosen after aeration in a milk chamber. Potassium dichromate was used for sample preservation. Milk analysis was conducted by the Rajzhan Ltd. milk laboratory (Karaj, Iran), using infrared technology (MilkoScan FT6000 spectrometer, Foss, Hillerød, Denmark) according to ISO 9622:2013 (ISO, 2013). Milk fatty acid profiles were also determined by the method of Folch et al. [26]. To prevent fatty acid oxidation, lipid extraction was performed thrice using chloroform/methanol (C/M, 2/1, v/v) until reaching a final volume of 100 mL, conducted under an argon gas blanket. Following each extraction stage, the flasks underwent centrifugation at 1800 g for 10 min, and the resulting organic portion was isolated and introduced into a 100 mL volumetric flask. Subsequently, they underwent dehydration using anhydrous sodium sulfate and were later evaporated using a rotary evaporator (Büchi, Switzerland) at 40 °C under vacuum conditions.

2.6. Statistical analysis

A standard deviation of 300 g for the daily milk production of ewes was presumed, considering the minimum significant difference in milk production to be 135–150 g/day, as per previously reported values [27–30]. A power analysis was executed with a significance level (α) of 0.05 and a power of 0.80. The established sample size of 12 ewes per treatment was reached for the evaluation of feed intake and milk production, recognized as the most resilient parameters in the power analysis. Before the analysis, all of the data residuals were screened for normality using the UNIVARIATE procedure of SAS. Data was analyzed in a completely randomized design with 4 treatments. The statistical model applied to once-measured parameters encompassed the fixed effects of dietary treatments and random animal effects. Variables involving repeated measurements (dry matter intake (DMI), BW, BCS, milk yield, milk composition, and plasma metabolites) within individual ewes. The analysis included fixed effects for treatment, time of measurement (days), the interaction between treatment and time, along with the random effect of ewes nested within treatments. Data was analyzed using the MIXED procedure of SAS version 9.4 (SAS Institute, USA). In the case of repeated measurements, auto-regressive type 1, variance-covariance structures that minimized the Schwarz's Bayesian information criterion were selected. The data were presented as least squares means, and to address multiple comparisons, the Tukey-Kramer adjustment was implemented. The separation of treatment means was carried out using the PDIFF procedure of SAS. The significance level was determined at $P \leq 0.05$, and trends were considered for $0.05 < P \leq 0.10$.

3. Results

All of the ewes in the experimental groups remained healthy during the experimental period and did not require medication or drug administration.

3.1. Dry matter intake, body weight and BCS changes

The greatest daily dry matter intake (DMI) was observed in the WLE and WL + GT ewes *in pre-partum*, and daily DMI did not significantly differ in GTE compared to the CON. The supplemental phytochemicals did not affect mean body weight (BW) and live weight change in the pre-

partum period. However, the effect of time on these parameters was significant ($P < 0.05$; the time effect presents different measurements over before and after parturition) in the *pre-partum*. Ewes supplemented with WL + GT had the greatest body condition score (BCS), but the differences between WLE and GTE ewes were not significant in the *pre-partum*. In the *post-partum* period, the WL + GT and WLE ewes had greater ($P < 0.001$) daily DMI compared to the GTE and CON groups. There was an interaction ($P < 0.001$) between treatments and time on DMI of *post-partum* ewes. Ewes of WLE, GTE, and WL + GT groups had greater ($P < 0.05$) BW on 30 days after parturition compared to the CON. Ewes supplemented with WL + GT had the greatest BCS, but the differences between WLE and GTE groups were not significant. The CON group exhibited the highest average daily weight loss, while no notable distinction was observed among the experimental groups receiving plant extract supplements. There was not a significant difference in functional characteristics of fat-tail ewes during the experiment period (Table 2). The effects of WLE, GTE, and their combination (WL + GT) on DMI and daily weight change of ewes during 30 days before and 30 days after parturition are illustrated in Figs. 1 and 2. In Fig. 1, it is evident that DMI declines as the time of parturition approaches, reaching its lowest point on the day of lambing. After lambing, all groups showed an increase in DMI from day 7–30 *post-partum*. The rise in DMI from 7 days after lambing was faster in the WLE and WL + GT ewes *versus* other groups. A significant treatment × time interaction was observed ($P < 0.01$) during the peripartum period. Ewes showed a considerable loss in BW from lambing until one-week *post-partum* and a continuing decrease until 30 days. There was a difference ($P < 0.05$) in daily weight loss of ewes fed different plant extracts *versus* CON ewes (Fig. 2). The highest and lowest loss in BW was observed in CON and WL + GT groups, respectively.

3.2. Blood collection and analysis

Table 3 shows the blood metabolites of pregnant ewes during the transition period. The feeding of the plant extracts did not change the least square means of plasma metabolites *pre-* and *post-partum*, except for insulin, non-stratified fatty acids (NEFA), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Plasma insulin concentration was not affected by feeding extracts in *pre-partum*. In the *post-partum* period, however, WL + GT ewes had the greatest ($P < 0.01$) insulin concentrations among experimental treatments. Surprisingly, in the case of insulin concentration, separate supplementation of extracts did not result in an effective change. Plasma NEFA concentration was reduced ($P < 0.01$) by WL + GT *versus* CON ewes. There was an interaction ($P < 0.01$) between treatments and time on NEFA concentration of *post-partum* ewes. Plasma AST concentration did not differ among treatments at *pre-partum*, but *post-partum*, AST concentration was lowest in the WL + GT ewes compared to other treatments. The combinations of extracts (WL + GT) resulted in the lowest plasma ALT concentration, lower ($P < 0.05$) than that of the CON ewes, both pre-partum and post-partum. ALT concentration in WLE and GTE *versus* CON ewes. The use of extracts had no significant effect on plasma ALP concentration.

Changes in plasma concentration of glucose, insulin, and NEFA during 30 days before and after parturition are shown in Fig. 3 (A–C). The blood glucose level increased slightly ($P < 0.05$) near parturition and dropped suddenly postpartum. Plasma concentration of glucose response to extracts was not different between treatments. A time effect ($P < 0.001$) was observed for plasma glucose caused by a decreasing concentration after parturition. Ewes had higher Insulin concentration before parturition compared to after parturition. A time ($P < 0.001$) and treatment effect ($P < 0.001$) were observed for plasma insulin caused by a decreasing concentration over time. Treatment ($P < 0.001$) and time ($P < 0.001$) and a trend for treatment × time interaction ($P = 0.09$) for

Table 2
Effect of feeding leaf extracts of walnut, green tea and their combination on dry matter intake, body weight change, body condition score and functional characteristics of fat-tail.

Parameters	Experimental treatments[a]					*P*-value[f]		
	CON (n = 12)	WLE (n = 12)	GTE (n = 12)	WL + GT (n = 12)	SEM[e]	Treatment	Time[b]	Treatment × Time
Pre-Partum (30 days before parturition)								
Dry matter intake, kg/d	1.53[b]	1.60[a]	1.54[b]	1.68[a]	0.09	0.001	0.001	0.630
BW, kg	59.0	58.2	57.1	58.5	1.11	0.100	0.004	1.000
Live weight change, kg/d	0.22	0.21	0.23	0.22	0.05	0.526	0.011	0.180
Body condition score	2.5[c]	2.75[b]	2.75[b]	3.00[a]	0.09	0.001	0.001	0.700
Post-Partum (0–30 days after parturition)								
Dry matter intake, kg/d	2.04[c]	2.26[a]	2.10[b]	2.27[a]	0.10	0.001	0.001	0.001
BW at parturition, kg	55.3	54.1	53.5	53.9	0.89	0.100	0.120	0.200
BW 30 days after parturition, kg	49.4[b]	51.4[a]	51.5[a]	53.2[a]	6.70	0.001	0.001	0.990
Body condition score	2.25[c]	2.50[b]	2.50[b]	2.75[a]	0.08	0.001	0.001	0.900
Live weight change, kg/d	−0.36[a]	−0.28[b]	−0.26[b]	−0.25[b]	0.06	0.050	0.001	0.450
Functional characteristics of fat-tail								
volumetric weight, kg						0.25		
30 day[c]	4.53	4.45	4.55	4.52	0.02		–	–
+30 day[d]	4.24	4.28	4.22	4.15	0.01			
Length, cm						0.19	–	–
30 day	31.0	32.7	30.4	29.7	2.23			
+30 day	31.7	31.8	30.3	30.7	3.76			
Wide, cm						0.38	–	–
30 day	26.0	26.9	26.3	26.1	2.63			
+30 day	26.5	26.8	27.3	27.6	1.41			
Diameter, cm						0.11	–	–
30 day	67.0	67.0	67.4	66.3	3.57			
+30 day	66.3	66.9	66.0	65.0	5.13			

[a] CON: Control; WLE: Walnut leaf extracts; GTE: Green tea leaf extracts; WL + GT = combination of both of the extracts at 100 mg per kg ewes body weight (BW).
[b] The time effect presents different measurements over before and after parturition.
[c] -30 days: 30 days before parturition.
[d] +30 days: 30 days after parturition.
[e] SEM: standard error of the mean.
[f] Data in each row with different superscripts were statistically different ($P \leq 0.05$).

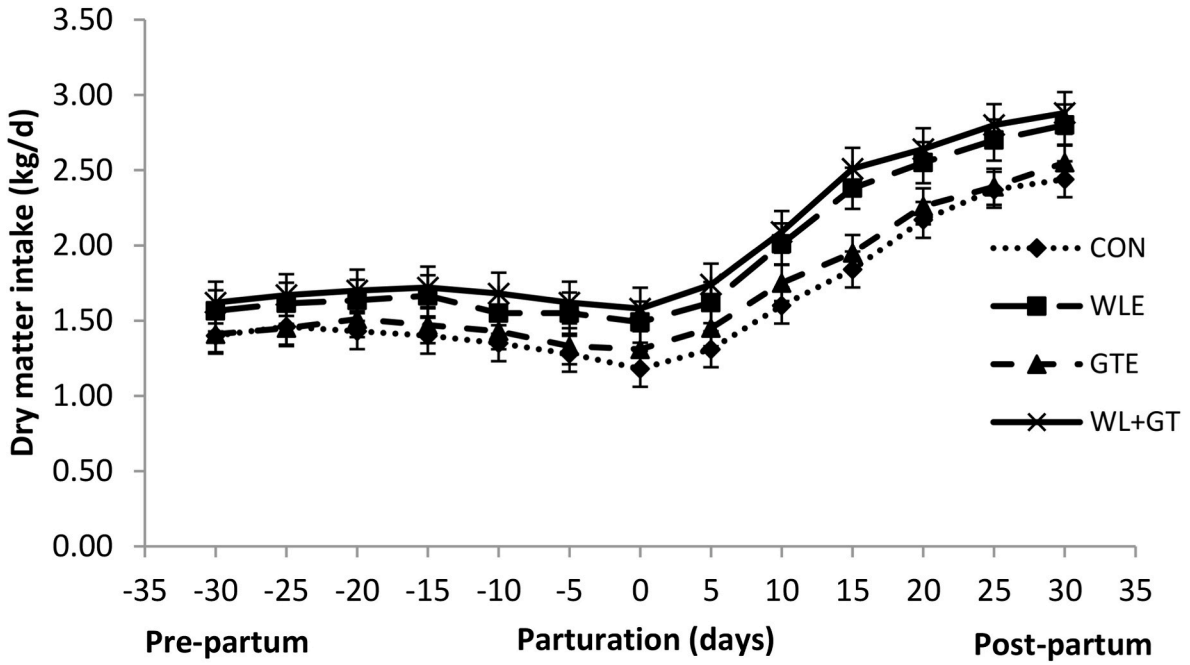

Fig. 1. Effect of WLE and GTE or a combination of both (WL + GT) on daily dry matter intake during *pre-* and *post-partum* period of ewes. Results are given as means of groups (n = 12); Con, WLE, GTE, and WL + GT represent groups of ewes fed 100 mg per kg of extracts by oral gavage. Error bars indicate the standard deviation of the mean values. *pre-partum*: Treatment effect: $P < 0.001$; Time effect: $P < 0.001$ and Treatment * time effect: $P < 0.001$. *post-partum*: Treatment effect: $P < 0.001$; Time effect: $P < 0.001$ and Treatment*time effect: $P > 0.63$. CON = control; WLE = walnut leaf extracts; GTE = green tea leaf extracts; WL + GT = combination of both of the extracts.

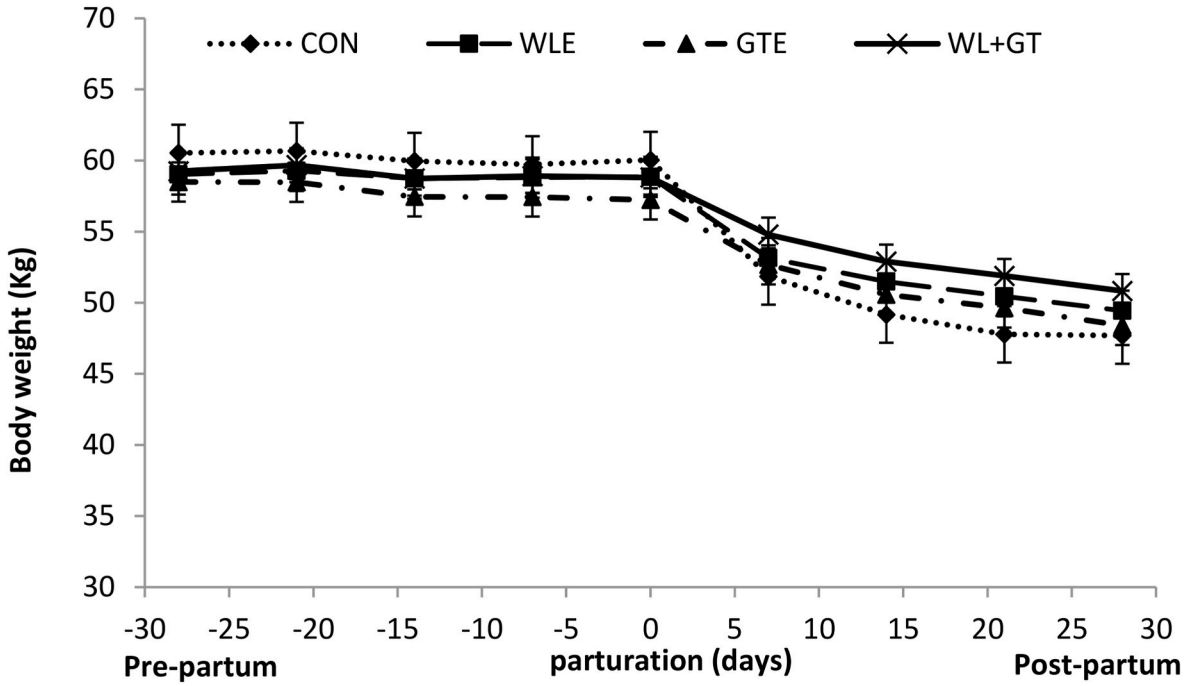

Fig. 2. Effect of WLE and GTE or a combination of both (WL + GT) on daily BW change during *pre-* and *post-partum* period of ewes. Results are given as means of groups (n = 12); CON, WLE, GTE, and WL + GT represent groups of ewes fed 100 mg per kg of extracts by oral gavage. Error bars indicate the standard deviation of the mean values. *pre-partum*: Treatment effect: $P > 0.08$; Time effect: $P < 0.001$ and Treatment * time effect: $P > 0.75$. *post-partum*: Treatment effect: $P < 0.001$; Time effect: $P < 0.001$ and Treatment*time effect: $P > 0.99$. CON = control; WLE = walnut leaf extracts; GTE = green tea leaf extracts; WL + GT = combination of both of the extracts.

plasma NEFA were detected, largely because peak elevations in plasma NEFA concentrations occurred 5 days after parturition. The highest and lowest ($P < 0.05$) NEFA concentrations were observed in CON and WL + GT ewes, respectively.

3.3. Plasma fatty acid profiles

In *pre-partum* ewes, supplementation with WL + GT decreased ($P < 0.05$) the 16:0 and increased 16:1 cis-9 compared to the CON ewes and

Table 3
Effect of feeding leaf extracts of walnut, green tea and their combination on plasma metabolites in transition ewes.

Parameters[b]	Experimental treatments[a]					*P-value*[c]		
	CON (n = 12)	WLE (n = 12)	GTE (n = 12)	WL + GT (n = 12)	SEM[d]	Treatment	Time[c]	Treatment × Time
Triglycerides, mg/dl								
pre-partum	29.6	28.9	29.1	28.1	0.23	0.55	0.94	0.18
post-partum	29.6	28.1	28.4	27.8	0.22	0.32	0.15	0.39
Cholesterol, mg/dl								
pre-partum	62.6	62.2	62.5	62.0	0.36	0.99	0.29	0.99
post-partum	60.7	60.3	60.4	60.0	0.28	0.98	0.82	0.38
Albumin, mg/dl								
pre-partum	3.57	3.53	3.51	3.62	0.02	0.84	0.95	0.98
post-partum	3.66	3.61	3.60	3.69	0.02	0.96	0.43	0.87
Blood Urea Nitrogen, mg/dl								
pre-partum	25.0	24.3	24.4	23.2	0.27	0.50	0.51	0.82
post-partum	22.7	22.2	22.6	21.7	0.22	0.74	0.88	0.86
Total Protein, mg/dl								
pre-partum	7.20	7.27	7.45	7.21	0.02	0.67	0.02	0.69
post-partum	7.25	7.32	7.17	7.49	0.04	0.35	0.52	0.36
Creatinine, mmol/li								
pre-partum	0.76	0.75	0.75	0.75	0.21	0.90	0.88	0.51
post-partum	0.75	0.73	0.74	0.76	0.29	0.12	0.63	0.52
Glucose, mg/dl								
pre-partum	64.2	64.5	65.2	63.2	0.29	0.48	0.01	0.85
post-partum	60.2	62.1	61.3	62.7	0.35	0.42	0.85	0.72
Insulin, q/li								
pre-partum	9.05	9.84	9.00	10.2	0.13	0.12	0.01	0.93
post-partum	3.83^{b}	3.53^{b}	3.05^{b}	6.01^{a}	0.09	0.01	0.01	0.40
NEFA, mmol/l								
pre-partum	0.17^{a}	0.14^{ab}	0.12^{ab}	0.11^{b}	0.03	0.03	0.03	0.92
post-partum	0.35^{a}	0.24^{bc}	0.31^{ab}	0.16^{c}	0.04	0.01	0.01	0.01
Liver enzymes parameters								
AST, UI/L						0.002	0.86	0.003
pre-partum	71.6	72.6	73.0	71.8	0.71			
post-partum	74.6^{a}	73.6^{ab}	72.0^{b}	68.4^{c}	0.64			
ALT, UI/L						0.001	0.002	0.170
pre-partum	23.6^{a}	21.6^{ab}	22.6^{ab}	20.9^{b}	0.52			
post-partum	22.8^{a}	21.4^{a}	21.4^{a}	18.5^{b}	0.41			
ALP, UI/L						0.24	0.001	0.313
pre-partum	133.6	132.0	132.7	131.4	0.58			
post-partum	130.6	129.8	130.6	130.7	0.76			

[a] CON: Control; WLE: Walnut leaf extracts; GTE: Green tea leaf extracts; WL + GT = combination of both extracts at 100 mg per kg ewes body weight (BW).
[b] NEFA: non-stratified fatty acids; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase.
[c] The time effect presents different measurements over before and after parturition.
[d] SEM: standard error of the mean.
[e] Data in each row with different superscripts were statistically different ($P \leq 0.05$).

there was no significant difference between WLE, and GTE. Furthermore, 18:2n-6 concentration was highest ($P < 0.05$) in WL + GT and GTE ewes. WL + GT resulted in the highest ($P < 0.05$) EPA, DPA and DHA content in WLE and GTE *versus* CON ewes. The MUFA and PUFA decreased and increased ($P < 0.05$) with WL + GT *versus* CON ewes, respectively. However, there was no significant difference in WLE and GTE ewes *versus* CON. Omega-6 concentration was highest in WL + GT and GTE and WLE did not differ with CON ewes.

In the *post-partum* period, feeding a combination of extracts (WL + GT) resulted in the greatest number of differences from control in reduction of 16:0 and yielding more in 16:4n-3, 18:0. Moreover, feeding WL + GT reduced ($P < 0.05$) 18:1 cis-9 and increased 18:2n-6, EPA, DPA, and DHA contents compared with that in the CON ewes. Feeding extracts influenced ($P < 0.05$) the MUFA, PUFA, Omega-3, and Omega-6. The content of MUFA showed more reduction, while PUFA, Omega-3, and Omega-6 contents showed more yielding by WL + GT than that in the CON (Table 4).

3.4. Milk and colostrum yield, composition and fatty acid profiles

Colostrum production, fat, and protein percentages were not different between experimental groups. However, experimental treatments affected milk yield, and the ewes that received WL + GT (1.25 kg/day) had the greatest ($P < 0.05$) amount of milk produced compared with the CON, WLE, or GTE ewes. Nevertheless, milk fat and milk protein percentages were not affected by the experimental treatments. The WL + GT ewes had the highest ($P < 0.05$) milk lactose concentration compared to the other treatments, but there was no significant difference in milk non-fat solids and milk protein yield between treatments. The ewes that received WL + GT had greater ($P < 0.05$) milk fat yield *versus* CON, but there was no difference between WLE, GTE, and WL + GT (Table 5).

Milk production through the experimental period was graphed against the days in milk in Fig. 4. As shown, on day 5 of the lactation, the ewes reached their lactation peak. The WL + GT ewes showed the highest ($P < 0.05$) lactation peak, followed by the WLE, GTE, and CON groups, respectively. In general, the WL + GT ewes had ($P < 0.05$) the higher ($P < 0.05$) milk production levels than other groups after day 5 of lactation.

Milk of WL + GT ewes resulted in a noteworthy elevation ($P < 0.05$) in the C6:0 content in contrast to the CON ewes, but the contents of C12:0 and C16:0 decreased ($P < 0.05$) with the WL + GT and GTE supplementation. Concentration of C16:1, C18:2n6c, and C18:3n3 was increased ($P < 0.05$) in WLE, GTE, and WL + GT ewes *versus* CON. 20:5n-3 (EPA) and 22:6n-3 (DHA) concentration increased by WL + GT compared with that in the CON. Greatest concentration $P < 0.05$) of short-chain fatty acid (SCFA) and medium-chain fatty acid (MCFA) was observed in WLE and GTE milk ewes. Moreover, the lowest

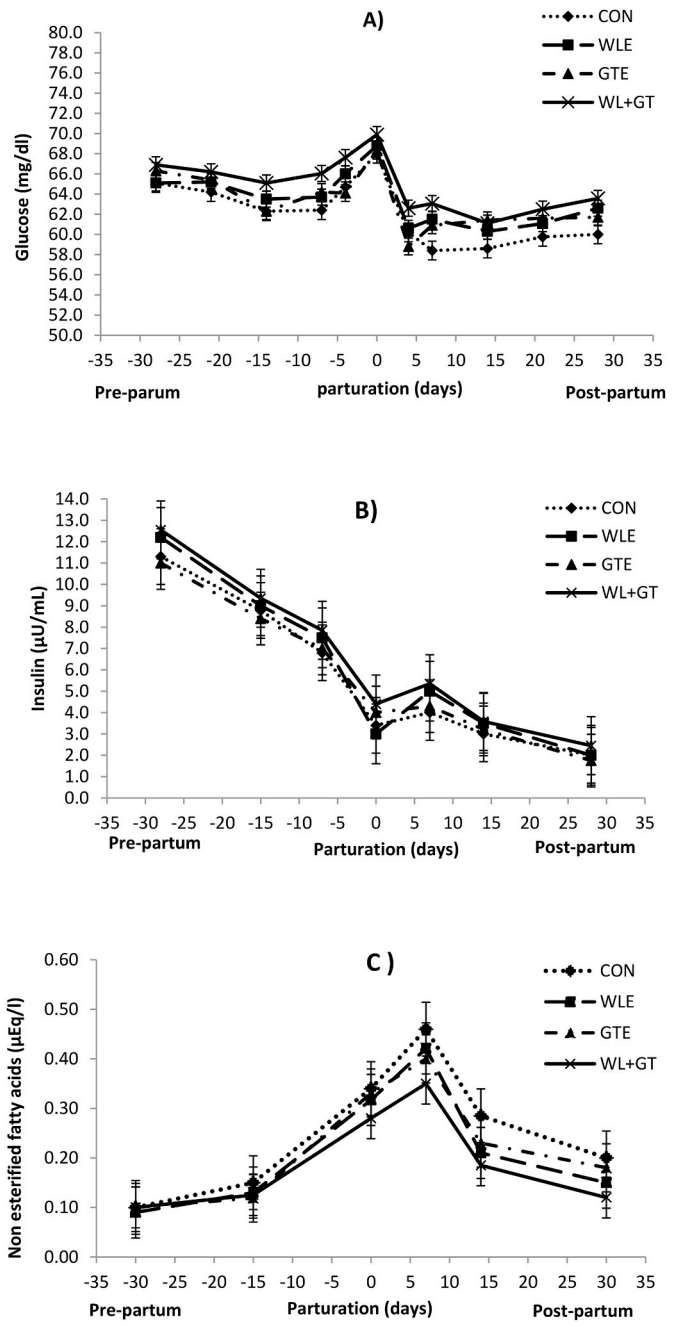

Fig. 3. Effect of feeding ethanolic extracts of WLE and GTE or a combination of both (WL + GT) on plasma glucose (Treatment effect: $P > 0.42$; Time effect: $P > 0.17$ and Treatment * time effect: $P > 0.76$), insulin (Treatment effect: $P < 0.001$; Time effect: $P < 0.004$ and Treatment*time effect: $P > 0.98$) and non-esterified fatty acids (Treatment effect: $P < 0.001$; Time effect: $P < 0.001$ and Treatment*time effect: $P > 0.09$) concentrations during the transition period. Results are given as means of groups (n = 12); Con, WLE, GTE, and WL + GT represent groups of ewes fed 100 mg per kg of extracts by oral gavage. Error bars indicate the standard deviation of the mean values. CON = control; WLE = walnut leaf extracts; GTE = green tea leaf extracts; WL + GT = combination of both of the extracts.

concentration $P < 0.05$) of saturated fatty acids (SFA) and greatest concentration ($P < 0.05$) of unsaturated fatty acids (USFA) were observed in WL + GT and GTE respectively. Supplementation of the ewes with GTE or the combination of both extracts (WL + GT) increased ($P < 0.05$) PUFA content and PUFA/SFA ratio *versus* CON ewes. Furthermore, milk concentration of trans fatty acids was reduced ($P < 0.05$) in WL + GT ewes (Table 6).

4. Discussion

4.1. Dry matter intake, body weight changes and body condition score

Conflicting reports exist on the impact of plant extracts on performance and dry matter intake in ruminants. Some studies suggest a decrease, while others indicate an increase [31], and there are also instances where no effect was observed [32,33] on dry matter intake. These variations may stem from differences in applied dosage, duration of consumption, processing methods of various plants, or diverse livestock conditions [34]. Chen et al. [35] reported that tea supplementation improved weight gain in Dorset sheep. In the current research, the increase in final weight, dry matter intake (DMI), and body condition score (BCS) may be attributed to improved digestion and nutrient absorption through alterations in the animal's gut microflora [18,19]. These changes enhance nutrient utilization and feed efficiency, as supported by findings on the extract's effects [35]. Bioactive components have been reported to improve feed intake, growth performance, nutrient intake and absorption, immunity, mammary gland development, and increased lactation [5,36]. Feeding extracts had no significant effect on the functional characteristics of the fat-tail. It can be concluded that feeding extracts did not affect the mobilization of energy reserves in fat-tail. According to the results of Olagaray and Bradford [37] the number and position of the phenolic hydroxyl group in the structure of polyphenolic compounds can affect the affinity of related enzymes and transporters, which in turn affects the intestinal absorption and metabolism of nutrients.

4.2. Blood collection and analysis

The WLE and GTE had no significant effect on most of the analyzed plasma biochemical parameters except for non-stratified fatty acids (NEFA) and insulin concentrations. According to Pesántez-Pacheco et al. [38], the level of triglycerides and cholesterol in the plasma increased during the gestation of ewes, and the level of triglycerides in the plasma decreased *post-partum*. Blood triglycerides are considered a source of energy [39], which are utilized when more energy is needed, such as in late gestation. The triglyceride and cholesterol reduction were not significant in the present study because the ewes did not need more energy than those provided by the diet. In the research conducted by Gessner et al. [32], adding GTE tended to reduce plasma triglyceride and cholesterol levels. No significant differences were observed in blood glucose levels across the various treatments. This was consistent with earlier research in dairy cattle [1] and goats [40] but contradicting the findings of Anassori et al. al [41]. in fattening lambs. However, the blood urea nitrogen (BUN) values in those investigations displayed variability. In this study, BUN concentration was not influenced by the extracts. According to Zhong et al. [42], green tea polyphenols in lamb diet suppressed BUN concentration. The BUN level reflects the increased breakdown of proteins, resulting in the generation of excess ruminal NH_3-N. Subsequently, this compound enters the bloodstream and undergoes conversion to urea in the liver [43]. The NEFA originates from adipose tissue, and its presence in the plasma predominantly signifies the mobilization of body fat due to a negative energy balance [44]. The decline in plasma NEFA concentrations noted in ewes supplemented with WL + GT during *pre-* and *post-partum* periods could be attributed to mitigating negative energy balance compared to the CON ewes. The ewes supplemented with WL + GT probably had a reduced delivery of NEFA to the liver compared to the control ewes. Although the precise mechanism through which GTE and WLE regulate negative energy balance remains unclear, it is possible that the combination of extracts reduced stress, potentially linked to enhanced fatty acid utilization in the liver in ewes supplemented with WL + GT [45]. Furthermore, the elevation in serum insulin levels following extract consumption aligns

Table 4
Effect of feeding leaf extracts of walnut, green tea and their combination on plasma fatty acid profile in peripartum ewes.

Parameters[b]	*Pre-partum*						*Post-partum*					
	Experimental treatments[a]						Experimental treatments[a]					
	CON (n = 12)	WLE (n = 12)	GTE (n = 12)	WL + GT (n = 12)	SEM[c]	*P*-value[d]	CON (n = 12)	WLE (n = 12)	GTE (n = 12)	WL + GT (n = 12)	SEM[c]	*P*-value[d]
12:0	0.26	0.31	0.35	0.34	0.035	0.248	0.44	0.29	0.51	0.41	0.043	0.85
14:0	0.35	0.32	0.35	0.38	0.047	0.345	0.49	0.31	0.28	0.40	0.043	0.74
15:0	0.53	0.49	0.55	0.53	0.054	0.741	0.79	0.47	0.30	0.54	0.045	0.21
16:0	18.6^{a}	19.0^{a}	17.6^{ab}	16.1^{b}	0.492	0.035	19.6^{a}	18.1^{b}	19.0^{a}	17.4^{b}	0.357	**0.04**
16:1 cis-9	0.34^{b}	0.44^{ab}	0.48^{a}	0.50^{a}	0.041	0.048	0.66	0.54	0.53	0.54	0.056	0.82
16:2	0.20	0.22	0.26	0.24	0.025	0.473	0.32	0.28	0.38	0.29	0.004	0.15
16:4n-3	0.24	0.26	0.29	0.29	0.087	0.341	0.37^{b}	0.33^{b}	0.55^{a}	0.66^{a}	0.066	**0.02**
17:0	0.54	0.68	0.77	0.74	0.044	0.543	1.02	0.84	0.84	0.80	0.077	0.34
18:0	22.4	21.7	21.4	22.5	0.825	0.374	21.2^{b}	22.8^{ab}	23.2^{a}	24.0^{a}	0.665	**0.04**
18:1 cis-9	13.0	12.8	12.5	11.1	0.660	0.079	14.8^{a}	14.1^{a}	14.6^{a}	12.1^{b}	0.593	**0.04**
trans-11 C18:1	1.10	0.97	1.05	0.94	0.082	0.762	1.05	0.91	0.98	0.86	0.161	0.28
trans-10 C18:1	0.55	0.84	0.78	0.68	0.193	0.814	0.84	1.04	1.19	0.88	0.068	0.43
18:2n-6	35.3^{b}	34.8^{b}	37.2^{a}	38.4^{a}	0.797	0.032	31.9^{b}	31.6^{b}	31.2^{b}	33.3^{a}	0.475	**0.04**
18:3n-3	1.11	1.19	1.24	1.46	0.131	0.135	1.18	1.22	1.02	1.40	0.112	0.32
18:4n-3	0.49	0.54	0.52	0.48	0.156	0.647	0.84	0.55	0.25	0.66	0.134	0.76
20:00	0.45	0.62	0.52	0.54	0.042	0.347	0.46	0.57	0.96	0.51	0.028	0.63
20:1cis	0.31	0.41	0.38	0.40	0.045	0.298	0.53	0.41	0.61	0.39	0.033	0.96
C20:3n-6	0.29	0.33	0.28	0.27	0.077	0.263	0.39	0.31	0.30	0.28	0.058	0.58
20:4n-3	0.15	0.21	0.19	0.31	0.049	0.224	0.15	0.23	0.26	0.28	0.035	0.69
20:4n-6	0.57	0.56	0.53	0.51	0.089	0.291	0.72	0.58	0.64	0.80	0.166	0.57
20:5n-3(EPA)	0.04^{b}	0.05^{ab}	0.06^{a}	0.06^{a}	0.004	0.042	0.10^{b}	0.15^{b}	0.14^{b}	0.21^{a}	0.024	**0.04**
22:5n-3 (DPA)	0.47^{b}	0.56^{a}	0.52^{ab}	0.59^{a}	0.026	0.032	0.40^{b}	0.43^{b}	0.46^{ab}	0.56^{a}	0.033	**0.03**
22:6n-3 (DHA)	0.75^{b}	0.83^{a}	0.83^{a}	0.91^{a}	0.023	0.024	0.55^{b}	0.52^{b}	0.44^{c}	0.74^{a}	0.019	**0.01**
24:0	0.36	0.45	0.36	0.39	0.110	0.299	0.39	0.36	0.41	0.32	0.082	0.24
SFA	43.5	43.6	41.9	41.5	0.985	0.298	44.4	43.7	45.5	44.4	0.894	0.24
MUFA	15.2^{a}	15.7^{a}	15.2^{a}	13.6^{b}	0.425	0.004	17.8^{a}	17.0^{a}	17.9^{a}	14.7^{b}	0.594	**0.01**
PUFA	39.6^{b}	39.8^{b}	41.9^{ab}	43.2^{a}	0.760	0.002	37.0^{b}	36.3^{b}	35.7^{b}	39.2^{a}	0.814	**0.01**
Omega-3	3.25	3.89	3.65	3.78	0.187	0.063	3.59^{b}	3.43^{b}	3.12^{b}	4.52^{a}	0.194	**0.005**
Omega-6	36.4^{b}	35.9^{b}	38.3^{a}	39.4^{a}	0.831	0.003	33.4^{ab}	32.8^{b}	32.5^{b}	34.7^{a}	0.461	**0.001**

[a] CON: Control; WLE: Walnut leaf extracts; GTE: Green tea leaf extracts; WL + GT = combination of both extracts at 100 mg per kg ewes body weight (BW).
[b] EPA: Eicosapentaenoic acid; DPA: Docosapentaenoic acid; DHA: Docosahexaenoic acid; SFA: Saturated Fatty acids; MUFA: Mono unsaturated fatty acids; PUFA: Poly unsaturated fatty acids.
[c] SEM: standard error of the mean.
[d] Data in each row with different superscripts were statistically different ($P \leq 0.05$).

Table 5
Effect of feeding leaf extracts of walnut, green tea and their combination on milk and colostrum production and composition of ewes.

Parameters	Experimental treatments[a]					*P*-value[d]		
	CON (n = 12)	WLE (n = 12)	GTE (n = 12)	WL + GT (n = 12)	SEM[c]	Treatment	Time[b]	Treatment × Time
Colostrum Production, kg	0.37	0.38	0.38	0.44	0.07	0.99	–	–
Colostrum fat, %	11.5	11.6	11.7	11.8	0.16	0.82	–	–
Colostrum protein, %	12.6	12.5	12.5	12.5	0.13	0.94	–	–
Milk								
milk yield, kg/day	0.93^{c}	1.12^{b}	0.92^{c}	1.25^{a}	0.11	0.01	0.86	0.60
Milk fat, %	5.24	5.22	5.33	5.35	0.04	0.37	0.08	0.99
Milk protein, %	6.42	6.50	6.47	6.34	0.10	0.35	0.12	0.97
Milk lactose, %	5.53^{b}	5.53^{b}	5.52^{b}	5.75^{a}	0.08	0.03	**0.05**	0.99
Milk non-fat solids, %	9.63	9.63	9.62	9.61	0.05	0.98	0.98	0.99
Milk fat yield, g/day	48.7^{b}	58.4^{b}	49.0^{b}	66.8^{a}	2.59	0.04	0.38	0.60
Milk protein yield, g/day	59.7	72.8	59.5	79.2	1.25	0.75	0.64	0.98

[a] CON: Control; WLE: Walnut leaf extracts; GTE: Green tea leaf extracts; WL + GT = combination of both extracts at 100 mg per kg ewes body weight (BW).
[b] The time effect presents different measurements over before and after parturition.
[c] SEM: standard error of the mean.
[d] Data in each row with different superscripts were statistically different ($P \leq 0.05$).

with previous research [46], indicating improved insulin secretion and sensitivity in dairy cows with intra-duodenal supplementation of quercetin (a major flavonoid found in mulberry leaves). Additionally, it has been shown that the bioactive components in green tea can enhance the number of pancreatic islets, responsible for insulin secretion [47]. According to the results of extract analysis in our previous study [18,19], benzimidazole and caffeine were the highest concentrations in green tea and walnut leaf extract respectively. Patagar et al. [48] reported that benzimidazole provides regeneration of β cells through activation of peroxisome proliferator-activated receptor gamma (PPARγ) and 5′ adenosine monophosphate-activated protein kinase in the treated group compared to diabetes group.

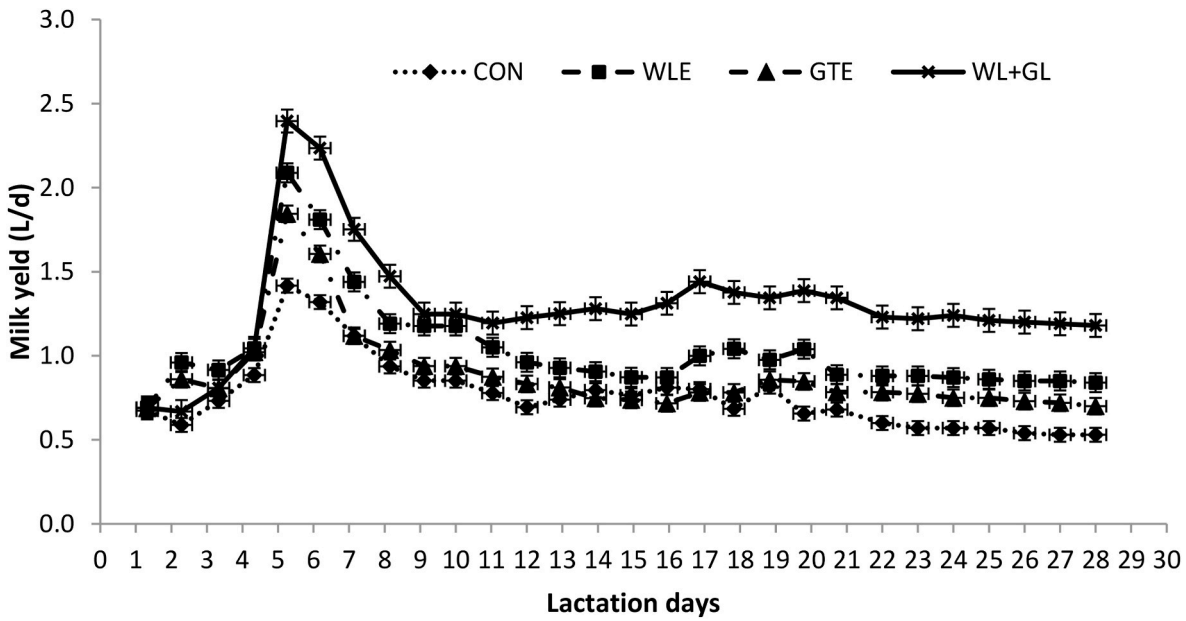

Fig. 4. Effect of WLE and GTE or a combination of both (WL + GT) on daily milk production during lactation of ewes. Results are given as means of groups (n = 12); Con, WLE, GTE, and WL + GT represent groups of ewes fed 100 mg per kg of extracts by oral gavage. Error bars indicate the standard deviation of the mean values. Treatment effect: $P < 0.001$; Time effect: $P < 0.001$ and Treatment * time effect: $P > 0.53$. CON = control; WLE = walnut leaf extracts; GTE = green tea leaf extracts; WL + GT = combination of both of the extracts.

The reduction in AST and ALT activity in our findings is consistent with the results of Ma et al. [49], who noted improved liver performance in cows with hyperketonemia following supplementation with green tea extract. Also, the protective function of green tea polyphenols against cellular damage mediated by reactive oxygen species is well recognized, at least in non-ruminant species [50]. In the present research, the observed reduction in AST (*post-partum*) and ALT (*pre- and post-partum*) activity in ewes treated with both extracts can be attributed to the bioactive components present in GTE (especially benzimidazole) and WLE (especially caffeine). These components demonstrate the ability to mitigate hepatocyte damage in response to changes in hepatocyte permeability caused by free radicals, ultimately leading to an improvement in liver function. Therefore, the absence of elevation in albumin, triglyceride, and cholesterol levels in the ewes receiving supplements might be indicative of an incomplete restoration of liver function to a healthy state. However, additional research is needed to evaluate the exact processes involved in liver metabolism influenced by WLE and GTE.

4.3. Plasma fatty acid profiles

Ruminant adipose tissues are predominantly characterized by abundant levels of 16:0, 18:0, and 18:1 cis-9 fatty acids. In the current study supplementation with GTE and WL + GT significantly lowered the serum 16:0 and 18:1 cis-9 concentrations but also increased 18:2n-6 and long-chain fatty acids in *pre-partum* ewes. 18:3n-3 concentration shows a trend for increase with WL + GT. This could be because of reducing lipolysis and fat mobilization by WL + GT. Anassori et al. [25] reported that garlic oil supplementation in feed-restricted ewes did not alter the levels of 18:3n-3 and 18:0. However, in a study carried out by He et al. [51], the addition of garlic oil to the adipose tissue resulted in a decrease in the levels of 18:1 cis-9, cellular 18:0 18:0, C16:0, and 16:1. They reported that garlic oil enhanced the activity of glycerol-3-phosphate dehydrogenase, leading to the observed changes in fatty acid profiles.

4.4. Milk and colostrum yield, composition and fatty acid profiles

Published information on the effects of plant extracts on sheep milk yield and composition is limited. Various studies explain the influence of using plant extracts on the production and composition of milk. Some studies show an increase in milk production [44], while others report a decline or the absence of a significant impact on milk yield [1,52]. In the present study, greater milk yield in ewes with WL + GT supplementation was observed compared with other groups. Ewes receiving WL + GT supplementation exhibit higher dry matter intake (DMI), indicating that the observed rise in milk yield may be partially linked to an increase in DMI. However, it is possible that an enhanced milk yield was attributed to the expedited recovery of animals from parturition stress and the antioxidant and anti-inflammatory properties exhibited by WLE and GTE, as suggested by Pennacchio [53]. Our findings align with Winkler et al. [44] observation of a rise in milk production in cows supplemented with herbal products incorporating green tea and turmeric extract. Gessner et al. [32] reported that supplementing dairy cattle diets with green tea did not affect milk production while reducing milk fat, lactose yield and concentration. Vizzotto et al. [1] also reported that green tea extract and Oregano plant did not influence the milk production, fat, and lactose content in Jersey cow's milk. They observed that the lack of impact on milk production from oregano and green tea extract when compared to the control group can be attributed to similar nutrient ingestion and moderate levels of milk production. The increase in milk fat content in our study was contrary to those of Gessner et al. [32] while it was in agreement with the results of Winckler et al. [44]. It is possible that bioactive compounds found in WLE and GTE, by acting as inhibitors of enzymes in metabolic pathways in ewes, can potentially increase the activity of enzymes involved in synthesizing fats. This heightened enzymatic activity may contribute to the three distinct pathways: (I) synthesis of fatty acids within the mammary gland through de novo fatty acid synthesis, (II) acquisition of fatty acids from lipoproteins rich in triacylglycerol released by lipoprotein lipase activity, and (III) extraction of fatty acids resulting from the hydrolysis of adipose tissue and subsequent absorption into the mammary gland [32] and resulted in the augmentation of the fat content. Moreover, inflammatory damage to the mammary gland in the transition period enhances the blood-breast barrier permeability, leading to a reduction in milk fat content [54,55].

The WL + GT increased the content of C6:0 and C14:0 and decreased the contents of C12:0. This fatty acids originate from butyrate,

Table 6
Effect of feeding leaf extracts of walnut, green tea and their combination on milk fatty acid profile.

Fatty acid profile, g/100 g FA[b]	Experimental treatments[a]					
	CON (n = 12)	WLE (n = 12)	GTE (n = 12)	WL + GT (n = 12)	SEM[c]	*P*-value[d]
C4:0	0.710	0.648	0.696	0.694	0.03	0.152
C6:0	1.983[b]	2.409[b]	2.313[b]	2.857[a]	0.16	0.040
C8:0	1.937	2.254	2.041	2.028	0.13	0.183
C10:0	8.796	8.892	8.223	8.282	0.32	0.234
C12:0	4.088[a]	4.644[a]	3.327[b]	3.609[b]	0.31	0.040
C14:0	5.684[b]	5.998[a]	6.733[a]	6.852[a]	0.28	0.040
C14:1	1.109	1.122	1.105	1.191	0.21	0.367
C15:0	0.572	0.508	0.628	0.569	0.09	0.241
C16:0	27.34[a]	26.53[a]	26.84[a]	24.28[b]	0.82	0.001
C16:1	0.642[b]	0.895[a]	0.919[a]	0.905[a]	0.07	0.023
C17:0	1.372	1.145	1.422	1.162	0.15	0.750
C18:0	14.25	14.57	13.81	14.27	0.38	0.580
C18:1n9t	0.201	0.161	0.160	0.158	0.03	0.340
C18:1n9c	26.41	26.38	26.17	25.34	0.74	0.080
C18:2n6c	2.416[b]	3.096[a]	3.404[a]	3.556[a]	0.16	0.003
C18:3n6	0.153	0.151	0.220	0.187	0.03	0.260
C18:3n3	0.610[b]	0.748[a]	0.811[a]	0.827[a]	0.04	0.040
Total CLA 1	0.441	0.379	0.421	0.463	0.08	0.370
C20:0	0.364	0.388	0.451	0.355	0.14	0.760
20:5n-3 (EPA)	0.123[b]	0.123[b]	0.150[ab]	0.227[a]	0.03	0.040
22:5n-3 (DPA)	0.086	0.104	0.110	0.138	0.02	0.075
22:6n-3 (DHA)	0.029[b]	0.028[b]	0.040[a]	0.049[a]	0.01	0.036
SCFA	17.51[ab]	18.85[a]	16.60[b]	17.47[ab]	0.43	0.040
MCFA	36.75[ab]	36.02[bc]	37.65[a]	34.96[c]	0.57	0.030
LCFA	45.08	46.13	45.75	45.57	1.17	0.690
SFA	67.10[a]	67.99[a]	66.48[ab]	64.96[b]	0.85	0.040
USFA	32.25[b]	33.02[ab]	33.52[a]	33.04[ab]	0.27	0.040
MUFA	28.39	28.38	28.36	27.59	0.42	0.160
PUFA	3.858[b]	4.629[b]	5.157[a]	5.447[a]	0.25	0.030
PUFA/SFA	0.057[c]	0.068[b]	0.078[a]	0.084[a]	0.002	0.001
MUFA/SFA	0.423	0.417	0.427	0.425	0.03	0.670
n-3	0.848[c]	1.003[b]	1.111[b]	1.241[a]	0.02	0.002
n-6/n-3	2.848	3.085	3.063	2.865	0.04	0.370

[a] CON: Control; WLE: Walnut leaf extracts; GTE: Green tea leaf extracts; WL + GT = combination of both extracts at 100 mg per kg ewes body weight (BW).
[b] SCFA = short-chain fatty acid; MCFA = medium-chain fatty acid; LCFA = long-chain fatty acid; SFA = saturated fatty acids; USFA = unsaturated fatty acids; MUFA = mono unsaturated fatty acids; PUFA= Poly unsaturated fatty acids.
[c] SEM: standard error of the mean.
[d] Data in each row with different superscripts were statistically different ($P \leq 0.05$).

synthesized in the rumen. Potential variations in rumen butyrate synthesis could impact milk C4:0 content. The alterations in fatty acids composition are likely linked to rumen fermentation and, consequently, lipid metabolism. Aguiar et al. [10] observed changes in milk fatty acids composition with the addition of phenolic compounds from propolis-based products. Moreover, Aguiar et al. [10] documented the *in vitro* antimicrobial properties of propolis against rumen bacteria. The ability of propolis to modify both *in vitro* and *in vivo* rumen microbial fermentation has been detailed by Özturk et al. [56]. *In vitro* studies by Sahebi et al. [18] and Ala et al. [19] revealed that WLE and GTE have the ability to modify rumen microbial fermentation, leading to the inhibition of *Butyrivibrio fibrisolvens* and *Ruminococcus albus* growth. The phenolic compounds from WLE and GTE might be influencing these bacterial species during the biohydrogenation process, consequently affecting the content of conjugated linoleic acid isomers in milk fat. Some studies propose that no single type of bacteria in the rumen completes the entire process of biohydrogenation [57]. This makes it difficult to understand how the phenolic compounds from WLE and GTE alter milk fatty acids composition. In the present study, there was a notable increase in C22:6n-3 and C20:5n-3. The increased presence of these fatty acids in the milk is probably linked to the elevated availability of their precursors, C18:2n-6 and C18:3n-6 (refer to Ref. [58], or the increase in accessibility of precursors for fatty acid elongation in the liver (see Szczechowiak et al. [59], and Bryszak et al., [60]. In general, the active compounds in green tea and walnut leaves can potentially enhance milk production and improve milk composition through various mechanisms. Polyphenols (e.g., Catechins) are active compounds in green tea extract reducing oxidative stress in body tissues and improving the function of milk-producing cells. Increasing blood flow to mammary glands, delivering more nutrients to these tissues. Stimulating lipogenesis (fat synthesis) enzymes, which may enhance milk fat content [61]. Active compounds in walnut leaves tannins and flavonoids, enhance feed digestibility and nutrient absorption, leading to a sufficient energy supply for milk production. Reducing potential inflammations in the body, which might otherwise disrupt mammary gland function. Essential fatty acids (e.g., linoleic acid): These compounds can contribute to the synthesis of milk fats. Increasing milk fat content by improving fat metabolism. These compounds support the growth of beneficial gut bacteria, enhancing nutrient absorption and energy availability for milk production [62].

5. Conclusions

Dietary inclusion of WL + GT at 100 mg/kg body weight enhances dry matter intake, body weight, and body condition score while reducing postpartum weight loss. It increases milk yield, lactose, and fat, improves milk fat quality by raising unsaturated fatty acids like C18:3n3, and decreases saturated fatty acids. Additionally, it reduces lipolysis without negatively affecting energy balance during the transition period. The findings of this study highlight the potential for WL + GT supplementation to improve feed efficiency, enhance milk yield and composition, and support better energy balance during the critical transition period. These benefits could translate into improved profitability for livestock producers, especially in systems where the nutritional quality of milk is prioritized for human consumption. Future research should focus on examining the long-term effects of WL + GT supplementation on the health and productivity of ewes and their offspring. Additionally, exploring the impact of varying doses and forms of walnut leaf and green tea leaf extracts could help optimize their use for maximum benefit.

CRediT authorship contribution statement

Maryam Sahebi Ala: Conceptualization, Investigation, Formal analysis, Software, Writing – original draft, Writing – review & editing. **Hamed Khalilvandi-Behroozyar:** Supervision, Project administration, Conceptualization, Validation, Writing – review & editing. **Rasoul Pirmohammadi:** Supervision, Validation. **Ehsan Anassori:** Writing – review & editing. **Marc Drillich:** Writing – review & editing. **Abdelfattah Zeidan Mohamed Salem:** Writing – review & editing. **Pedro Enrique Hernández Ruiz:** Writing – review & editing. **Mona M.M.Y. Elghandour:** Writing – review & editing. **Susanne Kreuzer-Redmer:** Writing – review & editing. **Abdelfattah Zeidan Mohamed Salem:** Writing – original draft, Validation, Conceptualization.

Ethics approval and consent to participate

The research protocol was approved by the university's Animal Care and Use Committee (IACUC Protocol #IR2018011), following the guidelines of the Iranian Council of Animal Care (1995).

Consent for publication

Not applicable.

Availability of data and materials

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

Funding

This study was partially supported by the Iran National Science Foundation under Grant no. 96001700 and by Urmia university PhD program by Grant no. 1413.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to appreciate Dr. Ramin Mazaheri-Khameneh, assistant professor of the department of radiology, Urmia University of Medical Sciences, Iran, for his technical assistance in this study.

Data availability

Data will be made available on request.

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