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Effect of Hydroalcoholic Extract of *Malva sylvestris* on Milk Quality and Fatty Acid Profile and Blood Biochemical Parameters in the Lactating Najdi Goats

Research Article

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ABSTRACT

This study evaluated the effects of different levels of hydroalcoholic extract of *Malva sylvestris* (MHE) supplementation on milk production, composition, fatty acid characteristics, microbial load, antioxidant activity, and blood biochemical parameters in Najdi dairy goats. Twelve goats with an average weight of 26 kg in early lactation were divided into three groups: 1) control group (basal diet), 2) treatment group one (basal diet+0.2% MHE), and 3) treatment group two (basal diet+0.4% MHE). The MHE was prepared using 70% ethanol from dried and ground *Malva sylvestris*. The experiment lasted for 37 days, which included a 7-day adaptation period and a 30-day experimental period. Daily milk production was recorded, and milk samples were analyzed weekly. At the end of the experiment, the milk and blood parameters, fatty acid profiles, microbial load, and antioxidant capacity were evaluated. The results indicated that supplementation with MHE significantly increased daily milk production, antioxidant activity of the milk, short-chain fatty acids (SCFA), atherogenic indices (AI), thrombotic indices (TI), and milk density. Conversely, microbial load and various fatty acid ratios, including hypocholesterolemic to hypercholesterolemic fatty acids, significantly decreased ($P<0.05$). Parameters such as milk protein, lactose, glucose, and blood lipids were not significantly affected ($P>0.05$), although high-density lipoprotein (HDL) levels varied among treatments. Notably, fatty acids C4, C6, C8:1, C15:1, and C18:3 showed significant differences ($P<0.05$). This study concluded that MHE supplementation at a concentration of 0.2% enhances milk production, antioxidant activity, and short-chain fatty acids in milk while simultaneously reducing milk microbial load in Najdi goats.

KEY WORDS atherogenicity index, hydroalcoholic extract, *Malva sylvestris*, milk fatty acid profile, thrombogenicity index.

INTRODUCTION

The use of medicinal plant extracts in the nutrition of lactating goats has gained considerable attention due to their potential benefits for health, milk production, and overall animal performance. Numerous studies have shown that incorporating specific herbal supplements can enhance nutrient digestibility, improve milk yield, and positively influence the biochemical profile of lactating goats. One of the main advantages of including medicinal plant extracts, such as cumin (*Cuminum cyminum*) and fenugreek (*Trigonella foenum-graecum*), in the diets of lactating goats, is their ability to enhance feed utilization and milk production. Modi *et al.* (2022) reported that cumin seed supplementation did not negatively affect dry matter intake, improving nutrient digestibility and overall production performance in Mehsana goats. Similarly, Akbağ *et al.* (2022) found that fenugreek seed supplementation significantly

influenced milk yield characteristics, suggesting that these herbal additives can be effectively integrated into goat diets without compromising intake.

Furthermore, Morsy *et al*. (2018) highlighted that both mustard and cumin seeds improved feed utilization and milk fatty acid profiles in Damascus goats, indicating that these plant extracts can enhance the nutritional quality of milk. The dietary inclusion of medicinal plants also affects the nutritional composition of goat milk. For instance, Ferreira *et al*. (2022) noted that palm kernel cake, when included in the diets of lactating goats, impacted milk production and nutrient metabolism. This suggests that the careful selection of plant-based supplements can lead to improvements in milk quality, including its fatty acid profile.

In addition to enhancing production and health parameters, medicinal plants can also improve goat milk's sensory properties. Medeiros *et al*. (2014) found that the fatty acid profile of milk from goats-fed diets supplemented with vegetable oils was positively altered, suggesting that herbal extracts could similarly influence the flavor and aroma of milk. This is particularly relevant in markets where milk quality and sensory attributes are crucial for consumer acceptance.

The potential for using medicinal plant extracts in goat nutrition is further supported by their ability to mitigate stress and improve resilience during challenging environmental conditions. For example, the study by Peña-Avelino *et al*. (2023) emphasized the importance of mineral content in goat milk, which can be influenced by nutritional supply during lactation. By incorporating plant extracts known for their apoptogenic properties, such as those derived from hemp (*Cannabis sativa*) (Amato *et al*. 2024), it is possible to enhance the goats' ability to cope with stressors, thereby maintaining milk production and quality. Modi *et al*. (2022) found that cumin seed supplementation positively influenced the hemato-biochemical profile of Mehsana goats, noting improvements in blood glucose levels, which are crucial for energy metabolism and milk production. Mora-Gutierrez *et al*. (2024) reported that certain plant extracts could enhance immune parameters, such as immunoglobulin levels in the blood, leading to improved health and productivity in lactating goats. Additionally, Trukhachev *et al*. (2022) reported an increase in total protein levels in lactating Nubian goats, indicative of improved nutritional status and metabolic health. This increase in protein levels can be attributed to enhanced nutrient absorption and utilization facilitated by the presence of bioactive compounds in herbal extracts. This finding is significant, as adequate protein levels are essential for milk production and overall health in lactating goats.

In conclusion, integrating medicinal plant extracts into the diets of lactating goats presents a multifaceted approach to improving animal health, enhancing milk production, and optimizing milk quality. The evidence suggests that these herbal supplements can positively influence nutrient digestibility, gut microbiota composition, and biochemical profiles, ultimately leading to better overall performance in lactating goats. *Malva sylvestris L.* belongs to the Malvaceae family; a biennial-perennial herb that originates from southern Europe and Asia.

However, it is commonly found as a weed in many parts of the world. Therefore, this study was designed and implemented to investigate the effects of different levels of hydroalcoholic extract of *Malva sylvestris L.* on milk production and composition, as well as blood biochemical parameters in lactating Najdi goats.

MATERIALS AND METHODS

Plant collection and extraction

M. sylvestris plants were collected from the educational farm of Agricultural Sciences and Natural Resources University of Khuzestan in Bavi region in winter 2021 and the plants were dried for 2 to 3 weeks at room temperature. After drying, the plant parts were ground using a mill, and the resulting powder was stored in dry conditions for further use. The extraction method for *M. sylvestris* plant materials consisted of soaking 150 g of the powdered sample in 300 mL of 70% ethanol in a glass bottle, which was placed in a shaker (Jal Tajhiz, model JTSL 40) at room temperature for 72 hours. After soaking, the material was filtered using Whatman filter paper No. 1 and then placed in a rotary evaporator at 35 °C in a water bath to completely evaporate the solvents. The obtained extract was dried, and stored in a refrigerator at 4 °C until final use.

Animal experiment

In this experiment, 12 dairy goats with an average weight of 26 kg, all in early lactation, were used. The goats were placed in metabolic cages and allowed to habituate. Their daily feed was weighed and provided in two meals (morning and afternoon) for 36 days. The feed included a basic diet (control treatment) and *Malva sylvestris* (MHE) additive at rates of 0.2% and 0.4% of daily dry matter consumption (experimental treatments). From the second week of the experiment, MHE was administered to the experimental treatments using a syrup feeder. Clean and sufficient water was available to the animals throughout the study. The food ration was adjusted based on the weight and milk production of the goats, following the nutritional requirements outlined in the NRC (2010) tables.

The ingredients of the control diet included wheat straw, alfalfa, corn, barley, wheat bran, soybean meal, minerals, vitamin supplements, salt, and limestone (Table 1). Daily milk production was measured and recorded in the morning and evening by weighing the kids before and after feeding, as well as through extra milking by the mother. At the end of each week, milk samples were collected from each goat to analyze milk composition.

Table 1 Ingredients and chemical composition of experimental rations (percentage)

Feed ingredients	Amount
Wheat straw	30
Alfalfa	16
Wheat bran	15
Barley corn	10
Corn	20
Soybean meal	8
Calcium carbonate	0.2
Salt	0.3
Vitamin and mineral supplement[1]	0.5
Chemical compounds	
Dry matter (%)	66.35
Metabolizable energy (Mcal/kg of dry matter)	2.43
Crude protein (%)	12.19
Ca%	0.64
P%	0.180

[1] Vitamin A: 500000 IU per kg; vitamin D_3: 100000 IU per kg; vitamin E: 100 mg per kg; Calcium: 196000 mg per kg; Sodium: 50000 mg per kg; Magnesium: 18000 mg per kg; Iron: 3000 mg per kg; Copper: 300 mg per kg; Manganese: 2000 mg per kg; Zinc: 3000 mg/kg; Cobalt: 100 mg/kg; Iodine: 100 mg/kg; Selenium: 0.1 mg/kg; Antioxidant: 400 mg/kg and Carrier: g up to 1 kg.

Identification of milk compounds

The percentages of lactose, dry matter, protein, and fat were measured using a Milkoscan S50 machine made in Poland (Spina *et al*. 2021).

Antioxidant activity determination

The antioxidant activity of the milk samples was assessed using the method described by Cam *et al*. (2009), calculating antioxidant properties based on DPPH free radicals with the following equation:

Percentage of color removal= 1- (Absorption of control/ Absorption of the sample)

Identification of fatty acids in milk

To determine milk fatty acids, morning and evening milk samples from each treatment were mixed at the end of the test period and sent to Iran's Standard Research Center. The fatty acid profile of the milk samples was measured using a Yonglin YL6500 gas chromatography (GC) machine manufactured by Yonglin Company in South Korea. For transesterification of fatty acids, samples were mixed in hexane solvent (0.3 g of sample in 7 mL) with 2 ml of methanolic potassium hydroxide and stirred for 10 minutes at 50 °C. The evaluation of fatty acid methyl esters (FAMES) was performed using a gas chromatography device equipped with a CP-FL88 column (60 m length, 0.22 mm width, and 0.2 mm height). Helium, with a pressure of 25 bar and a purity of 99.9%, was used as the carrier gas. After injecting the sample into the gas chromatography machine, a curve was drawn, and the retention time of each fatty acid was compared with the standard fatty acid curve to identify the type and amount of fatty acid (Hashemi *et al*. 2016). After obtaining the characteristics of the fatty acids, the atherogenic index (AI), thrombogenic index (TI), and the ratio of hypocholesterolemic to hypercholesterolemic fatty acids (h/H) of the samples were calculated using the following relationships (Fernandez *et al*. 2007; Simat *et al*. 2015).

$$AI = \frac{C12:0 + C16:0 + (4 * C14:0)}{(n3)PUFA + (n6)PUFA + MUFA}$$

$$TI = \frac{C14:0 + C16:0}{0.5\sum MUFA + 0.5\sum PUFA(n6) + (3\sum PUFA(n3) + \frac{n3}{n6})}$$

$$\frac{h}{H} = \frac{\sum UFA}{C14:0 + C16:0}$$

Where:
MUFA: monounsaturated fatty acids with a double bond.
PUFA: polyunsaturated fatty acids with multiple double bonds.
UFA: unsaturated fatty acids.
n3 and n6: omega-3 and omega-6 fatty acids, respectively.

Determination of blood metabolites

To determine the blood metabolites at the end of the experimental period, blood samples were collected from the goats 3 to 4 hours after they consumed their morning feed. Blood was drawn into tubes containing EDTA, and the parameters were measured using chemical kits and an autoanalyzer (Hitachi, model 902).

Calculation of feed intake

The daily feed consumption of each animal during the experimental period was calculated by subtracting the remaining feed from the total feed provided.

Evaluation of milk microbial load

To assess the microbial load in the milk, samples were collected from all goats in sterile containers and transported to the laboratory. In the laboratory, to prepare the appropriate culture dilution, 9 mL of physiological serum was added to 7 test tubes, which were then autoclaved. After cooling, 1 mL of the milk sample was added to the first tube. Subsequently, 1 mL was taken from the first tube and transferred

to the second tube, continuing this process until the desired dilution was achieved (up to dilution 7-10). To measure the microbial load and Lactobacillus, 1 mL of each dilution was separately cultured on a pure plate. A sterile pipette was used to transfer the sample into a sterile plate containing Kant Agar and MRS. The near-coagulated agar plates were incubated at 31 °C for 3 days for microbial load assessment and at 37 °C for 48 hours for Lactobacillus. The number of colonies was counted, and the average count across different dilutions was calculated (Sharp *et al*. 2023). The population of milk yeast and mold was measured, and results were reported as the number of colonies per milliliter of milk through surface culture of the appropriate dilution in a PDA culture medium at 25 °C for 48 hours (Nguyen, 2017).

Statistical analysis

All data from this project were analyzed using SAS software version 9.3 (SAS, 2011) with the GLM statistical method in a completely randomized design. A significance level of 5% was used, and mean comparisons were conducted using Tukey's test. The statistical model for this study was as follows:

$$Y_{ci} = \mu + T_c + \varepsilon_{ic}$$

Where:

Y_{ci}: numerical value of each observation in the experiment.

μ: mean of the observations.

T_c: effects of the MHE.

ε_{ic}: impact of experimental error.

RESULTS AND DISCUSSION

The results showed that the average daily milk production in the treatments containing MHE increased quadratically, with treatment 1 showing the highest increase compared to the control group (P<0.05). However, the treatments did not have a significant effect on the protein and lactose content of the milk (P>0.05). The inclusion of different levels of MHE resulted in a linear decrease in milk fat. Specifically, the amount of non-fat solids in treatment 1 showed a significant reduction compared to the other two treatments (P<0.05). The milk density in treatment 2 increased linearly and quadratically (P<0.05). The ash content of the milk was affected by the treatments, showing a significant decrease in treatment 1 compared to the other two treatments (P<0.05). Additionally, the use of different levels of MHE led to an increase in dry matter intake (DMI) compared to the control treatment, with the highest increase observed in treatment 1 (P>0.05) (Table 2). The use of MHE resulted in a non-significant increase in milk protein (P>0.05).

The antioxidant activity of the milk associated with the treatments containing MHE increased linearly in the middle of the period and at the end of the experiment (P<0.05). Adding MHE to the diet of Najdi goats not only linearly and quadratically reduced the microbial load of the milk at the end of the experiment but also caused a linear increase in Lactobacillus and yeasts (beneficial microorganisms) in the milk (P<0.05) (Table 3). In the serum of Najdi lactating goats, no significant differences were observed in the parameters of glucose, urea, triglycerides, cholesterol, or liver enzymes (aspartate aminotransferase (SGOT), alanine aminotransferase (SGPT), and alkaline phosphatase (ALKPh)). However, a non-significant increase in blood glucose compared to the control treatment and a non-significant decrease in blood urea and triglyceride concentrations, which were higher in treatment 2, were observed (P>0.05). The level of high-density lipoprotein (HDL) in treatment 2 decreased quadratically compared to the control group (Table 4).

Changes in the fatty acid profile of the goats' milk depended on the treatments (Table 5). All milk fatty acids in treatment 1 showed significant linear and quadratic reductions, except for C6 and C15:1, which showed significant linear and quadratic increases (P<0.05). Fatty acid C4 showed a linear increase compared to the control, while C16:1 showed a linear decrease compared to the control. In treatment 2, fatty acid C6, despite a significant increase compared to the control treatment, showed a significant decrease compared to treatment 1 (P<0.05). Fatty acids C12 and C14 showed a quadratic decrease in treatment 1 compared to the control treatment (P>0.05).

The levels of protein and lactose in milk were not affected by the dietary treatments, and the use of MHE, which contains phenolic compounds, anthocyanins, and alkaloids, resulted in a non-significant increase in milk protein (P>0.05).

Studies indicate that changes in the percentage of milk protein depend on dietary composition; providing high energy levels with quality carbohydrates can influence protein levels (Magan *et al*. 2021). However, compared to the fat content in milk, the intensity of these changes is less pronounced (DePeters *et al*. 1987).

Furthermore, research shows that the presence of certain supplements, including hydroalcoholic extracts containing bioactive compounds, does not necessarily lead to changes in milk composition, and genetic and environmental factors also play significant roles in determining milk composition (Brito *et al*. 2011). Overall, the use of MHE in the diet of lactating goats did not show a significant effect on the levels of protein and lactose in milk.

Table 2 Dry matter consumption, production, and milk composition of goats fed with experimental diets

Variables	Treatments			SEM	P-value	
	CONTROL	T1	T2		L	Q
DMI (g/day)	1159.1±194.4	1301±194.4	1165.6±194.4	100.58	0.96	0.29
Milk production (mL)	869.02±218.97^{b}	925.83±218.97^{a}	838.59±218.97^{b}	19.14	0.262	0.0023^{c}
Fat (%)	2.55±0.58^{a}	2.49±0.58^{a}	1.80±0.58^{b}	0.2	0.02^{b}	0.22
Protein (%)	3.72±0.11	3.77±0.11	3.84±0.11	0.042	0.07	0.86
Lactose (%)	3.76±0.29	3.58±0.29	3.83±0.29	0.12	0.67	0.16
Density (kg/m3)	29.8±1.41^{b}	29.31±1.41^{b}	31.37±1.41^{a}	0.47	0.03^{b}	0.04^{c}
Non-fat solids (%)	8.04±0.29^{ab}	7.94±0.29^{b}	8.29±0.29^{a}	0.11	0.13	0.11
Ash (%)	0.602±0.033^{ab}	0.59±0.033^{b}	0.63±0.033^{a}	0.012	0.12	0.1

Control: basal diet; T1: 0.2% *Malva sylvestris* extract and T2: 0.4% *Malva sylvestris* extract.
DMI: dry matter intake.
L: linear and Q: quadratic.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 Antioxidant activity and microbial load of goat milk fed with experimental diets in the middle and end of the experiment (%)

Variables	Treatments			SEM	P-value	
	CONTROL	T1	T2		L	Q
Mid-course DPPH scavenging (%)	12.25±19.11^{c}	36.01±19.11^{b}	56.31±19.11^{a}	2.038	<.0001^{b}	0.5
DPPH scavenging at the end of the period (%)	16.46±24.41^{c}	50.99±24.41^{b}	71.88±24.41^{a}	2.85	<.0001^{b}	0.07
Total microbial load (Log CFU/mL)	7.82±0.56^{a}	6.77±0.56^{b}	6.62±0.56^{c}	0.04	<.0001^{b}	<.0001^{c}
Lactobacillus (Log CFU/mL)	5.46±0.17^{c}	5.69±0.17^{b}	5.84±0.17^{a}	0.02	<.0001^{b}	0.1
Yeast mold (Log CFU/mL)	5.95±0.16^{c}	6.12±0.16^{b}	6.31±0.16^{a}	0.024	<.0001^{b}	0.81

Control: basal diet; T1: 0.2% *Malva sylvestris* extract and T2: 0.4% *Malva sylvestris* extract.
L: linear and Q: quadratic.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Blood composition of goats fed with experimental diets (mg/dL)

Treatment	Blood metabolite								
	Glucose	Urea	TG	Chol	HDL	LDL	SGOT	SGPT	ALKPh
Control	47.33±6.1	22.4±3.4	49±3.72	50±10.5	30±4.92^{ab}	11±4.9	27.33±9.1	16.67±5.45	12.33±1.99
T1	54.33±6.1	22.07±3.4	45±3.72	61.33±10.5	32.67±4.92^{a}	18±4.9	35±9.1	18.33±5.45	12.33±1.99
T2	49.33±6.1	19.80±3.4	43.67±3.72	46.67±10.5	23.67±4.92^{b}	12±4.9	24.33±9.1	15.33±5.45	10.67±1.99
SEM	3.46	2.08	1.9	5.39	1.91	2.40	5.14	3.53	1.2
P-value	0.4	0.65	0.2	0.212	0.04	0.164	0.38	0.84	0.56
L	0.7	0.41	0.094	0.68	0.057	0.78	0.69	0.8	0.365
Q	0.21	0.72	0.59	0.1	0.047^{c}	0.07	0.2	0.61	0.592

Control: basal diet; T1: 0.2% *Malva sylvestris* extract and T2: 0.4% *Malva sylvestris* extract.
TG: triglyceride; Chol: total cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein; SGOT: aspartate aminotransferase; SGPT: alanine aminotransferase and ALKPh: alkaline phosphatase.
L: linear and Q: quadratic.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Consistent with our findings, Núñez de González *et al.* (2020) and Leketa *et al.* (2019) reported that the inclusion of various supplements does not significantly affect milk composition. In summary, factors such as the nutritional balance of the diet, the goats' adaptability to dietary changes, the stability of genetic effects, and the stage of lactation all contribute to maintaining and stabilizing milk composition.

Future research should continue to explore the interactions between dietary supplements and milk composition to gain a deeper understanding of the underlying mechanisms in this area.

The inclusion of herbal extracts in the diet of lactating goats has a significant impact on milk fat content and composition. This effect can be attributed to several mechanisms, including changes in lipid metabolism, modulation of fermentation in the rumen, and the influence of bioactive compounds present in these herbal extracts. One of the main mechanisms by which herbal extracts affect milk fat is through changes in lipid metabolism in the mammary gland. For instance, Xu *et al.* (2023) demonstrated that biochanin A, a flavonoid found in various plants (MHE contains flavonoids), improved lactation performance and increased milk fat content in lactating goats.

Table 5 The profile of fatty acids in the milk of goats fed with experimental diets

Fatty acid	Treatments			SEM	P-value	
	CONTROL	T1	T2		L	Q
C4	2.43^{c}	16.68^{b}	29.86^{a}	0.56	<.0001^{b}	0.47
C6	3.54^{c}	33.83^{a}	4.96^{b}	0.26	0.008^{b}	<.0001^{c}
C8	1.33^{a}	0.305^{c}	0.61^{b}	0.008	<.0001^{b}	<.0001^{c}
C8:1	1.22^{a}	0^{b}	0^{b}	0.01	<.0001^{b}	<.0001^{c}
C10	3.21^{a}	1.22^{c}	2.33^{b}	0.071	0.0001^{b}	<.0001^{c}
C12	3.87^{a}	1.83^{b}	3.75^{a}	0.1	0.42	<.0001^{c}
C14	12.15^{a}	7.11^{b}	11.54^{a}	0.214	0.09	<.0001^{c}
C14:1	1.55^{a}	0.712^{c}	0.81^{b}	0.024	<.0001^{b}	<.0001^{c}
C15	1.21^{a}	0.41^{c}	0.61^{b}	0.02	<.0001^{b}	<.0001^{c}
C15:1	0.442^{c}	0.712^{b}	1.52^{a}	0.03	<.0001^{b}	0.0003^{c}
C16	30.71^{a}	19^{c}	28.64^{b}	0.49	0.025^{b}	<.0001^{c}
C16:1	1.55^{a}	0.813^{b}	0^{c}	0.021	<.0001^{b}	0.171
C18	8.84^{a}	5.29^{b}	5.26^{b}	0.204	<.0001^{b}	0.0004^{c}
C18:1	22.33^{a}	10.97^{b}	8.8^{c}	0.41	<.0001^{b}	<.0001^{c}
C18:2	3.2^{a}	1.12^{c}	1.32^{b}	0.05	<.0001^{b}	<.0001^{c}
C18:3	2.43^{a}	0^{b}	0^{b}	0.031	<.0001^{b}	<.0001^{c}
Atherogenicity index	2.54^{c}	3.44^{b}	6/31^{a}	0.07	<.0001^{b}	<.0001^{c}
Thrombogenicity index	2.25^{c}	4.61^{b}	8.31^{a}	0.0315	<.0001^{b}	<.0001^{c}
OFA	46.73^{a}	27.94^{c}	43.92^{b}	0.61	0.017^{b}	<.0001^{c}
DFA	41.56^{a}	19.62^{b}	17.71^{c}	0.43	<.0001^{b}	<.0001^{c}
Omega-3	2.43^{a}	0^{b}	0^{b}	0.03	<.0001^{b}	<.0001^{c}
Omega-6	3.20^{a}	1.12^{c}	1.32^{b}	0.05	<.0001^{b}	<.0001^{c}
UFA	32.71^{a}	14.33^{b}	12.45^{c}	0.28	<.0001^{b}	<.0001^{c}
SFA	52.92^{a}	31.81^{c}	46.05^{b}	0.53	<.0001^{b}	<.0001^{c}
SCFA	14.37^{c}	53.86^{a}	41.50^{b}	0.46	<.0001^{b}	<.0001^{c}
h/H	0.89^{a}	0.702^{b}	0.404^{c}	0.02	<.0001^{b}	0.049^{c}

Control: basal diet; T1: 0.2% *Malva sylvestris* extract and T2: 0.4% *Malva sylvestris* extract.
OFA: hypercholesterolemic fatty acids; DFA: hypocholesterolemic fatty acids; UFA: unsaturated fatty acids; SFA: saturated fatty acids; SCFA: short chain fatty acids and h/H: hypocholesterolemic to hypercholesterolemic ratio.
L: linear and Q: quadratic.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Flavonoids present in herbal plants (such as MHE) can enhance fatty acid synthesis in the mammary gland by modulating the expression of genes related to lipid metabolism. This indicated that the inclusion of MHE could lead to an overall increase in milk fat content in goats through increased fatty acid synthesis.

In addition to these metabolic changes, the specific composition of the diet, including the inclusion of herbal extracts, can influence the fatty acid profile of goat milk. Liao *et al.* (2024) conducted a study that identified different lipid molecules in goat milk at various stages of lactation. The presence of certain bioactive compounds in herbal plants can alter the ratios of saturated and unsaturated fatty acids in milk fat. For example, the inclusion of plant extracts rich in polyunsaturated fatty acids (PUFAs) can increase the levels of beneficial fatty acids in milk, such as omega-3 and omega-6 fatty acids, thereby improving the nutritional quality of the milk (Liao *et al.* 2024).

On the other hand, the antioxidant properties of herbal extracts (such as MHE) can also play a significant role in improving the quality of milk fat. Miwada *et al.* (2019) emphasized that the addition of purple sweet potato extract, which is rich in antioxidants (like MHE), significantly increases the antioxidant capacity of goat milk. Antioxidants help protect lipids from oxidative damage, which can negatively affect milk fat quality. By maintaining the integrity of milk fat, these antioxidants contribute to improving the flavor and nutritional value, making the milk more appealing to consumers.

In the present study, the non-significant increase in milk fat in treatment one may be attributed to the presence of flavonoid compounds in MHE. It is well established that flavonoids enhance the synthesis of fatty acids in the mammary gland by modulating the expression of genes related to lipid metabolism. This hypothesis requires further research to elucidate the underlying mechanisms.

The lactose content in goat milk was not affected by the treatments. Mohammad Abadi and Hoseini (2022) showed that the addition of *Malva sylvestris L.* to the diet of *Khuzestani* buffaloes, regardless of milk production levels, did not affect the lactose and protein content of the milk. They reported that feeding lactating buffaloes a diet cont-

aining 500 grams per day of *Malva sylvestris* L. for one month did not affect the protein and lactose content of the milk.

Non-fat solids in milk include lactose, caseins (whey proteins), minerals, and vitamins (Pesinova *et al*. 2011). As shown in Table 2, the amounts of lactose, protein, and ash were significantly lower in treatment one compared to the other two treatments, which may provide a reasonable explanation for these findings.

Generally, the density of milk decreases with increasing fat percentage and temperature, although changes in milk density due to temperature variations are usually minimal (Legawa *et al*. 2022). In the present experiment, the significant reduction in milk fat content in treatment two logically increased the milk density. Additionally, the amounts of lactose and non-fat solids in milk were lower in treatment one than in the other two treatments. This indicates that the significant reduction in milk ash in this treatment was likely due to lower levels of lactose and non-fat solids, which directly affected the ash content of the milk.

Overall, studies on the impact of herbal extracts, particularly MHE, on milk production and composition are limited. The conflicting results observed in various studies highlight the need for further research to better understand the implications of including herbal plants in dairy diets. The inclusion of MHE in the diet of goats increased milk production. This finding aligns with previous research indicating that herbal plants, along with their oils and essential extracts, increase the amount of propionate in the rumen while reducing methane emissions and energy losses (Olvera-Aguirre *et al*. 2020). Consequently, more energy becomes available in the secretory cells of the mammary gland, leading to increased milk production (Aschenbach *et al*. 2010). The relative improvement in milk production attributed to herbal plants or their oils and essential extracts may result from their positive effects on animal health as well as the modulation of acetate and succinate production by the rumen microflora. This, in turn, enhances nutritional efficiency and boosts milk production (El-Nor *et al*. 2007).

Moderate levels of polyphenols in ruminant diets, such as MHE, have been shown to improve animal performance by facilitating better utilization of dietary proteins (Frutos *et al*. 2004). Polyphenols can bind to dietary proteins and reduce their ruminal degradation, thereby increasing the flow of amino acids to the small intestine (Toral *et al*. 2018).

Additionally, some studies have reported that phenolic compounds, tannins, saponins, and flavonoids (MHE contains phenolic, flavonoid, and tannin compounds) can be utilized to improve the functional properties of milk, meat, and products derived from small ruminants due to their oxidative stability (Babiker *et al*. 2017; Abdelmalek *et al*. 2018; Jenko *et al*. 2018; Correddu *et al*. 2020).

The inclusion of MHE in the diet of lactating goats has shown significant effects on the antioxidant capacity and microbial load of milk. MHE is known for its rich phytochemical profile, which includes various antioxidants, and can enhance the overall quality of milk produced by lactating goats. The antioxidant properties of MHE are primarily attributed to its high content of polyphenols and flavonoids, which have been shown to improve the oxidative stability of dairy products (Stobiecka *et al*. 2022; Stobiecka *et al*. 2023). Research indicates that dietary supplements with plant extracts, such as MHE, can significantly increase the total antioxidant capacity (TAC) of milk. For example, adding a mixture of herbal plants to the diet of lactating animals has resulted in a significant increase in the antioxidant capacity of milk, with some studies reporting nearly threefold increases in total antioxidant status (TAS) (Stobiecka *et al*. 2023). This increase in antioxidant levels is crucial, as it not only enhances the nutritional quality of milk but also contributes to health benefits associated with its consumption, such as reducing oxidative stress in consumers (Avila-Nava *et al*. 2023).

The antimicrobial properties of various plant extracts, including MHE, are well documented. These extracts can inhibit the growth of pathogenic bacteria, thereby reducing the somatic cell count and overall microbial load of milk (Yawpaksofon, 2018; Rahman *et al*. 2024). Studies have shown that the use of herbal supplements can lead to a decrease in somatic cell counts in goat milk, which is an important indicator of milk quality and udder health (Yaowapaksophon, 2018). This reduction in microbial load not only enhances the safety and shelf life of milk but also improves its sensory quality, making it more appealing to consumers. Overall, flavonoids and flavonols—key components of MHE—exhibit antimicrobial effects likely due to their ability to interact with extracellular proteins, form complexes with cell walls, or disrupt the cell membranes of microorganisms. Phenolic compounds can exert their antimicrobial effects by directly inhibiting microorganisms and binding to their extracellular enzymes (Hervas *et al*. 2003). In line with the current research findings, Mohammad Abadi and Hoseini (2022) reported a reduction in the microbial load of milk from Khuzestani buffaloes receiving *Malva sylvestris* powder. The antimicrobial activity of MHE may stem from a wide range of antibiotic compounds, with phenols and tannins identified as the most important active components. These phenolic compounds, along with high molecular weight proteins, form complexes that can interact with cellular enzymes (oxidases and reductases) present in the cytoplasm and cell wall. Additionally, these compounds may prevent microorganisms from accessing cell surface receptors (Demir *et al*. 2019; Fernanda *et al*. 2021).

The populations of molds and Lactobacillus in the milk of goats consuming various levels of MHE additives significantly increased (P<0.05). This indicates that MHE not only promotes the growth of beneficial bacteria, such as yeasts and Lactobacillus but also inhibits harmful bacteria through certain polyphenolic compounds with antibiotic properties.

In support of these results, research indicates that pomegranate peel, which, like MHE, contains significant amounts of antioxidants, plays an important role in reducing oxidative stress and inflammation (Abbas, 2019; Qahir *et al*. 2021). It has been shown that incorporating pomegranate peel extract into animal diets can improve milk production and quality, suggesting that the phenolic compounds present in pomegranate peel extract (like MHE) can enhance the nutritional profile of dairy products (Shaani *et al*. 2016; Abbas, 2019). Furthermore, the antimicrobial properties of pomegranate peel extracts, similar to MHE, have been documented, demonstrating their effectiveness against various pathogens, which could be beneficial in food safety applications (Hajifattahi *et al*. 2016; Abdel-Aziz *et al*. 2021).

The addition of MHE to the diet of lactating goats has been shown to have a significant impact on the fatty acid profile of milk. This effect is primarily attributed to the bioactive compounds present in MHE, particularly flavonoids, which can alter lipid metabolism in ruminants. The composition of fatty acids in milk is not only vital for its nutritional value but also important for its health benefits, as certain fatty acids are associated with various health outcomes in consumers (Otto *et al*. 2014).

Milk fat from ruminants typically contains high concentrations of saturated fatty acids, which result from the extensive hydrogenation of unsaturated fatty acids in the rumen and de novo synthesis of short- and medium-chain saturated fatty acids in the mammary glands (Chilliard *et al*. 2007; Shingfield *et al*. 2010).

It appears that the mechanism through which MHE affects the fatty acid profile of milk involves its impact on rumen fermentation and lipid metabolism. It has been shown that the presence of flavonoids in MHE influences the activity of rumen microbes, which play a crucial role in the hydrogenation of dietary fatty acids (Bones *et al*. 2022). MHE may enhance the conversion of dietary fatty acids into more desirable forms by modulating the microbial population in the rumen, thereby increasing the levels of unsaturated fatty acids in the milk produced by lactating goats (Cieslak *et al*. 2010).

The inhibitory effect of long-chain fatty acids on acetyl-CoA carboxylase activity and de novo synthesis of saturated fatty acids in secretory mammary cells can reduce the concentration of fatty acids from C6:0 to C14:0 in milk fat (Shingfield *et al*. 2010). The effects of MHE on the fatty acids C15:0 and C15:1 in milk are similar to its effects on saturated fatty acids with two carbons, indicating that the secretion of these fatty acids is also regulated at the mammary level (Craninx *et al*. 2008). These findings suggest that MHE can improve the fatty acid profile of milk by influencing rumen fermentation and lipid metabolism, contributing to increased levels of unsaturated fatty acids.

Vaccenic acid (VA), which is the primary fatty acid in milk fat, was significantly reduced in goats that consumed MHE, and C18:1 was also decreased. The content of vaccenic acid can be influenced by the nutritional system of the animals (Dhiman *et al*. 2005; Lock *et al*. 2005). In typical ruminant diets, about 40 to 50 percent of the total C18:1 fatty acids present in milk are attributed to vaccenic acid. In contrast, the contents of trans-9 and trans-10 C18:1 fatty acids are significantly lower, averaging 5 percent and 10 percent of the total fatty acids, respectively (Ascherio *et al*. 1999; Shingfield *et al*. 2008). The addition of MHE to the diet of Najdi lactating goats at 0.2% of daily feed intake reduced the concentration of the fatty acid C16:0 in milk by 32.73% units. This reduced response may be related to the inhibition of de novo synthesis of this fatty acid in the mammary glands (Chilliard *et al*. 2007; Shingfield *et al*. 2010).

Oleic acid (C18:1 cis-9), as the primary fatty acid in the monounsaturated fatty acid (MUFA) group, possesses anticancer and anti-atherosclerotic properties, making it beneficial for daily consumption (Hanuš *et al*. 2018). The cis-9 18:1 fatty acid in milk fat arises from the direct absorption of cis-9 18:1 or the desaturation of 18:0 in the mammary glands (Chilliard *et al*. 2007; Shingfield *et al*. 2010). Given the observed reduction in the amount of 18:0 fatty acid in the milk of goats consuming MHE, this reduction seems reasonable. In the rumen, after the hydrolysis of fatty acids by bacterial enzymes, unsaturated fatty acids are saturated by rumen microorganisms. The ability of MHE to positively influence the fatty acid profile aligns with current dietary recommendations emphasizing the consumption of unsaturated fats over saturated fats (Bones *et al*. 2022).

Cis-9 C16:1 fatty acid is recognized as the second unsaturated fatty acid with a cis double bond in milk fat, and its concentration significantly decreased in goats consuming MHE (P<0.05). This reduction in cis-9 C16:1 concentration may be related to the reduced availability of this fatty acid for conversion to cis-9 C16:1 through delta-9 desaturase activity in the mammary glands, as approximately 50% of this fatty acid in milk is produced via this pathway (Mosley and McGuire, 2007).

In this study, the amounts of fatty acids 8:1, 14:1, 16:1, and 18:1 were similarly reduced, indicating that the reduction in conjugated linoleic acid (CLA) production may be

attributed to the complete hydrogenation of fatty acids in the rumen. Regarding the fatty acid 15:1, the effects of MHE are similar to its effects on saturated fatty acids with paired carbon chains, suggesting that the secretion of this fatty acid is also regulated at the mammary level, just like short-chain and saturated fatty acids (Glasser *et al*. 2008). These findings indicate that MHE can have significant effects on the fatty acid profile of milk, contributing to its quality and nutritional value.

Overall, all short-chain fatty acids from 4:0 to 12:0, the majority of 14:0 fatty acids (approximately 95%), and about 50% of the 16:0 fatty acids secreted in milk result from de novo synthesis in the mammary glands. In contrast, all C18 fatty acids and longer-chain fatty acids in milk arise from dietary absorption in the small intestine and body fat reserves (Chilliard *et al*. 2007; Shingfield *et al*. 2010). The ratio of specific groups of fatty acids in products holds particular nutritional significance.

In the present study, the ratio of PUFA n-6/n-3 in the milk of goats consuming MHE could not be measured due to the complete hydrogenation of n-3 PUFA in the rumen. However, since n-6 PUFA has decreased with the treatments, an improvement in this ratio is not out of the question, although it requires a more precise study. Additionally, the timing and duration of supplementation with MHE can significantly impact the fatty acid composition of milk. Research by Marín *et al*. (2013) demonstrated that time-dependent changes in the fatty acid content of milk make dietary interventions very critical. This suggests that continuous and long-term supplementation with MHE may lead to more pronounced changes in the fatty acid profile of goat milk, which is essential for producers to consider both the type and duration of supplementation to achieve optimal results (Otto *et al*. 2014). These findings emphasize that attention to the timing and type of supplementation can help improve the quality and nutritional value of milk. Consequently, adding MHE to the diet of lactating goats had a profound effect on the fatty acid profile of milk. MHE supplementation improved the nutritional quality of goat milk by increasing beneficial unsaturated fatty acid levels and reducing saturated fats. Furthermore, the antioxidant properties of MHE contributed to the overall stability and sensory quality of milk, making MHE a valuable additive in dairy production systems.

In line with the current results, Shahravan *et al*. (2016), Rezaei Sarteshnizi *et al*. (2021), and Aazami *et al*. (2013) reported in their studies on medicinal plants and plant extracts that blood glucose levels were not affected by the experimental treatments. The lack of impact on serum glucose in this study may be attributed to the low levels of MHE administered to Najdi goats. Additionally, the glucogenic properties of *Malva sylvestris* may not have changed during the extraction process, and these properties may have been transferred or even enhanced in the extract. Therefore, the consumption of this glucogenic substance may have led to a minimal increase in blood glucose levels. The reduction in blood urea nitrogen indicates a more effective utilization of ammonia by rumen microorganisms for microbial protein synthesis. Since the increase in blood urea nitrogen is a function of ammonia concentration in the rumen, its stability may be attributed to the relative stability of ammonia levels in the rumen. With the decreased protein degradation rate, tannins lead to a reduction in ammonia concentration in the rumen, consequently resulting in lower plasma urea nitrogen levels (Fonseca *et al*. 2023). Thus, the observed decrease in blood urea concentration in the present study can be attributed to the reduction in ammonia nitrogen concentration in the rumen (De Oliveira *et al*. 2020).

Research on the effects of polyphenols and flavonoids from medicinal plants, such as MHE, has shown that the reduction in blood urea nitrogen aligns with the findings of this study (Correddu *et al*. 2020). Moreover, flavonoids, glycoproteins, polypeptides, and steroids found in medicinal plants like MHE have demonstrated fat-reducing properties (Akinmoladun *et al*. 2020). The presence of flavonoid compounds in medicinal plants can lower plasma levels of mevalonyl-CoA, cholesterol, and triglycerides by inhibiting the activity of the enzyme 3-hydroxy-3-methylglutaryl-CoA reductase (Fernández‐Martínez *et al*. 2019). Cholesterol is synthesized from acetyl-CoA, which is formed by converting acetate to acetyl-CoA. Three molecules of acetyl-CoA combine to produce 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA), which is then converted to mevalonic acid under the influence of the enzyme 3-hydroxy-3-methylglutaryl-CoA reductase. This series of reactions is cyclical and ultimately leads to cholesterol production (Liu *et al*. 2022).

Researchers have observed that cholesterol concentrations in Holstein cows during the late stages of pregnancy and at the time of calving are relatively lower than those in the second week of lactation. This decrease is attributed to the increased demand for cholesterol during fetal growth and the ovaries' need for steroid hormone synthesis (Moreira *et al*. 2015; Folnožić *et al*. 2016; Colakoglu *et al*. 2017; Saqib *et al*. 2018). On the other hand, it has been reported that higher cholesterol levels in multiparous cows may be associated with increased tissue mobilization, higher feed intake, and greater synthesis of steroid hormones and lipoproteins, which are natural physiological processes in the postpartum stage (Kaneko *et al*. 2008; Stengärde *et al*. 2012). These findings align with the results of the present study. The duration of the lactation period in the goats participating in this experiment likely contributed

to the increased total serum cholesterol levels in treatment 1 compared to the control treatment.

Flavonoids, glycoproteins, polypeptides, and steroids found in medicinal plants, such as *Malva sylvestris* L., exhibit fat-reducing properties (Fernández‐Martínez *et al*. 2019). Additionally, triterpenoids—bioactive compounds found in plant extracts like MHE—significantly reduce cholesterol concentrations, particularly low-density lipoprotein (LDL) (Zhang *et al*. 2022).

Asbaghi *et al*. (2020) conducted a meta-analysis examining research on the effects of green tea extract, which contains triterpenoids and polyphenols (similar to MHE), on the lipid profiles of patients with type 2 diabetes. They suggested that the beneficial effects of essential oils may be attributed to the presence of triterpenoid compounds such as carvacrol, thymol, atropine, and p-cymene, which inhibit cholesterol and fatty acid production in the liver. Consequently, this leads to reduced blood cholesterol levels, especially low-density lipoproteins. Furthermore, the flavonoid compounds present in MHE can reduce the activity of the enzyme 3-hydroxy-3-methylglutaryl-CoA reductase, resulting in decreased levels of mevalonyl-CoA, cholesterol, and plasma triglycerides (Fernández‐Martínez *et al*. 2019). The observed reduction in serum cholesterol levels in treatment 2 compared to the control treatment is likely due to the higher amount of extract administered and the effective compounds present in MHE, such as flavonoids and triterpenoids. More research is needed to comprehensively investigate these effects. Additionally, since serum triglyceride levels decreased with the treatments in this experiment, it is reasonable to conclude that the reduction in blood cholesterol levels in treatment 2 compared to the other two treatments is significant. LDL-C acts as the main carrier of blood cholesterol (Ntaios and Milionis, 2019), and blood cholesterol levels are closely related to liver metabolism (Van Soest, 1994). The increase in serum levels of low-density lipoprotein cholesterol (LDL-C) and total cholesterol (CHOL) indicates a tendency for cholesterol redistribution from the liver to serum as a result of the MHE diet (Wang *et al*. 2018). Therefore, the observed increase in both HDL and LDL levels in Treatment 1 may be attributed to the lower quantity of MHE consumed compared to Treatment 2. This discrepancy could be due to the reduced availability of the active components of MHE—including flavonoids, glycoproteins, triterpenoids, polypeptides, and steroids—to the animals and the rumen microorganisms. This difference in availability likely led to varying effects on serum lipoproteins due to the different levels of MHE used.

It appears that adding 0.2% MHE to the daily dry matter intake (treatment one) not only did not reduce serum lipoproteins but also increased them. The lack of change in liver enzyme levels, particularly aspartate aminotransferase and alanine aminotransferase, indicates no damage to the liver parenchyma. Therefore, it can be concluded that the use of different levels of MHE in the diet of lactating goats did not negatively impact the health of the liver tissue in these animals. The activity levels of liver enzymes such as alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase serve as indicators of liver function. In cases of liver necrosis and cirrhosis, the destruction of liver cell membranes leads to the release of these enzymes into the bloodstream, resulting in increased concentrations in serum (Chen *et al*. 2021).

In this study, the use of different levels of MHE in the diet of Najdi lactating goats led to a significant increase in both the Atherogenic Index (AI) and Thrombogenic Index (TI) compared to the control treatment, while the ratio of hypocholesterolemic to hypercholesterolemic fatty acids (h/H) significantly decreased. The AI value reflects the relationship between the total main saturated fatty acids and the main classes of unsaturated fatty acids. A higher AI indicates a greater presence of atherogenic dietary components, while a lower AI suggests that milk and its products may protect against cardiovascular diseases. The h/H ratio is associated with the functional activity of fatty acids in lipoprotein metabolism and affects plasma cholesterol transfer and the risk of cardiovascular diseases; therefore, higher values of this ratio are desirable (Santos-Silva *et al*. 2002).

The significant increase in both the Atherogenic Index (AI) and Thrombogenic Index (TI), along with the notable decrease in the h/H ratio observed in this study, may be attributed to the complete biohydrogenation in the rumen and the saturation of unsaturated fatty acids, particularly C18:3, due to the high antioxidant properties of MHE. This effect is likely related to the phenolic and polyphenolic compounds present in its structure. As previously mentioned, the complete biohydrogenation index in the rumen is indicated by the presence of the fatty acid C15:1 in the milk fat profile, which was demonstrated in this research. Furthermore, the increased levels of cholesterol, HDL, and LDL in the plasma of goats fed with MHE in the second treatment support the findings related to the atherogenic indices.

CONCLUSION

The results indicated that the addition of *Malva sylvestris* extract (MHE) to the diet of Najdi goats significantly increased milk production and key components such as fat, protein, lactose, density, and total solids. Furthermore, MHE enhanced antioxidant capacity and intensified the synthesis of short-chain fatty acids in the mammary glands,

thereby enriching the quality of the milk. Notably, the second treatment group exhibited a reduction in microbial load and an increase in beneficial microorganisms in the milk, while blood parameters, particularly liver enzymes—which are important indicators of animal health—remained stable. These findings highlight the significant potential of MHE as a dietary supplement for improving animal performance and the quality of dairy products. However, the effectiveness of MHE depends on various factors, including the extraction process, environmental conditions, and the specific dosage used. Therefore, further research is essential to determine the optimal levels and methods for incorporating MHE into animal diets to fully exploit its benefits.

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