Effect of breed, age and pasture periods on milk yield, milk components, somatic cell counts and lipid profiles of raw milk from morkaraman and tushin sheeps

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SUMMARY

The aim of this study was to investigate the effects of breed, age of sheep, and pasture periods on the milk yield, milk components, somatic cell counts (SCC), and lipid profiles of forty-one Morkaraman and forty-seven Tushin sheep maintained in a traditional grazing system of natural pastures. Milk samples were collected for milk component, SCC, and lipid profiles analysis at the beginning of the pasture, mid-pasture, and the end of the pasture. Data were analyzed as repeated measurements by two-way ANOVA in the General Linear Model (GLM) procedure. The mathematical model included the effects of breed, pasture periods, ages, and all interactions. Bivariate correlations procedure was used to determine the relationship between milk components and lipid profiles. It was determined that breed was not significant for lactation traits. Milk yield, daily average milk yield ($p<0.05$), and lactation length ($p<0.01$) were significantly affected by the age of sheep. Solids non-fat (SNF), density, and protein were affected by breed. It has been determined that the milk of Tushin sheep has higher SNF, density, and protein values than milk obtained from Morkaraman sheep. The pasture periods are significant in milk composition. The SNF, density, protein, lactose, and ash increased with the passing of the season. The effect of breed, pasture period, and age on SCC was found to be insignificant. Except for cholesterol (COL), the milk lipid profiles such as triacylglycerol (TAG), free fatty acid (FFA), monoacylglycerol (MON), and phospholipid (PL) in studied breeds were similar. The pasture periods had a highly significant effect on lipid profile. The interactions breed x age, breed x pasture period, and pasture period x age were not significant for milk components and lipid profiles except for breed x age interaction in the milk fat. Consequently, the pasture is more effective on the milk composition and lipid profiles rather than the breed and age of sheep.

KEY WORDS

Milk yield; Milk component; SCC; Milk lipid profile; Sheep.

INTRODUCTION

The significance of milk and dairy products in relation to human dietary requirements in terms of protein, vitamins, minerals, and energy is indisputable. Milk, the first source of nutrition after birth, has been a valuable and desirable product for centuries. Sheep (and goats) have been milked for thousands of years, probably before any other animal. However, cow milk is the most commonly consumed milk, dominating the world's milk production with 683 million tons in 2018[1,2]. Milk production in Turkey in 2018 was around 22.9 million tons[3].The sheep population of the eastern and northeastern parts of Turkey consists predominantly of the Morkaraman breed. The Tushin breed, which is bred in the same region, is unfortunately in danger of extinction due to uncontrolled crossbreeding. These fat-tailed breeds are thought to have evolved through natural selection under harsh environmental conditions[4,5].

There are some differences in physical-chemical characteristics between sheep and cow milk. Sheep milk is much richer, containing higher amounts of fat, solids, and protein. Sheep milk contains almost twice as much protein as goat and cow milk[1]. The smaller fat globules size of sheep milk (65% globules less than 3 μm) is advantageous for digestibility and a more efficient lipid metabolism compared to cow's milk fat. Dairy products obtained from sheep have gained market size due to their quality, high yield, and nutritional value of the product[6,7]. The components of sheep milk differ over time and among animals depending on several factors, such as genotype, age, parity, season, environmental temperature, udder structure lambing interval, type of birth, and nutrition[7].

Milk quality is very important in controlling the quality of dairy products produced from it. Therefore the somatic cell count (SCC) and the microbiological quality of the milk are correlated. SCC is a known indicator of sheep health and milk quality. Only 10% of the somatic cells are mammary gland cells (eosinophils, epithelial cells), secreted with milk as a result of cellular turnover. The remaining 90% of the somatic cells are blood cells such as macrophages, leukocytes, and lymphocytes[8].

Milk lipids are important because of their high nutritional value as well as their effect on physicochemical, sensory, and production properties of dairy products. Triacylglycerols (TAG), whose composition is very complex, make up the largest group (about 98%), including a large number of esterified fatty acids. The lipid composition of sheep milk, along with TAG, consists of other simple lipids such as diacylglycerols, monoacylglycerols, cholesterol esters, complex lipids such as phos-

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pholipids, and liposoluble compounds such as sterols, cholesterol esters, hydrocarbons[9,10,11,12].

Studies conducted to determine the milk characteristics of sheep breeds such as Morkaraman and Tushin sheep are quite limited. Therefore, the aim of this study was to evaluate the influence of the pasture periods on milk yield, milk components, SCC, and milk lipid profiles of these indigenous non-dairy sheep breeds.

MATERIALS AND METHODS

Animals and management

This research was carried out at the Food and Livestock Application and Research Center of Ataturk University in accordance with the permit of the Local Ethics Committee (E.2100058743, 2021/1/1) on Animal Experiment considering the project (BAP-2016/262) supported by Atatürk University in Erzurum, Turkey. Forty-one Morkaraman sheep and forty-seven Tushin sheep between 2nd and 6th ages were used in order to determine milking performance. The sheep were held under semi-extensive conditions. Morkaraman and Tushin sheep were mated in October and December with their own breed ram. The sheep were fed 2 kg/day (± 0.2 kg) of roughage and 300 g/day (± 50 g) of a concentrated mixture (13% crude protein, 8.7% crude cellulose, 2% crude fat, 9% crude ash and 2400 Kcal ME per kg) during the pregnancy period. After the pregnancy of sheep, the births occurred between April and May. The lambs stayed with the ewes for two months. After two months, the lambs were weaned and sheep left to graze on pasture. Lambs and sheep were grazed on the pasture as separate herds. In the pasture, primary forage plants were *Festuca ovina*, *Bromus tomentalus*, *Medicago* sp., *Koleleria cristata* and *Onobrychis sp*. Feeding and management practices were applied equally to all sheep. Fresh water and mineralized salt blocks were freely available all time. About two months old, the lambs were weaned and all of the flock started to pasture by June. In order to determine lactation milk yield characteristics, control milking was started after weaning lambs and continued until the end of pasture in 15-day intervals. The lactation length was determined by drying off the sheep which were below 50 g milk yield per day. According to the regional climate conditions, the pasture period was terminated taking account of the vegetation period. Lactation milk yields from each control milking during the lactation period were calculated according to the Trapeze II (Dutch Method) method used by International Registry Commission (ICAR).[13].

Samples measurement and data collection

Milk samples were collected for milk components and some chemical compositions, SCC, and lipid profiles analysis at the beginning of the pasture (Begin-pasture), mid-pasture, and the end of the pasture (End-pasture). The milk samples were analyzed to determine protein, solids non-fat (SNF), fat, density, lactose, ash, and freezing point (FP) using LactoScan (MCC). The somatic cell counts (SCC) were determined by using DeLaval (DCC). Lipid profiles such as triacylglycerol (TAG), phospholipid (PL), free fatty acid (FFA), monoacylglycerol (MON), and cholesterol (COL) were analyzed by high-performance thin-layer chromatography (HPTLC). Five hundred µl of *n*-hexane: isopropanol 3:2 (v/v) mixture was added to 1000 µl of milk. After, the tubes were centrifuged at 5000 g for 5 min at 40 °C, and the upper phase was used for HPTLC analysis[14]. All measurements were performed in duplicate.

Statistical analysis

Data on milk yield, milk compositions, SCC, and lipid profiles were analyzed as repeated measurements by two-way ANOVA in the General Linear Model (GLM) of the SPSS 20.0 statistical package (SPSS Inc., Chicago, IL). Data sets were analyzed for normality using the Shapiro-Wilk test and for homogeneity of variance using Levene's test. The mathematical model included the effects of breed, pasture periods, ages, and all interactions. Bivariate correlations procedure was used in order to determine the relationship between milk components and lipid profiles (SPSS 20.0). Duncan's multiple comparison test was applied in order to find the statistical differences between significant means. Significant differences were considered at $p<0.05$.

RESULTS AND DISCUSSION

Lactation traits

The lactation traits are given in Table 1. It has been determined that breed is not significant for lactation traits. Milk yield, daily average milk yield ($p<0.05$), and lactation length ($p<0.01$)

Table 1 - The lactation traits of Morkaraman and Tushin sheep.

		Milk yield (kg)	Lactation length (day)	Daily average milk yield (kg)
	n	Mean±S.E.	Mean±S.E.	Mean±S.E.
Breed (B)		0.355	0.059	0.902
Morkaraman	41	42.65±3.38	125.22±4.86	0.329±0.019
Tushin	47	42.20±3.15	133.64±4.52	0.301±0.017
Age (A)		0.042	0.028	0.041
2	24	$31.57±4.22^{c}$	$114.80±6.06^{b}$	$0.256±0.023^{b}$
3	13	$43.26±5.73^{abc}$	$139.18±8.23^{a}$	$0.298±0.031^{ab}$
4	16	$32.45±5.19^{bc}$	$106.94±7.46^{b}$	$0.303±0.028^{ab}$
5	16	$52.99±5.19^{a}$	$142.56±7.46^{a}$	$0.366±0.028^{a}$
≥ 6	19	$51.86±5.37^{ab}$	$143.68±7.71^{a}$	$0.352±0.029^{ab}$
B X A		0.244	0.599	0.081

*: $p<0.05$; **: $p<0.01$; ns: non-significant; a,b,c: Means with different superscript in the same column are significantly different ($p<0.05$).

were significantly affected by the age of sheep. The results were in agreement with previous reports[15]. The lactation milk yield in the Morkaraman and Tushin breeds was found lower than the results of some authors[4,5,16]. The interaction breed x age is not significant for lactation traits.

Milk composition and SCC

Sheep's milk is very important in Turkey because it has high in fat and protein and is mainly used in quality cheeses and yogurts. Sheep milk has a lower freezing point than cow's milk, while viscosity, specific gravity, titratable acidity, and refractive index are higher. Additionally, sheep milk contains higher total solids and more nutrient contents than cow's milk[7,8]. The composition of sheep milk depends on the genotype, environmental conditions, sanitary characteristics, social and economic environment, and farming methods (feeding and milking). These factors contributed directly or indirectly to the synthesis of milk components by secretory cells of the mammary gland[8]. Table 2 shows the milk composition and some physical characteristics of Morkaraman and Tushin sheep milk during the different pasture periods. SNF, density, and protein were affected by breed. There were no significant differences between the breeds in fat, lactose, ash, freezing point, and SCC. It has been determined that the milk of Tushin sheep has higher SNF, density and protein values than milk obtained from Morkaraman sheep. Similar to this study, breed-related changes in SNF, density, and protein were observed in the previous studies[13]. The interactions breed x age, breed x pasture period and pasture period x age are not significant for any of the traits studied except for breed x age interaction in the milk fat. The effect of breed by age interaction on milk fat in Morkaraman and Tuj sheep is presented in Figure 1.

The milk composition was affected by the pasture periods. All parameters examined, at the beginning of the pasture were lower than at the end of the pasture except for fat. The SNF, density, protein, lactose, and ash increased as the season progressed. Results of this study show that seasonal changes had a significant impact on milk composition. Similar to this study, the lower levels of fat and protein in early lactation milk was reported by some authors[17,18,19,20].

As the season progressed, milk fat decreased because of increased milk production during the pasture period. Total fat content in the sheep milk was higher at the end of the pasture than at the beginning of the pasture[17,20]. Fat is the component that can easily vary in milk composition, and diet is one of the factors that can promote this change. While the fat decreases with the addition of concentrated fed in the diet, pasture feeding with high relation fiber/energy increases the fat in milk[7]. The fluctuations were observed during the grazing season in terms of milk composition and they were similar to Tsiplakou et al.[19], however, they differed from Pavic et al.[18]. The higher SNF, fat, protein, density, ash, and lactose of the end of pasture were, in part, the more attributed to the concentration effect caused by the decline in milk yield[21]. This is most likely due to poorer pastures resulting in lower solids milk. The density was significantly different for the breed and pasture periods. The density of sheep milk increased until the end of the pasture period, reaching a density of 1030 kg/m^3. In the present study, the density was lower than that observed by some researchers[20]. The freezing point was affected by pasture periods. The freez-

Table 2 - The chemical composition and some physical characteristics of Morkaraman and Tushin sheep milk.

Components	N	Fat %	SNF %	Density (kg/m^3)	Protein %	Lactose %	Ash %	FP °C	SCC (cell*1000/ml)
	88	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.
Breed (B)		0.065	0.008	0.001	0.001	0.078	0.100	0.080	0.799
Morkaraman	41	9.27±0.25	9.40±0.10	1028.55±0.38	2.98±0.04	5.51±0.05	0.92±0.01	-0.735±0.007	252.1±85.9
Tushin	47	8.84±0.25	9.92±0.10	1030.70±0.38	3.19±0.04	5.77±0.05	0.96±0.01	-0.747±0.008	357.8±87.8
Pasture period (PP)		0.004	0.004	0.05	0.043	0.005	0.004	0.002	0.142
Begin-pasture	88	9.25±0.28^a	9.42±0.11^b	1028.64±0.43^b	2.99±0.04^b	5.52±0.06^b	0.92±0.01^b	-0.727±0.009^a	254.3±96.3
Mid-pasture	88	8.03±0.27^b	9.56±0.10^b	1029.90±0.41^a	3.10±0.04^a	5.54±0.06^b	0.93±0.01^b	-0.728±0.008^a	368.4±112.1
End-pasture	88	9.89±0.36^a	9.99±0.14^a	1030.34±0.55^a	3.17±0.05^a	5.86±0.08^a	0.98±0.01^a	-0.768±0.011^b	292.2±108.4
Age (A)		0.947	0.466	0.572	0.672	0.444	0.409	0.696	0.461
2	24	8.97±0.39	9.87±0.15	1 030.46±0.59	3.17±0.06	5.75±0.08	0.96±0.01	-0.741±0.012	386.7±165.6
3	13	8.86±0.40	9.50±0.15	1 029.19±0.61	3.04±0.06	5.55±0.09	0.93±0.01	-0.733±0.012	201.8±91.9
4	16	8.97±0.43	9.51±0.16	1 029.16±0.65	3.04±0.06	5.56±0.09	0.93±0.01	-0.734±0.013	908.6±313.5
5	16	9.17±0.38	9.68±0.15	1 029.65±0.57	3.09±0.06	5.66±0.08	0.94±0.01	-0.745±0.011	437.2±242.3
≥6	19	9.32±0.35	9.71±0.13	1 029.67±0.53	3.09±0.05	5.68±0.07	0.95±0.01	-0.753±0.011	408.2±196.6
B X PP		0.630	0.091	0.084	0.086	0.214	0.153	0.539	0.661
B X A		0.019	0.190	0.438	0.434	0.075	0.085	0.073	0.412
PP X A		0.109	0.570	0.908	0.762	0.179	0.287	0.299	0.689
B X PP X A		0.506	0.550	0.256	0.395	0.640	0.590	0.702	0.732

*: p<0.05; **: p<0.01; ns: non-significant; ^{a,b}: Means with different superscript in the same column are significantly different (p<0.05).
SNF: Solids non-fat, FP: Freezing point, SCC:Somatic cell counts.

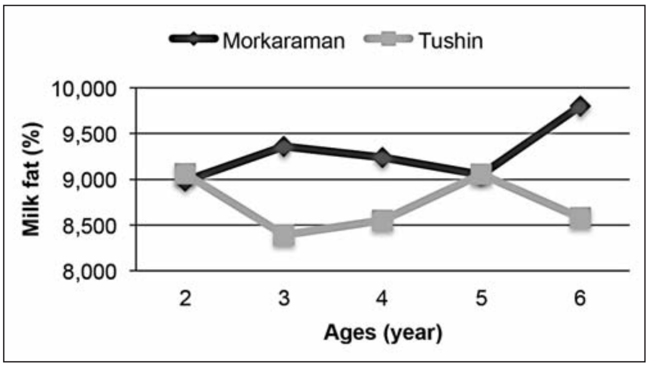

Figure 1 - The effects of breed by age interaction on milk fat in Morkaraman and Tushin sheep.

ing point value of sheep milk at the end of the pasture period was significantly higher ($P < 0.01$) in relation to the beginning and mid-pasture periods. While the lowest freezing point value was obtained at the beginning of the pasture, this value increased towards the end of the pasture period and reached its highest level. The freezing point of sheep milk compared to cow's milk is lower due to a higher content of solids-non-fat[18]. The freezing point values obtained from the present study were found to be higher than the values reported by Assenat[22], and lower than the values reported by Mayer and Fiechter[23].
Small ruminants, such as sheep and goats, present higher SCC base levels than those observed in healthy cows[7]. In our study, the effect of breed, pasture period, and age on SCC was found to be insignificant.

Lipid profile

The lipid profiles of milk obtained from Morkaraman and Tushin sheep are presented in Table 3. Studies on the lipid profile in sheep milk are quite insufficient. Lipids are present in sheep and goat milk as characteristically abundant globules in sizes less than 3.5 µm. This is advantageous for digestibility and a more efficient lipid metabolism compared with cow's milk fat[6,7]. Lipids in milk are mainly composed of TAG, FFA, COL, MON, and PL. TAG has a complex composition and is mainly found in the core of globules[14]. In this study, TAG constitutes the biggest group (nearly 90%). It was observed that there was no effect of breed and age on TAG in the sheep milk, while the effect of the pasture period on this ratio was found to be very significant ($p<0.01$). The TAG was found lower than reported by Miller et al.[24] for the sheep milk.
It was determined that the amount of COL obtained from the milk of Tushin was significantly higher ($p <0.05$) compared to those obtained from the milk of Morkaraman. It was observed that the pasture period was a very significant effect ($p <0.01$) on the COL. While the COL ratio in sheep milk was high at the beginning of the pasture, it decreased significantly in the mid and end-pasture. PL is associated with the milk fat globule membrane and accounts for 0.2–1 % of all milk lipids. PL plays an important role in the milk fat globule membrane due to its highly surface-active properties. According to some studies, PL may be involved in anti-cancer and anticholesterolemic functions[25]. The PL is reported to have tumor-suppressing properties by influencing cell proliferation and are highly bioactive compounds with bacteriostatic and cholesterol-lowering properties[16]. The level of PL in milk of both breeds was similar. The differences obtained were not due to the influence of the breed and age but to the influence of different pasture periods. The effect of the pasture period on the PL was found to be significant ($p < 0.05$). The PL was found lower than the values reported by some researchers[26]. While there was no effect of breed and age on FFA and MON, it was observed that there was a significant ($p < 0.01$) effect of the pasture period on these variables. FFA and COL values were found higher than the values reported by Miller et al.,[24] but found lower than the values reported by some researchers[25]. Lipids, as well as other milk components, are subjected to variability due to genetics, physiology, and environmental factors (that is, season). As determined in the study, the pasture period has become extremely important because of seasonal and climatic changes that interfere with animal feed nutrients and consequently in animal physiology, affecting milk quality[1,9,10]. Changes in the lipid profile (seasonal variations) in milk during the pasture periods have been reported in many studies[9,10,11,26]. The interaction breed x age, breed x pasture period and pasture period x age are not significant for lipid profiles.
The phenotypic correlation between milk components and lipid profiles is shown in Table 4. As observed in Table 4, TAG had a significant ($p <0.01$) positive correlation with density and protein while it had a significant negative correlation ($p <0.05$) with the fat ratio. A very significant ($p <0.01$) positive correlation was observed between FFA and fat ratio. While COL had a very significant ($p <0.01$) negative correlation with fat, SNF, density, protein, lactose, and ash ratios, a very significant ($p <0.01$) positive correlation was found between the freezing point and COL. MON had a significant negative correlation ($p<0.05$) with density and protein ratios.

CONCLUSION

There were no significant differences were observed in lactation traits between Morkaraman and Tushin breeds. The effect of the age of sheep on milk yield was observed to be significant. The present study demonstrates that there was not a large variation in milk composition and lipid profile between Morkaraman and Tushin breeds except for SNF, density, protein, and COL. However, the milk composition and lipid profile were affected by pasture periods except for SCC. Most parameters were higher at the end of the pasture than at the beginning of the pasture. On the other hand, there were no differences in the milk composition and lipid profiles between the age of sheep. In this study, the milk fat was generally determined above the literature reports. It is thought that milk production with high-fat content may be more beneficial and profitable for producers and consumers due to the rich nutrient content. Literature studies on the detection of SCC in sheep are limited and the studies concentrate on cow milk.
This study has taken a step to fill this gap. Consequently, the pasture is more effective on the milk composition and lipid profiles rather than breed and age. In order to manipulate the milk composition and lipid profile, which has important and functional properties in terms of human nutrition, it is recommended to perform new studies.

Data availability

The original data are available upon request to corresponding author.

Table 3 - The lipid profiles of Morkaraman and Tushin sheep milk.

Sources	N	Triacylglycerol %	Free fatty acid %	Cholesterol %	Monoacylglycerol %	Phospholipid %
	88	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E
Breed (B)		0.194	0.114	0.043	0.267	0.056
Morkaraman	41	90.40±0.22	2.41±0.09	2.67±0.08	3.41±0.10	1.12±0.05
Tushin	47	90.12±0.23	2.47±0.09	2.94±0.08	3.42±0.10	1.06±0.06
Pasture Period (PP)		0.001	0.001	0.007	0.001	0.013
Beg-pasture	88	88.40±0.27^{b}	3.13±0.12^{a}	3.09±0.10^{a}	4.25±0.12^{a}	1.13±0.07^{a}
Mid-pasture	88	91.49±0.26^{a}	1.94±0.11^{b}	2.74±0.09^{b}	2.88±0.12^{b}	0.96±0.06^{b}
End-Pasture	88	90.89±0.28^{a}	2.25±0.12^{b}	2.58±0.10^{b}	3.10±0.13^{b}	1.18±0.07^{a}
Age (A)		0.193	0.110	0.826	0.195	0.754
2	24	90.15±0.34	2.44±0.15	2.87±0.12	3.41±0.149	1.14±0.08
3	13	89.66±0.36	2.63±0.16	3.04±0.13	3.52±0.159	1.17±0.09
4	16	90.22±0.40	2.43±0.17	2.82±0.14	3.43±0.178	1.11±0.10
5	16	90.70±0.36	2.36±0.15	2.64±0.13	3.28±0.157	1.01±0.09
≥6	19	90.57±0.31	2.35±0.13	2.65±0.11	3.42±0.135	1.01±0.07
B X PP		0.391	0.248	0.734	0.126	0.567
B X A		0.191	0.467	0.167	0.448	0.375
PP X A		0.243	0.058	0.069	0.666	0.178
B X PP X A		0.588	0.829	0.474	0.904	0.627

*: p<0.05; **: p<0.01; ns: non-significant; a,b: Means with different superscript in the same column are significantly different (p<0.05).

Table 4 - Correlations between lipid profile and milk components.

	TAG	FFA	COL	MON	PL
Fat	-0.230*	0.253**	0.171	0.171	0.011
SNF	0.163	-0.049	-0.332**	-0.143	0.086
Density	0.241**	-0.146	-0.374**	-0.200*	0.075
Protein	0.231**	-0.132	-0.371**	-0.193*	0.077
Lactose	0.103	0.013	-0.280**	-0.098	0.085
Ash	0.113	-0.004	-0.294**	-0.102	0.097
FP	-0.147	0.014	0.340**	0.118	-0.05

*: p<0.05; **: p<0.01; SNF: Solids non-fat, FP: Freezing point.
TAG: Triacyglcerol, FFA: Free fatty acid, COL: Cholesterol, MON: Monoacylglycerol, PL: Phospholipid.

Conflicts of Interest

The authors declare no conflicts of interest.

Ethics approval

All procedures were approved by the Local Ethics Committee (E.2100058743, 2021/1/1) of the Ataturk University.

Author contribution

NE designed the study. The trials were executed by UD while NE supervised. Statistical analysis of the study was done by NE. All authors wrote and approved the manuscript.

Supporting Information

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