



Effect of Birth Type on Production, Characteristics and Somatic Cell Count in Milk of Bafra and Karayaka Ewes

Mehmet Akif Cam^{1,*} and Koray Kirikci²

¹Department of Animal Science, Faculty of Agriculture, Ondokuz Mayıs University, 55270, Samsun, Türkiye

²Department of Animal Science, Faculty of Agriculture, Ahi Evran University, 40100, Kırşehir, Türkiye

ABSTRACT

The aim of this study was to determine the effects of the birth type on milk yield (MY), milk characteristics and milk somatic cell count (SCC) in two Turkish sheep breeds. In this study, 3.0-3.5 years old Karayaka (n=35) and Bafra ewes (n=45) were used. Animals were kept until their milk yield was reduced to 50 g/d. The lactation milk yields (LMY) of single and multiple bearing ewes in Karayaka and Bafra breeds were 99.48±9.66 and 126.01±10.75 kg (P<0.05), and 104.89±8.57 and 165.62±10.79 kg (P<0.05), respectively. Lactation lengths (LL) of single and multiple bearing ewes in Karayaka and Bafra sheep breeds were 114.89±1.95 and 110.92±1.07 and 131.25±1.54 and 131.70±2.99 d (P<0.01), respectively. The log₁₀ SCC in milk in Bafra ewes with single, twin and triplet + quadruplet were 5.21±0.15, 5.14±0.06 and 5.003±0.05 and single and twin bearing for Karayaka ewes were 4.94±0.06 and 5.04±0.07, respectively. The SCC (≥7.5×10⁵) affected MY (P<0.05) and milk components (MC, P<0.01) significantly. With the increase in the number of sucking lambs in the Bafra breed, the SCC in the milk decreased (P<0.05), but not in Karayaka.

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Authors' Contribution

MAC designed and implemented the research and analysed the data. MAC and KK wrote and edited the manuscript.

Key words

Sheep milk, Somatic cell count, Lactation length, Milk composition, Lactation stage, Litter size.

INTRODUCTION

The production of sheep milk and its products derived from sheep and are becoming increasingly important all over the world (Paschino *et al.*, 2019). In countries with higher milk production, the milk is usually derived from native sheep genotypes (Riggio and Portolano, 2015) and the large part of the milk obtained is evaluated by cheese (Pazzola *et al.*, 2018). On the other hand, problems related to hygiene during the period from milking to consumption lead to significant losses in this valuable product (Riggio and Portolano, 2015; Suter *et al.*, 2018).

Although, there are native sheep breeds such as Chios and Awassi (Sakiz and Ivesi) and synthetic crossing genotypes such as Tahirove, Sonmez, Bafra known for their milk yield, the dairy sheep farming is not adequately developed in Turkey. The share of Turkey in total worldwide production of ovine milk is about 12.56%

(FAO, 2019; TUIK, 2018). Some farmers have to raise sheep due to the topographic and environmental characteristics of the area where they are located in Turkey. The local sheep breeds that are adapted to the region they are grown all over the world are low-yield breeds (Riggio and Portolano, 2015).

The average milk yield of Karayaka breed, which is the dominant breed of the Black Sea region, is nearly 40-45 kg besides lamb suckling (Kaymakci, 2016). Bafra sheep breed is newly developed in the Black Sea region and has been registered as a synthetic breed with high milk and reproductive performance (Cam *et al.*, 2018).

Lactation milk yield (LMY), lactation length (LL) and resistance to agents threatening mammary gland health vary according to genotypes, environment and management applications (Caboni *et al.*, 2018; Albenzio *et al.*, 2019). It is important to be able to use alternative sources in animal production and to increase LMY, LL, milk characteristics and somatic cell count (SCC) values which are an important criteria for quality milk production. Therefore, it is suggested that the SCC should be used as a trait for the selection of dairy ruminants, which are less prone to mastitis (Rainard *et al.*, 2018). The breed of animal, care and environmental conditions has an impact on udder health (Riggio and Portolano, 2015). In this context, it is important to determine the resistance of different breeds or genotypes in terms of udder health or milk quality. So,

* Corresponding author: makifcam@omu.edu.tr
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in this study, it was aimed to determine i) milk yields and milk characteristics and ii) the relationship between milk yields, birth type and milk hygiene in Karayaka and Bafra breeds.

MATERIALS AND METHODS

The study was carried out with 45 heads of Bafra and 35 heads of Karayaka ewes, aged 3.0-3.5, at the Agricultural Research and Application Center of Ondokuz, Mayıs University (41.2° N and 36.15° E) in 2018. Ewes were housed with their lambs until they were weaned. Forages (mixed of oat, clover and wheat straw) and fresh water were provided *ad libitum* and 450 g/d of concentrated feed per animal was given. All experimental procedures and animal management were performed according to the Animal Care and Use Guidelines of Local Ethical Committee Ondokuz Mayıs University.

Milk yields were based on control day average milk yields (morning milking in approximately 30-day intervals delivery after days. Control milking was carried out by hand milking at 8:00 in the morning after the lambs were separated from their mothers at 20:00 the previous day. Each single morning milking was multiplied by 2, and daily milk yield was determined (Kaymakci, 2016).

The Dutch method [$LMY = ([Cd] / 2 \times L)$] was used to determine lactation milk yield (LMY, lactation milk yield; Cd, control days, LL, lactation length). The lactation length was calculated by the formula $LL = n \cdot a - (a / 2 - A)$ (n, the number of control milking; a, the interval time between control days; A, days between birth and first control milking).

The milk samples (about 30 ml per ewe) were immediately transferred to laboratory and stored at -20°C until analysis. In the milk samples, the effects of single and twin lamb breastfeeding and breed differences on milk composition were investigated, and routine milk composition (not fat dry matter (NFDm), protein, fat, lactose, freezing point (FP) and density) analyses were performed.

The raw milk samples were heated up to 36-38°C in water bath and component analyses were performed for NFDm, protein, lactose, density and freezing point. Lactostar automatic milk analyser (Funke Gerber, Germany) was used for raw milk tests (Davut and Atasever, 2017). Somatic cell counts (SCCs) were determined from the total 252 samples taken on milk control days. Somatic Cell Counter DCC (DeLaval Group, Sweden) portable test device was used for the determination of SCC in milk samples. During the test, each raw milk sample was transferred to the counting cassette and placed in

the counting device and automatic counting was realized (Kirac, 2014).

All statistical evaluations were performed using the SPSS software package version 24.0 for Windows. All tests were conducted at the $P < 0.05$ level of significance. The values given after the numbers represent the standard error of the mean. In the analysis of the data, the conformity to normal distribution was evaluated with Kolmogorov-Smirnov Z test and the data were determined to be normal distribution ($P > 0.05$). The homogeneity of the variances was evaluated by the Levene test. SCC data revealed that the variances were not homogeneous ($P < 0.05$). SCC related to the homogenization of the data is made by transforming the \log_{10} to homogenize the variances.

The data related to LMY, LL, milk components and the physical characteristics of milk in the evaluation of the breed, the number of lambs at the birth, lactation stage as fixed factors were taken.

General linear method was used to determine the differences between two breeds related to data in LL, LMY, milk component, milk physical characteristics and SCC. The breed, the number of offspring at the birth (litter size, LS) and lactation stage as fixed factors were taken. Results are presented as the mean \pm standard error of the means. LSD and Duncan multiple comparison tests were used to compare the differences between the means.

Correlation analysis was done to reveal relationship between \log_{10} SCC and milk yield and milk components; regardless of breed of ewes (Oravcova *et al.*, 2018).

RESULTS AND DISCUSSION

Bafra ewes had higher LL and more LMY than Karayaka ewes. While there was no triplet or quadruplet births in the Karayaka breed, most of the births were triplets and quadruplets in the Bafra breed, and its effect on milk yield was found to be significant (Table I). The effect of birth type on milk yield was not significant in Karayaka breed. These results are consistent with the findings that multiple birth increases milk yield (Cappio-Borlino *et al.*, 2004) for Bafra sheep, but not for Karayaka sheep in this study. On the other hand it might be explained that this kind of yield differences may change according to the yield aspects of sheep.

In sheep breeding, milk production is important in terms of raising the lambs with less cost and care and making more profit from dairy products. It is an advantage for enterprises that provide a large part of their income from milk or dairy products to have a long lactation period and high milk yield. MY and LL in between different breeds (Othmane *et al.*, 2002; Kaymakci, 2016).

Table I.- Lactation length and lactation milk yields in Bafra and Karayaka sheep breeds.

	Karayaka		Bafra		Sig. level
	LS 1 (n=14)	LS 2 (n=21)	LS 1 (n=10)	MLS 2-4 (n=35)	
LL (days)	114.89±1.95 (102-127)	110.92±1.08 (96-123)	131.25±1.55 (120-137)	131.71±2.99 (89-160)	P<0.003
Overall	112.8±1.53 ^{A*}		132.5±2.34 ^B		
VC (%)	8.514		12.84		
LMY (Kg)	99.48±9.66 (44.50-163.33)	104.89±8.58 (34.91-195.35)	126.01±10.75 (59.22-160.98)	165.62±10.79 (71.91-325.76)	P<0.000
Overall	101.176±6.36 ^A		153.823±11.94 ^B		
VC (%)	42.87		38.96		

*The differences between the means (A, B) indicated in the same line are significant. LL, lactation length; LMY, lactation milk yield; LS, litter size; VC, variation coefficient; n, number of milked ewes.

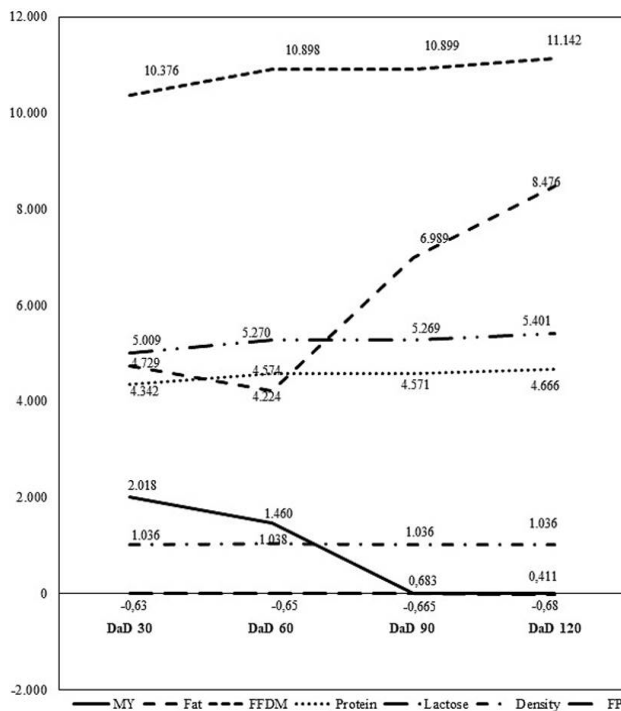


Fig. 1. Milk yield, milk components and milk physical traits according to lactation stages.

In this study, Bafra breed had higher LL (132.5 versus 112.8 days) and LMY (153.823 versus 101.176 kg) than Karayaka breed. These values are similar to findings reported by Isik (2010) and Unal *et al.* (2008) for Bafra breed. The values related to Karayaka breed were found to be higher than some earlier reports (Unal *et al.*, 2008; Kaymakci, 2016). However, it should be taken into consideration that the milk taken by the lamb is included in these values.

It was determined that the variation in LMY was high among individuals in Karayaka and Bafra breeds. This variation can be used as a good opportunity for selection in terms of LMY in these breeds.

In this study, no difference ($P > 0.05$) was found between Bafra and Karayaka breeds in terms of milk fat, NFD, protein and lactose contents in samples taken from hand milking on control days. In the month following the first lactation month, the increase in milk yield and decrease in the percentage of milk components was observed as a natural physiological phenomenon (Cappio-Borlino *et al.*, 2004). It was also determined that there was no difference in milk density and freezing point. The density did not change during all periods, but the freezing point increased slightly from the beginning to the end of lactation, although it was not significant. The fat milk samples in the 2nd month had lower fat contents compared to those in 1st month. In the 3rd and 4th months there was an increase of approximately twice the initial value towards the end of the lactation. The same trend was found for the NFD, protein and lactose contents after the first month (Fig. 1; Tables II, III). It is known that these changes in LMY and milk components during lactation are the result of a healthy physiological production and events of the mammary alveoli (Cappio-Borlino *et al.*, 2004; Rupp and Foucras, 2010). It was found that there was no difference between the milk components with the increase in the number of sucking lambs in both two sheep breeds.

There were significant differences between Bafra and Karayaka sheep breeds in terms of SCC (Table IV, $P < 0.05$). On the other hand, in terms of SCC determined in Karayaka and Bafra ewes' milk, the number of litter size in the Bafra breed had negative effect ($P < 0.01$) on the number of milk somatic cells, whereas it was found to be ineffective in Karayaka (Table IV).

Table II.- Milk components according to lactation stages in Karayaka and Bafra sheep breeds.

Milk component	Lactation stage (No. of ewes)	30 days after delivery		60 days after delivery		90 days after delivery		120 days after delivery		Overall	
		Bafra	Karayaka	Bafra	Karayaka	Bafra	Karayaka	Bafra	Karayaka	Bafra	Karayaka
Fat	1 (10-14)	5.61±1.23	5.44±0.41	4.47±0.37	4.45±0.64	6.26±0.44	6.80±0.46	6.77±0.76	7.84±0.32	5.78±0.41	6.14±0.30
	2 (15-21)	4.49±0.89	4.51±0.21	4.55±0.33	3.78±0.28	7.77±0.55	6.23±0.53	8.51±0.46	7.53±0.40	6.33±0.32	5.51±0.27
	3 (12)	4.78±0.32		4.01±0.24		6.74±0.39		8.94±0.40		6.12±0.29	
	4 (8)	4.07±0.42		3.47±0.65		5.96±0.58		8.54±0.32		5.51±0.63	
	Overall (45-35)	4.73±0.22	4.90±0.23	4.22±0.17	4.08±0.31	6.99±0.28	6.46±0.36	8.47±0.28	7.66±0.26	6.10±0.18	5.77±0.20
	VC (%)	29.15	22.67	25.79	35.60	25.32	27.45	20.38	17.03	37.48	34.53
NFDM	1 (10-14)	9.76±0.72	10.41±0.21	9.88±1.13	10.76±0.24	10.22±0.85	10.09±0.22	10.02±0.91	11.27±0.23	9.99±0.42	10.88±0.12
	2 (15-21)	10.55±0.22	10.39±0.16	10.88±0.29	10.43±0.27	11.27±0.21	10.96±0.36	11.25±0.17	11.06±0.37	10.99±0.12	10.71±0.15
	3 (12)	10.34±0.14		10.09±0.15		10.79±0.31		11.39±0.19		10.90±0.11	
	4 (8)	10.77±0.20		11.40±0.51		10.87±1.17		11.03±0.30		11.02±0.29	
	Overall (45-35)	10.37±0.13	10.39±0.12	10.89±0.19	10.57±0.19	10.90±0.20	11.01±0.22	11.14±0.16	11.15±0.23	10.83±0.09	10.78±0.10
	VC (%)	8.80	5.97	10.97	8.71	11.70	10.11	9.20	10.24	10.35	9.33
Protein	1 (10-14)	4.06±0.33	4.35±0.08	4.16±0.51	4.51±0.10	4.26±0.38	4.65±0.10	4.14±0.42	4.75±0.09	4.16±0.19	4.56±0.05
	2 (15-21)	4.42±0.09	4.34±0.07	4.55±0.13	4.34±0.12	4.75±0.10	4.58±0.16	4.71±0.07	4.63±0.16	4.61±0.05	4.47±0.06
	3 (12)	4.32±0.06		4.66±0.07		4.51±0.14		4.78±0.08		4.59±0.05	
	4 (8)	4.55±0.12		4.82±0.26		4.59±0.52		4.62±0.16		4.65±0.13	
	Overall (45-35)	4.34±0.06	4.34±0.05	4.57±0.08	4.41±0.08	4.57±0.09	4.61±0.10	4.66±0.07	4.68±0.10	4.54±0.04	4.51±0.04
	VC (%)	8.79	5.97	11.91	9.26	12.86	10.56	10.01	10.76	11.26	9.78
Lactose	1 (10-14)	4.69±0.38	5.02±0.09	4.80±0.58	5.19±0.11	4.91±0.43	5.36±0.11	4.79±0.48	5.50±0.11	4.80±0.22	5.27±0.06
	2 (15-21)	5.10±0.11	5.01±0.08	5.25±0.15	5.01±0.14	5.47±0.12	5.29±0.18	5.46±0.09	5.36±0.19	5.32±0.06	5.17±0.08
	3 (12)	4.98±0.07		5.37±0.08		5.20±0.16		5.54±0.09		5.27±0.06	
	4 (8)	5.24±0.13		5.55±0.29		5.29±0.60		5.34±0.17		5.35±0.15	
	Overall (45-35)	5.01±0.07	5.01±0.06	5.27±0.10	5.09±0.09	5.27±0.11	5.32±0.11	5.40±0.08	5.42±0.12	5.23±0.04	5.21±0.05
	VC (%)	8.78	6.02	11.85	9.25	12.78	10.48	9.96	10.78	11.22	9.81

VC, variation coefficient.

Table III.- Milk physical characteristics according to lactation stages in Karayaka and Bafra sheep breeds.

Milk physical characteristics	Lactation stage (No. of ewes)	30 days after delivery		60 days after delivery		90 days after delivery		120 days after delivery		Overall 30-120 days	
		Bafra	Karayaka	Bafra	Karayaka	Bafra	Karayaka	Bafra	Karayaka	Bafra	Karayaka
Density	1 (10-14)	1.03±0.01	1.03±0.01	1.03±0.01	1.03±0.01	1.03±0.01	1.04±0.01	1.03±0.01	1.03±0.01	1.03±0.01	1.03±0.01
	2 (15-21)	1.03±0.01	1.03±0.01	1.04±0.01	1.03±0.01	1.04±0.01	1.03±0.01	1.03±0.01	1.03±0.01	1.03±0.01	1.03±0.01
	3 (12)	1.03±0.01		1.04±0.01		1.03±0.01		1.03±0.01		1.03±0.01	
	4 (8)	1.04±0.01		1.04±0.01		1.03±0.01		1.03±0.01		1.03±0.01	
Overall (45-35)		1.03±0.01	1.03±0.01	1.03±0.01	1.03±0.01	1.03±0.01	1.03±0.01	1.03±0.01	1.03±0.01	1.03±0.01	1.03±0.01
VC (%)		0.35	0.25	0.44	0.34	0.44	0.38	0.36	0.38	0.40	0.34
Freezing point	1 (10-14)	-0.62±0.02	-0.65±0.08	-0.63±0.01	-0.66±0.03	-0.66±0.01	-0.66±0.02	-0.70±0.04	-0.63±0.08	-0.63±0.01	-0.65±0.06
	2 (15-21)	-0.62±0.02	-0.64±0.04	-0.67±0.03	-0.59±0.03	-0.69±0.01	-0.67±0.06	-0.68±0.01	-0.68±0.03	-0.67±0.01	-0.64±0.08
	3 (12)	-0.64±0.02		-0.65±0.02		-0.664±0.01		-0.68±0.01		-0.66±0.01	
	4 (8)	-0.59±0.05		-0.61±0.05		-0.583±0.05		-0.63±0.05		-0.60±0.05	
Overall (45-35)		-0.63±0.01	-0.64±0.09	-0.65±0.01	-0.62±0.07	-0.655±0.01	-0.67±0.05	-0.68±0.01	-0.66±0.06	-0.66±0.01	-0.65±0.04
VC (%)		12.05	9.25	12.22	13.29	8.15	7.36	7.75	9.24	10.46	11.90

VC, variation coefficient.

The SCCs in milk is considered to be an important criterion for quality of the milk (Reyes *et al.*, 2017; Sutera *et al.*, 2018). In this context, the SCC should be lower in raw milk or dairy products (Paape *et al.*, 2007). The number of somatic cells that can be contained in milk in developed countries is limited according to species (400-750 thousand for cows (Rodríguez *et al.*, 2019; Paschino, 2019); 1 million for sheep and goats for USA (Albenzio *et al.*, 2015; Paape *et al.*, 2007); 7.5×10^5 for EU (Pachino *et al.*, 2019; Albenzio *et al.*, 2019)). Milk SCC in Bafra breed was found as higher compared to those found in Karayaka breed (5.13 ± 0.04 vs 5.008 ± 0.04 ; $P < 0.05$). This indicates that there may be differences in terms of SCC between different breeds (Othmane *et al.*, 2002; Caboni *et al.*, 2018). In many studies involving many animal species, conflicting (positive or negative) relationships between milk SCC and MY and milk components are indicated (Tancin *et al.*, 2017; Caboni *et al.*, 2018; Sutera *et al.*, 2018). These contradictory results may be due to the different immunological or genotypic responses (such as increase or decrease plasmin activity, or increase or decrease of any milk component) to the stress factor occurring in the mammary gland on the basis of race or species (Albenzio *et al.*, 2019).

The SCC values determined in milk in our study were found within acceptable limits (Table IV). However, MY and milk components decreased with increasing SCC in milk (Table V). Ewes with SCCs above acceptable limits in their milk were found to have 24% lower milk yield, 11.31% lower milk fat, 8.76% lower NDFM, 10.60% lower protein, 10.48% lower milk lactose, 0.33% lower milk density and 5.66% higher milk freezing point compared to those with low SCC in their milk (Table V). These findings are in conformity with the many findings (Riggio and Portolano, 2015; Caboni *et al.*, 2017; Oravcova *et al.*, 2018; Sutera *et al.*, 2018). In addition, it is expected to investigate the developmental performance of lambs fed with high SCC content and their future milk quality values.

There are also studies showing that milk fat and protein levels increase with increasing number of somatic cells in milk (Oravcova *et al.*, 2018). This is because of the defence mechanism against agents threatening breast health accelerates alveolar physiology (Sutera *et al.*, 2018). This interpretation is thought to be speculative. Because, when compared with normal and high SSC content milk, it was reported that milk components decreased in high SCC milk, whereas milk immunoglobulins and lymphocytes and macrophages were increased (Riggio and Portolano, 2015; Albenzio *et al.*, 2019). It is understood that different defence mechanisms against pathogens that causing breast health disorder may have different results. Therefore, there is no consensus on the relationship between SCC and milk components. There is a negative and weak ($SCC = 292895 -$

36.20 MY, $r = -0.070$; or $\log_{10} \text{SCC} = 5.102 - 0.000017 \text{ LMY}$, $r = -0.026$) relationship amongst MY, milk components and SCC. Similar relationships were found between milk SCC and milk components (Table VI). These results obtained in our study are similar to that of findings of Oravcova

et al. (2018) and Kirikci (2012). On the other hand, there are other researches (Kirikci, 2012; Sutura *et al.*, 2008) indicating that there were strong and negative relationship between milk SCC and milk yield and milk components.

Table IV.- The content of SCC ($\log_{10} \text{SHS}$; $\times 10^5$) in different lactation stages in Karayaka and Bafra sheep breeds.

Breed	Litter size	30 DaD	60 DaD	90 DaD	120 DaD	Overall
Bafra	Single (10)	5.10±0.19	5.25±0.36	5.02±0.42	5.47±0.28	5.21±0.15B
	Twin (15)	5.30±0.13	5.15±0.14	4.95±0.13	5.18±0.12	5.14±0.07AB
	Triplet (12)	5.30±0.14	5.03±0.11	5.11±0.14	5.17±0.11	5.15±0.06AB
	Quadruplet (8)	4.93±0.20	4.62±0.06	4.87±0.04	5.01±0.14	4.86±0.07A
	Overall (45)	5.25±0.09a	5.07±0.08a	5.02±0.09a	5.19±0.08a	5.13±0.04a
Karayaka	Single (14)	4.85±0.16B	5.11±0.13	4.82±0.12	4.99±0.10	4.94±0.06
	Twin (21)	5.09±0.13A	5.06±0.17	4.93±0.15	5.09±0.13	5.04±0.07
	Overall (35)	4.99±0.16b	5.08±0.14b	4.88±0.15b	5.05±0.13b	5.01±0.05b

DaD, days after delivery. Means within the same column with different letters (a, b) differ significantly ($P < 0.05$). Means within the same column and the same breed with different letters (A, B) differ significantly ($P < 0.01$).

Table V.- Effect of SCCS levels on MY and milk components.

MY and MC	SCC Level			Significant level
	High SCC $\geq 7.5 \times 10^5$ cells/ml (n=21)	Medium SCC $7.5 \times 10^5 < X < 1.00 \times 10^4$ cells/ml (n=110)	Low SCC $\leq 1.00 \times 10^4$ cells/ml (n=121)	
TMY (gr)	826.67±142.63A	1094.89±81.87B	1106.40±66.93 B	0.036
Fat	5.37±0.45B	6.50±0.23A	5.61±0.17 AB	0.003
NFDM	9.86±0.41A	10.81±0.09B	10.81±0.08 B	0.000
Protein	4.08±0.18A	4.53±0.04 B	4.61±0.03 B	0.000
Lactose	4.72±0.21A	5.23±0.05B	5.31±0.04 B	0.000
Density	1.03±0.001A	1.03±0.0004B	1.037±0.0004B	0.000
Freezing point	-0.62±0.06	-0.65±0.007	-0.65±0.004B	0.281

TMY, the average of test day milk yields; NFDM, non-fat dry matter; MC, milk component. Means within the same line with different letters (A, B) differ significantly.

Table VI.- Relationships between milk SCC and milk components.

	MSCC	Milk yield	Fat	NFDM	Protein	Lactose	Density
Milk yield	-0.07	1					
Fat	-0.07	-0.43**	1				
NFDM	-0.43**	-0.13*	0.32**	1			
Protein	-0.43**	-0.12	0.31**	0.99**	1		
Lactose	-0.43**	0.12	0.32**	0.99**	0.99**	1	
Density	-0.42**	0.01	0.006	0.94**	0.95**	0.94**	1
Freezing point	0.15*	0.12	-0.17**	-0.29**	-0.25**	-0.26**	-0.25**

*, the relationship is significant ($P < 0.05$); **, very significant $P \leq 0.001$).

CONCLUSIONS

In this study, it was determined that the MY and milk components of the animals were inversely related to the number of SCC in the milk. The number of lambs at birth, the number of sucking lambs at the lactation stage and the lactation phase did not affect SCC. The effect of breed difference on somatic cell count and udder health was found to be important.

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IRB approval

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Funding

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Ethical statement

This study was conducted in accordance with the guidelines for animal research and were certified by the Ethics Committee of Animal Exp. of the University of 19 Mayıs (C. No.: 2018-08).

Statement of conflict of interest

The authors have declared no conflict of interest.

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