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INFLUENCE OF GRAZING PERIOD ON MILK FATTY ACIDS COMPOSITION IN AKKARAMAN EWE AND HAIR GOAT

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ABSTRACT

The present study aims to determine the seasonal change of fatty acid composition in pasture period, especially CLA (Conjugated linoleic acid), in the milk of Akkaraman ewe and Hair goat. During the pasture period, 10 Akkaraman ewe and 10 Hair goats were grazed only on pasture without additional feeding. Individual milk samples were obtained from each animal once a month from May to October. Plant samples were again obtained from the pasture once a month. The pasture period displayed effects on the CLA ratio in total milk fat acid in both species. CLA had the highest level of total milk fat acid in both species in May when pasture had the highest PUFA, especially linoleic acid (C18:2n6) and α-linolenic acid (C18:3n3). SFA: UFA ratio reached the highest level in June for Akkaraman sheep and Hair goat. Although the atherogenic index was at the lowest level in September ($P \leq 0.01$) in Akkaraman sheep milk, Hair goat milk had the lowest atherogenic index in May ($p \leq 0.001$). All Δ^9 desaturase activities in Akkaraman sheep milk (14:1/14, 16:1/16 and 18:1/18) were affected by the pasture period. The findings of the present study indicated a relationship between CLA ratio in the total milk fatty acid and C18:3n3ratio in pasture.

Key words: Sheep milk; milk fatty acid; CLA; pasture fatty acid; goat milk.

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INTRODUCTION

The fat in the composition of milk is a natural nutrient insoluble in water and synthesized in the animal body and mainly composed of carbon, hydrogen and oxygen. Thanks to the gas chromatography, the number of fatty acids in milk fat was found to be nearly 400. CLA denotes the overall conjugated isomers of C18:2n6, which are ω-6 fatty acids essential for the body and contain 18 carbon atoms and two double bonds (Koknaroglu 2007). A total of 28 isomers are reported in CLA. However, only biological activities of t-10, c-12 and c-9, t-11 (rumenic acid) isomers were analyzed in previous studies (Banni 2002). Rumenic acid is the most widespread isomer in food and it is also the most biologically active isomer due to its ability to combine with phospholipids present in the cell membrane (Aydin 2005).

CLA isomers are formed in two different ways in meat and milk of ruminant animals. First is the formation of conjugated octadecadienoic (C18:2, c-9, t-11) acid due to biological hydrogenation of C18:2n6by bacteria in rumen. Secondly, during the biological hydrogenation of C18:3n3, the part of trans-vassenic acid (C18:1, t-11) which does not undergo biological hydrogenation in rumen is absorbed from the gut tissues and transformed into CLA through Δ^9 desaturase enzyme in the mammary tissues (Lock and Garnsworthy 2003).

CLA has attracted attention in the academic community following the emergence of cancer-protective and body fat-reducing effects. Studies on many animal species demonstrated that CLA reduced breast, skin, stomach, colon, liver cancers (Lee *et al.* 2005) and that it prevented vascular stiffness in rabbits and hamsters.

As of early 2018, there are 388 705 head sheep and 46 298 head goats in Erzincan province of Turkey (Anonymous 2019). The dominant indigenous breeds in the region are the Akkaraman sheep and the Hair goat. In Erzincan, the small ruminant is usually milked and used for the production of Erzincan Tulum cheese, which is a geographically certificated product.

Thanks to the technological developments, the higher yields per unit animal in the large cattle farms make it difficult for the local farmers who live in rural areas to continue their husbandry activities. In addition, "fast food" eating habits give harm to the consumption of food obtained from animals, which constitute our genetic sources. In addition, although the Akkaraman breed has an important position in animal husbandry in the region as a component of Erzincan Tulum cheese, the breeders still express dissatisfaction and complaints about their commercial activities. Therefore, as the sheep breeding is losing importance day by day, it can be stated that the perception regarding the existence of CLA, which has important functional effects on human health, in Akkaraman sheep and Hair goat milk will create a positive effect on sheep breeding. Mountain farming

provides high-quality food products due to the peculiar characteristics of the raw materials combined with traditional processing conditions. However, these products and their intrinsic characteristics are not clearly recognized by consumers on the market. Because of all these reasons, in the present study, it is aimed to determine the seasonal changes in fatty acid composition in pasture period, particular in terms of CLA, in the milk of two local breeds grown under extensive conditions. It is thought that the results of the study will reveal the product quality of traditional breeding farmers with local breeds at high altitudes.

MATERIALS AND METHODS

General procedure of the study: The present study was conducted Erzincan province of Turkey (1850 meters above sea level, 39.91" N and 41.22" E). In the present study, 10 Akkaraman and 10 hair goats were used in 3 parities. Lambing and kidding were in February. They were kept indoors until the middle of April and then sent to the pasture as of April 20 following the weaning. During the pasture period, sheep and goats were grazed only on pasture without additional feeding. Individual milk samples were obtained from each animal once a month from May to October. The collected milk samples were kept in the cold chain and brought to the milk analysis laboratory. Average monthly rainfall and temperature data in the working calendar are shown in Figure 1.

Plant samples were collected from 3 different points which were determined randomly using 50 cm x 50 cm sized wooden frames to reflect the overall pasture on the days when milk samples were taken. The plants entering the quadrate were mown 7.5 cm above the soil surface. The plant samples were kept at room temperature for 24 hours and then dried in oven at 80°C for 2 days. Later, they were homogenized and were used for fatty acid analysis (Tsiplakou *et al.* 2006a).

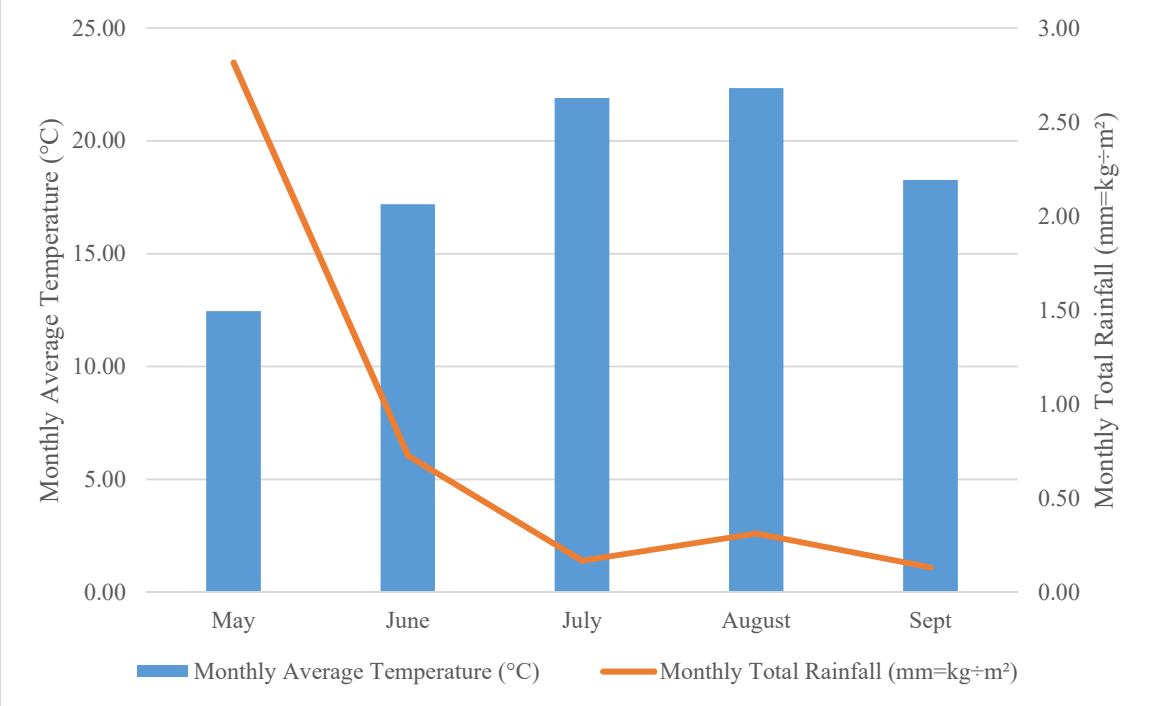

Figure 1. Monthly average rainfall and temperature data

Extraction of fat from milk: Jiang *et al*. (1996) method was modified as follows for extraction of fat from milk: 10 ml milk was put in 100 ml plastic tube and 22.5 ml isopropanol and 12.5 ml hexane were added. After each chemical had been added, the tube was shaken vigorously for 3 minutes. The mixture was put in the centrifuge machine at 5000 rpm for 4 minutes. Afterwards, the superior phase was put into a balloon, while the lower phase was washed using 12.5 ml hexane solvent twice. Finally, combined organic phases were dried using sodium sulfate, and then the resolvent was removed in the evaporator at 30°C.

Extraction of oil from plant samples: 0.25 g sample was taken into a capped tube. Then 200 µl pyrochatechol solution (prepared by dissolving 1 g pyrocatechol in 5 ml methanol) and 5 ml 0.5 M KOH (potassium hydroxide) solution in solved methanol were added to the mixture, which was mixed in the vortex for 20 seconds. The

mixture was put into the water bath and kept there for 15 minutes at 80°C and mixed with the vortex for 15 seconds every 5 minutes. Then, the tube was cooled in a mixture of icy water. 1 ml of fresh sterile distilled water and 5 ml hexane were added to the mixture, it was mixed for 1 minute and centrifuged for 2 minutes, and the supernatant was removed. It was washed using 2x5 ml hexane, and the obtained organic phases were dried over sodium sulfate. Finally, the hexane was removed in the evaporator at 30°C (Tsiplakou *et al.* 2006a).

Methylation: 0.1 g fat was put into a 15 ml tube. 1 ml 2N KOH solution (prepared in Methanol) was added and vortexed. Then 10 ml hexane was obtained and mixed well for centrifuge at 7000 rpm for 8 minutes. The samples were kept at -20 degrees for GC analysis. Finally, supernatant was taken and 1 microliter was injected to GC.

Procedure of GC-FID (flame ionization detector) in analyses of fatty acid: Fatty acids in the milk and plant were analyzed using Perkin Elmer Clarus 500 Chromatography and Restek capillary column (30 m x 0.25 mm x 0.2 µm). Operating conditions of the GC were as follows: Helium flow 1 ml/min; FID at 250°C; split–splitless injector at 250°C. Starting temperature of the oven was 120 °C. It was kept at this temperature for 2 minutes and increased by 2 °C per minute up to 180 °C. Finally, it was increased to 200 °C by 4 °C per minute and left at this temperature for 3 minutes.

A mixture of 37 fatty acids was used as usual in the determination of fatty acids (Food Industry FAME Mix-Restek). The standards offered by Cayman Chemical were employed to determine the standards for CLA. In addition, the amount of fatty acid is expressed as the percentage amount (%) of the fatty acid per total fatty acid.

Statistical analysis: Firstly, the obtained data were transferred to Microsoft Excel 2010 and for a statistical analysis on SPSS 17.0 package program. The data were analyzed using General Linear Model to adjust the monthly effect on milk fatty acids. Duncan test was used for multiple comparisons.

RESULTS

The pasture fatty acid composition is given in Table 1. The highest ratio of MUFA (% of fatty acids) in plants was determined in August, while the highest ratio of PUFA and UFA (% of fatty acids) were determined in May. During the pasture period, MUFA ratio was inversely proportional to the PUFA ratio decreased. SFA ratio (% of fatty acids) was found to be at the lowest and highest level in May and September, respectively. The SFA/UFA ratio increased by 147.3% from May to September.

CLA ratios in milk of Akkaraman sheep and Hair goat in total fatty acid are shown in Figure 1 and given in Table 2 and 3. In both species, the CLA ratio in total milk fat acid was affected by the pasture period, and the highest CLA ratio (% of fatty acids) was observed in May ($P \leq 0.001$). However, there were no statistical differences among other monthly periods. Again, the lowest CLA ratio (% of fatty acids) was determined in both species in July, along with but partial increases until the end of September ($P > 0.05$). The ratio of CLA in Akkaraman sheep total milk fat acid was higher than goat total milk fat acid in each month.

The results of the fatty acid ratio in Akkaraman sheep and Hair goat milk throughout the pasture period are given in Tables 2 and 3. C10:0, C12:0, C16:0, C18:0 and C18:1n9c were determined as the main fatty acids in milk of sheep and goat. C18:3n3 displayed a significant change in Akkaraman sheep's milk ($P \leq 0.001$) on a monthly basis, despite no statistically significant changes in the Hair goat's milk for α-linolenic acid. Additionally, major fatty acids such as myristic, palmitic and stearic acid were statistically affected by the month at $P \leq 0.05$ level in Akkaraman sheep, and these fatty acids did not display any insignificant changes in Hair goat's total milk fat acid. C18:2n6c and C18:3n3 ratios of total milk fat acid in both species did not yield statistical differences in the pasture period.

The highest ratio of the MCFA (% of fatty acids) was observed in Akkaraman sheep ($P \leq 0.05$) in May and for Hair goats in June ($P \leq 0.05$). The highest SFA ratio in both species was found in June, whereas the lowest SFA ratio was in September for Akkaraman sheep and in May for Hair goats ($P \leq 0.01$). The ratio of MUFA was at the lowest level in June for milk of Akkaraman sheep and Hair goat. Besides, for both species, the MUFA was found to be highest in September. At the beginning of the pasture period, the ratio of MUFA in the Hair goat total milk fat acid was higher compared to ewe milk in May, whereas the ratio of MUFA in the Akkaraman sheep total milk fat acid was found to be higher in the following periods. The major MUFA (% of fatty acids) was determined as oleic acid in both species.

SFA/UFA ratio reached the highest level in June for Akkaraman sheep ($P \leq 0.001$) and Hair goat ($P \leq 0.05$).The ratio of PUFA in Akkaraman sheep total milk fat acid was affected by the pasture period ($P \leq 0.001$) reached the highest level in July ($P \leq 0.001$), while pasture period did not have any effect on the PUFA in Hair goat total milk fat acid.

Although the atherogenic index (AI) was at the lowest level in September ($P \leq 0.01$) in Akkaraman sheep milk, Hair goat milk had the lowest atherogenic index in May ($P \leq 0.001$). In addition, while the thrombogenic index (TI) value was not affected by the pasture period in Akkaraman sheep milk, Hair goat milk had the lowest value in May ($P \leq 0.001$). Both indexes were lower in

Akkaraman sheep milk compared to Hair goat milk (Table 4-5).

Table 1. Change of fatty acids in plants by months (% of fatty acids).

	SFA	MUFA	PUFA	UFA	C18:2N6C	C18:3N3	SFA/UFA
May	42.45	9.93	47.51	57.44	9.31	29.96	0.74
June	44.84	11.99	43.15	55.14	10.09	31.04	0.81
July	62.65	24.16	13.15	37.31	5.33	4.01	1.68
August	58.53	34.37	7.10	41.47	5.24	1.30	1.41
Sept	64.60	13.47	21.92	35.39	6.30	9.00	1.83

Table 2. The fatty acid ratio in Akkaraman sheep milk according to months (% of fatty acids).

Symbol	May	June	July	August	Sept	Range	SEM	P
C8:0	2.87^{c}	2.13^{b}	1.64^{b}	1.05^{a}	1.04^{a}	0.55-3.47	0.310	**0.000**
C10:0	9.05^{c}	7.18^{b}	4.96^{b}	3.24^{a}	3.3^{a}	2.09-10.53	1.013	**0.000**
C11:0	0.11^{c}	0.07^{b}	0.04^{a}	0.04^{a}	0.03^{a}	0.02-0.18	0.013	**0.000**
C12:0	4.61^{c}	4.28^{c}	3.04^{b}	2.35^{a}	2.63^{ab}	2.03-5.35	0.403	**0.000**
C13:0	0.11^{b}	0.11^{b}	0.07^{a}	0.12^{b}	0.1^{b}	0.06-0.15	0.008	**0.009**
C14:0	10.4^{a}	12.2^{b}	11.4^{ab}	10.3^{a}	11.1^{ab}	8.94-12.68	0.312	**0.014**
C14:1	0.93^{a}	0.92^{a}	0.86^{a}	1.07^{ab}	1.15^{b}	0.63-1.33	0.048	**0.030**
C15:0	1.44^{a}	1.39^{a}	1.32^{a}	1.82^{b}	1.73^{b}	1.19-1.97	0.088	**0.000**
C16:0	25.8^{a}	28.2^{b}	29.5^{b}	29.8^{b}	28.8^{b}	23.68-32.44	0.636	**0.013**
C16:1	0.99^{a}	1.09^{ab}	1.48^{c}	1.39^{bc}	1.88^{d}	0.85-2.39	0.141	**0.000**
C17:0	1.03^{a}	1.0^{a}	1.21^{b}	1.46^{c}	1.51^{c}	0.86-1.62	0.095	**0.000**
C17:1	0.22^{a}	0.19^{a}	0.3^{ab}	0.29^{ab}	0.45^{b}	0.07-0.64	0.040	**0.015**
C18:0	13.4^{bc}	13.7^{bc}	11.7^{ab}	14.3^{c}	11.2^{a}	8.36-15.71	0.536	**0.026**
C18:1n9t	0.16^{b}	0.11^{b}	0.16^{b}	0.05^{a}	0.05^{a}	0.01-0.24	0.022	**0.000**
C18:1n9c	18.7^{a}	18.3^{a}	22.7^{b}	24.6^{bc}	27.6^{c}	16.48-33.56	1.581	**0.000**
C18:2n6t	0.86^{a}	0.56^{b}	0.81^{bc}	0.25^{a}	0.07^{a}	0-1.26	0.138	**0.000**
C18:2n6c	2.83	2.74	2.93	2.6	2.29	1.96-3.58	0.099	**0.148**
C18:3n6	0.06	0.02	0.03	0.01	0.03	0-0.15	0.007	**0.179**
C20:0	0.34^{a}	0.45^{b}	0.58^{c}	0.77^{d}	0.78^{d}	0.32-0.86	0.078	**0.000**
C18:3n3	1.64^{ab}	1.87^{b}	2.64^{c}	1.69^{b}	1.32^{a}	1.14-2.94	0.197	**0.000**
CLA_{C9t11}	3.25^{a}	2.16^{b}	1.35^{b}	1.39^{b}	1.64^{b}	1.18-4.39	0.316	**0.001**
C21:0	0.05^{a}	0.07^{abc}	0.06^{ab}	0.09^{b}	0.09^{bc}	0.02-0.1	0.007	**0.006**
C20:2	0.22^{c}	0.18^{bc}	0.11^{ab}	0.09^{a}	0.04^{a}	0.03-0.39	0.029	**0.001**
C20:3n6	0.08	0.03	0.03	0.01	0.01	0.01-0.2	0.011	**0.054**
C22:0	0.2^{a}	0.3^{b}	0.3^{b}	0.4^{c}	0.4^{c}	0.09-0.57	0.033	**0.000**
C22:1n9	0.18^{b}	0.15^{ab}	0.18^{b}	0.12^{a}	0.16^{b}	0.11-0.22	0.010	**0.013**
C23:0	0.08^{a}	0.14^{b}	0.17^{bc}	0.19^{c}	0.16^{bc}	0.05-0.21	0.017	**0.000**
C20:5n3	0.11^{a}	0.12^{ab}	0.15^{b}	0.09^{a}	0.11^{a}	0.05-0.18	0.000	**0.017**
C24:0	0.06^{b}	0.02^{a}	0.01^{a}	0.0^{a}	0.01^{a}	0-0.09	0.009	**0.000**
C24:1	0.03^{a}	0.14^{c}	0.12^{bc}	0.03^{a}	0.07^{ab}	0-0.19	0.021	**0.001**
MCFA	16.7^{d}	13.7^{c}	9.75^{b}	6.79^{a}	7.1^{a}	4.79-19.27	1.722	**0.000**
SFA	69.7^{bc}	71.3^{d}	66.1^{ab}	66.2^{ab}	63^{a}	56.24-72.51	1.310	**0.001**
MUFA	24.5^{ab}	23.1^{a}	27.1^{bc}	29.0^{c}	33.0^{d}	21.75-39.49	1.561	**0.000**
PUFA	5.79^{bc}	5.53^{b}	6.7^{c}	4.76^{ab}	3.86^{a}	3.26-7.35	0.430	**0.000**
UFA	30.3^{ab}	28.6^{a}	33.8^{bc}	33.7^{bc}	36.9^{c}	27.51-43.69	1.302	**0.001**
SFA:UFA	2.3^{b}	2.49^{b}	1.95^{a}	1.96^{a}	1.70^{a}	1.29-2.63	1.006	**0.000**

Table 3.The fatty acid ratio in Hair goat milk according to months (% of fatty acids).

Symbol	May	June	July	August	Sept	Range	SEM	P
C8:0	2.45^{ab}	3.1^{b}	2.89^{b}	2.59^{ab}	1.8^{a}	1.39-4.38	0.199	**0.017**
C10:0	8.32^{ab}	10.6^{b}	9.84^{b}	8.79^{ab}	7.03^{a}	6.21-13.98	0.552	**0.019**
C11:0	0.1^{bc}	0.12^{c}	0.08^{ab}	0.05^{a}	0.05^{a}	0.02-0.15	0.012	**.000**
C12:0	4.17b^{c}	4.51^{c}	3.79^{abc}	3.26^{a}	3.4^{ab}	2.67-5.09	0.208	**0.014**
C13:0	0.1	0.12	0.1	0.11	0.07	0.06-0.18	0.008	**0.084**
C14:0	8.75	10	9.8	9.25	9.84	7.46-12.26	0.208	**0.493**
C14:1	0.83^{b}	0.7^{b}	0.61^{a}	0.58^{a}	0.63^{a}	0.47-1.08	0.040	**0.022**
C15:0	1.3	1.29	1.12	1.14	1.09	0.87-1.46	0.040	**0.062**
C16:0	24.8	26.4	28.1	28.1	29.4	22.7-34.7	0.714	**0.067**
C16:1	0.96^{bc}	0.6^{ab}	0.64^{ab}	0.35^{a}	1.06^{c}	0-1.33	0.115	**0.03**
C17:0	0.89^{a}	1.0^{a}	1.09^{ab}	1.24^{b}	1.27^{b}	0.81-1.53	0.067	**0.03**
C17:1	0.2^{ab}	0.21^{b}	0.16^{a}	0.23^{b}	0.32^{c}	0.12-0.37	0.024	**0.000**
C18:0	14.8	16.3	15.8	16.2	13.3	8.42-21.02	0.502	**0.294**
C18:1n9t	0.15^{b}	0.08^{a}	0.02^{a}	0.02^{a}	0.04^{a}	0-0.19	0.022	**0.001**
C18:1n9c	22.6^{bc}	18.1^{a}	19.6^{ab}	22.7^{bc}	24.1^{c}	16.37-27.33	0.991	**0.004**
C18:2n6t	0.76	0.29	0.11	0.02	0.04	0-0.99	0.123	**0.000**
C18:2n6c	2.29	2.08	2.15	2.01	1.92	1.53-2.9	0.056	**0.389**
C18:3n6	0.03	0.01	0.01	0.01	0.01	0-0.07	0.004	**0.276**
C20	0.41^{a}	0.54^{ab}	0.52^{ab}	0.46^{a}	0.62^{b}	0.24-0.81	0.032	**0.029**
C18:3n3	1.52	1.84	2.03	1.33	1.62	0-2.83	0.109	**0.410**
CLA$_{C9t11}$	2.93^{a}	0.83^{b}	0.71^{b}	0.73^{b}	1.25^{b}	0-4.85	0.443	**0.000**
C21:0	0.05	0.05	0.05	0.04	0.06	0-0.09	0.003	**0.865**
C20:2	0.18^{b}	0.1^{ab}	0.06^{a}	0.1^{ab}	0.04^{a}	0-0.28	0.021	**0.012**
C20:3n6	0.04^{b}	0.04^{b}	-	0.02^{a}	0.01^{a}	0-0.08	0.007	**0.000**
C22:0	0.21	0.18	0.2	0.19	0.41	0-0.55	0.039	**0.110**
C22:1n9	0.15^{b}	0.12^{b}	0.08^{a}	0.08^{a}	0.08^{a}	0.03-0.19	0.013	**0.03**
C23:0	0.07	0.1	0.1	0.08	0.1	0.05-0.16	0.006	**0.396**
C20:5n3	0.11	0.1	0.07	0.08	0.08	0.06-0.14	0.006	**0.156**
C24:0	0.02^{b}	0.02^{b}	8.67^{a}	0.01^{a}	2.6^{a}	0-0.06	1.501	**0.000**
C24:1	0.1^{b}	0.13^{b}	0.04^{a}	0.02^{a}	0.04^{a}	0.01-0.18	0.019	**0.001**
MCFA	15.1^{abc}	18.4^{c}	16.7^{bc}	14.8^{ab}	12.3^{a}	11.06-23.76	0.911	**0.012**
SFA	66.6^{a}	74.6^{c}	73.6^{c}	71.6^{bc}	68.6^{ab}	64.35-77.84	1.345	**0.001**
MUFA	28.4^{d}	20.8^{a}	21.9^{ab}	24.7^{bc}	27.6^{cd}	17.48-30.47	1.344	**0.000**
PUFA	4.95	4.49	4.46	3.6	3.74	2.32-6.11	0.226	**0.118**
UFA	33.3^{c}	25.3^{a}	26.3^{a}	28.3^{ab}	31.3^{bc}	22.18-35.65	1.345	**0.001**
SFA:UFA	2.0^{a}	2.95^{c}	2.80^{c}	2.53^{bc}	2.19^{ab}	1.80-3.50	1.000	0.002

Table 4. Δ^9 desaturase activity, AI and TI in Akkaraman ewe milk according to months.

Symbol	May	June	July	August	Sept	Range	SEM	P
14:1/14	0.09^{a}	0.06^{a}	0.08^{a}	0.10^{b}	0.10^{b}	0.07-0.12	0.021	0.001
16:1/16	0.04^{a}	0.04^{a}	0.05^{a}	0.05^{a}	0.07^{b}	0.031-0.077	0.033	0.000
18:1/18	1.40^{a}	1.35^{a}	1.93^{b}	1.73^{ab}	2.57^{c}	1.10-3.28	0.127	0.000
Aİ	2.36^{a}	2.84^{b}	2.31^{a}	2.19^{a}	2.1^{a}	1.49-2.98	0.115	0.002
TI	2.15	2.41	2.20	2.24	2.10	1.68-2.59	0.297	0.087

All Δ^9 desaturase activities in Akkaraman sheep milk (14:1/14, 16:1/16 and 18:1/18) were affected by the pasture period. In Akkaraman sheep, the ratio of 14:1/14 reached the highest value in August and September ($P \leq 0.01$), as the ratio of 16:1/16 and 18:1/18 reached the highest value in September ($P \leq 0.001$). Similar to Akkaraman sheep, Δ^9 desaturase activities of Hair goat milk (14:1/14, 16:1/16 and 18:1/18) were affected by pasture periods, too. The highest 14:1/14 value was determined in May, where 16:1/16 and 18:1/18 were determined in September.

In Figure 2, the relationship among the changes in CLA ratio in total milk fat acid and the changes in C18:2n6 and C18:3n3 in plants are shown. CLA reached

the highest level in the total milk fat acid in both species in May when C18:2n6 and C18:3n3 were high in the pasture. Again, in July and August, when C18:2n6 and C18:3n3 ratios had the lowest value in pasture, CLA ratio was the lowest in the total milk fat acid for both species. In September, the ratio of C18:3n3 in pasture and the ratio of CLA in Akkaraman sheep and Hair goat total milk fat acid were similar.

Table 5. Δ^9 desaturase activity, AI and TI in Hair goat milk according to months.

Symbol	May	June	July	August	Sept	Range	SEM	P
14:1/14	0.10^{a}	0.07^{b}	0.06^{b}	0.07b	0.07^{b}	0.05-0.11	0.011	**0.000**
16:1/16	0.04^{c}	0.23^{ab}	0.02^{ab}	0.01^{a}	0.04^{bc}	0-0.041	0.031	**0.003**
18:1/18	1.53^{b}	1.13^{a}	1.24^{ab}	1.40^{ab}	1.88^{c}	0.91-2.60	0.197	**0.001**
AI	1.92^{a}	2.83^{b}	2.74^{b}	2.45^{ab}	2.33^{ab}	1.67-3.52	0.145	**0.000**
TI	2.04^{a}	2.74^{b}	2.83^{b}	2.72^{b}	2.60^{b}	1.91-3.20	0.397	**0.000**

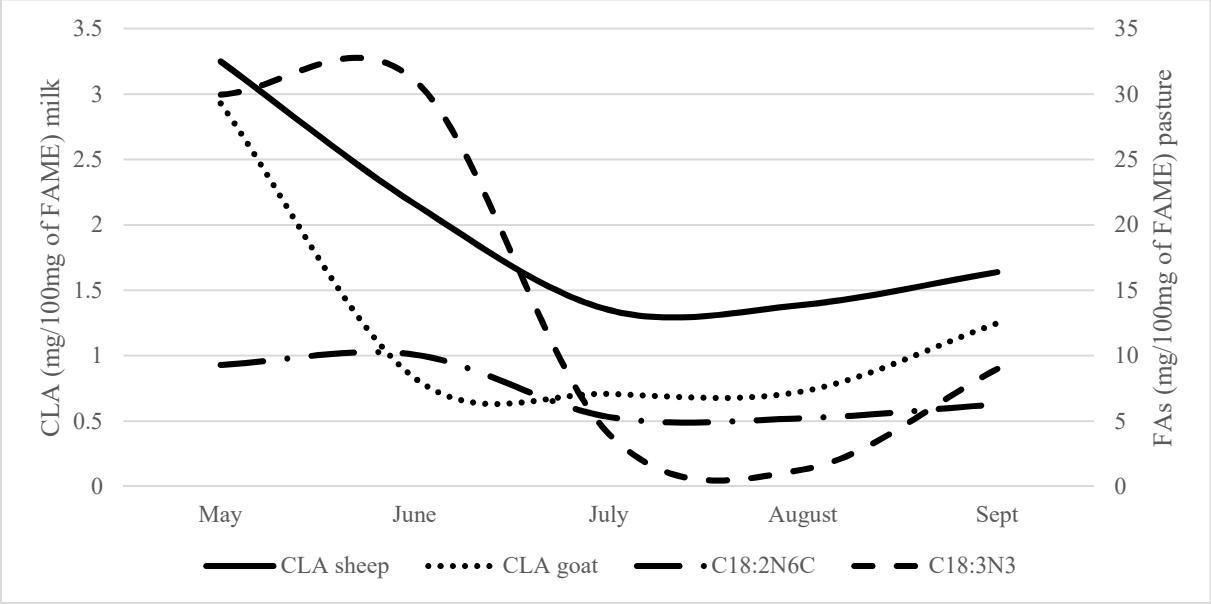

Figure 2. CLA ratios in Akkaraman sheep and Hair goat milk and C18:2n6c and C18:3n3 ratios in pasture

DISCUSSION

Piredda *et al*. (2002) and Tsiplakou *et al*. (2006b) suggested that the plant fatty acid content was more effective than the plant species in the pasture at the ratio of CLA. It was expected that the CLA ratio of total milk fat acid in both species would reach the highest and lowest level in May and August, respectively, because the region where the study was carried out was in the growth period of the plants until the middle of June and the remaining time was the period in which generative development of the plants occurred. It is also possible to explain the partial increment in the CLA ratio in the total milk fat acid of both species in September by the emergence of fresh shoots from the plants with the rainfall in August (Figure 1).

C18:2n6c ratio in the total milk fat of both species did not show any statistical differences during the pasture period and there was a lower change in C18:2n6c ratio compared to C18:3n3 ratio in pasture, indicating that CLA was synthesized mainly from C18:3n3 in mammary tissues. Moreover, the ratio of increase of CLA in Hair goat total milk fat acid from August to September and C18:3n3 in the pasture during the same months suggests that the source of CLA is especially α-linolenic acid. Similarly, Dhiman *et al*. (2000) in Bos tauraus, Tyagi *et al*. (2007) in buffaloes and Mel'uchova *et al*. (2008) in ovine reported that the source of CLA in milk is C18:3n3 during the pasture. The fact that the CLA ratio in Akkaraman sheep's total milk fat acid is higher compared to Hair goat's total milk fat acid overlaps the findings in Jahreis *et al*. (1999) and Talpur *et al*. (2009).

Lock and Garnsworthy (2003) reported a positive correlation between CLA ratio in cow and the ratio of 14:1/14. Desaturase activity is an important parameter that gives information about the source of CLA synthesis. Cabiddu *et al.* (2005) reported a decrease in C18: 2n6 and C18: 3n3 ratios in the transition from a vegetative to generative development in plants. They also demonstrated that this change in plant fatty acid composition reduced the formation of vaccenic acid in

milk, thus decreasing the ratio of CLA in milk. In the present study, however, only CLA ratio and 14:1/14 ratio in Hair goat milk increased in the same period.

Piredda *et al*. (2002) found out that ratio of SFA in total milk fat acid increased with the transition of plants to the generative period. Moreover, Biondi *et al*. (2008) stated that the SFA ratio in the total milk fat acid decreased when the sheep was grazing in the pasture, which overlap the findings of the present study. In both sheep and goat milk palmitic and oleic acids were higher in September. This is compatible with report of Mohapatra *et al.* (2019). The variation of linolenic acid ratio in sheep and goat milk and the variation of linolenic acid ratio in the pasture are in the same line with the Altomonte *et al*. (2019).

The risk of cardiovascular diseases was reduced by MUFA, especially by oleic acid (Kriss-Etherton 1999). The majority of MUFA in sheep and goat total milk fat acid is already oleic acid. Cabiddu *et al*. (2005) attributed this to the fact that Δ^9 desaturase enzymes preferred long chain fatty acids. Similar to the present study, Valvo *et al*. (2007) and Biondi *et al*. (2008) reported that the pasture period was decisive in MUFA, while Atti *et al*. (2006) in sheep argued that pasture period did not affect MUFA in total milk fat acid. Cabiddu *et al*. (2005) pointed out that the source of the change in MUFA in milk was the change in PUFA in plants. While the change curve of MUFA ratio in plants is similar to that of MUFA ratio in sheep total milk fat acid, it is not possible to reach the same conclusion for goat total milk fat acid.

PUFA cannot be synthesized by tissues in ruminants. It is synthesized either by ration or by PUFA escaping ruminal biohydrogenation (Chilliard *et al*. 2000). The changes in PUFA ratio in total goat milk fat acid and plants are similar. However, this finding is not valid for the changes in PUFA ratio in Akkaraman sheep's total milk fat acid. In a similar vein, Tsiplakou *at el*. (2006b) reported that the PUFA in goat's total milk fat acid was not affected by the pasture period, whereas the PUFA ratio in sheep's total milk fat acid was affected. It can be argued that the reason underlying this is that sheep and goats prefer different kinds of plants in the pasture.

Consumption of milk and products with low AI and TI has a lower effect on LDL and total cholesterol. (Mierlita 2016). The atherogenic index found for sheep's milk is similar to Mierlita *et al*. (2011) and Sojak *et al*. (2013). Thrombogenic index value in Hair goat milk is higher Osmari *et al*. (2011) and lower Sinanoglou *et al*. (2015). Similar to these results, Basdagiannia *et al*. (2019) reported that AI and TI were higher in goat milk than in sheep milk.

Conclusion: The present study demonstrated that there was a change in milk fatty acids during the pasture period. Changes of the fatty acid profile in sheep and goat milk due to pasture were related to variation in fatty acid concentration in the pasture. According to the findings of the present study, a relationship was observed between C18:3n3 ratio in pasture and CLA ratio in total milk fatty acid. Fatty acids in Akkaraman sheep milk were affected by the pasture period more compared to Hair goat milk fatty acids. Therefore, it is recommended that the increase the CLA ratio in milk and dairy products must be analyzed under more controlled conditions, especially in artificial pastures. Also, it is hoped that this study will be the source of new studies to investigate the hypothesis that the fatty acid composition of milk from sheep and goat bred on mountain farms is more beneficial for human health compared to milk from sheep and goat bred on valley farms.

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