

THE FATTY ACID COMPOSITION OF SHEEP'S MILK OF AN AUTOCHTHONOUS BREED

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Abstract: The study included a total of 127 sheep milk samples from two different areas (Livno and Travnik) in summer feeding period (July, August and September). Fatty acids in milk were determined by gas chromatography (GC). The animals were marked with the appropriate number of ear tags on the basis of which we always took samples from the same animals through different periods. Fatty acids in milk were determined by gas chromatography and the following fatty acids composition: butyric acid, caproic acid, caprylic acid, capric acid, stearic acid, oleic acid, linoleic acid, arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid, rumenic acid. The fatty acid content of sheep's milk in this study showed a tendency of variation, both within and between sampling areas, and characterized by its relatively high content of saturated fatty acid (SFA) during the period of harvest.

Key words: milk, fatty acid composition, sheep

Introduction

Milk and milk products are well balanced nutritious food in human diet. Milk fat contains approximately 400 different fatty acids, which make it the most complex of all natural fats (*Lindmark Mansson, 2008*). The premium nutritional quality of dairy products is highly correlated with milk fat quality and concerns: high concentration of fat soluble vitamins and n-3 fatty acids, as well as high content of conjugated linoleic acid (CLA) (*Markiewicz-Keszycka et al. 2013*). The large number of research studies with the aim of increasing the biological value of animal products - more specifically the milk of ruminants, comes from the FAO recommendations, which in 2003 established the consumption of SFA for humans. However, only two procedures can change the fatty acid profile of products derived from ruminants: modification of fatty acids during the processing, or change in the

fatty acid profile of the diet (*Palma Rennó et al. 2013*). Most studies focus primarily on the effect of feeding the sheep on the fatty acid profile (*Addis et al. 2005*). The influence of physiological factors (breed, lactation) is of less importance (*Tsiplakou et al. 2006*). The aim of this study is to determine the fatty acid composition of sheep's milk as well as to monitor the influence of diet on their composition.

Materials and methods

A total of 127 sheep were used to investigate the effects of different sampling period and areas on milk fatty acids (FA) profiles. The research was conducted during July, August, and September at Livno area (village Guber -724 m altitude) and Vlačić mountain (village Mudrike – 1300 m altitude) in Bosnia and Herzegovina (B&H). The animals were marked with numbered ear tags and the sampling was done through different sampling periods (July-I, August-II and September-III). In the area of Livno, two milk samples were taken - July (n = 20) and August (n = 20), while the samples for the third sampling were quantitatively insufficient for performing all the foreseen analyzes, and in the area of Travnik the milk was sampled in three terms - July (n = 25), August (n = 25) and September (n = 25). Fatty acids in sheep milk were determined by gas chromatography in the laboratory Vitas As Oslo Innovation Centre, Norway. Sample preparation was performed according to the procedure described in Luna et al. (2005), which includes the separation of milk fat by centrifugation and fatty acid methylation to produce fatty acid methyl esters (FAME) which are analyzed on a gas chromatograph. The following fatty acid composition was determined: butyric acid (C4:0), caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0), stearic acid (C18:0), oleic acid (C18:1 cis-9), linoleic acid (C18:2 n-3), arachidonic acid (C20:4 n-6, ARA), eicosapentaenoic acid (C20:5 n-3, EPA), docosahexaenoic acid (C22:6 n-3, DHA), rumenic acid (C18:2 cis 9, trans-11, CLA)). Statistical analysis was performed using the software package/SPSS 21.00. Nonparametric statistics were used for processing, Friedman (for the Travnik area) and Wilcoxon test (distribution free tests) (for the area of Livno) were used. The differences were considered statistically significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$.

Results and Discussion

The mean values of fatty acid in milk of sheep breed in the area of Livno and Travnik expressed in grams of each fatty acid per 100 g of total fatty acid (g / 100g FA) are shown in Tables 1 and 2, as well as the statistical significance of differences between sampling periods. Table 3 shows the statistical significance differences of the content of fatty acids in milk depending on the locality and the sampling period. By testing the differences in the fatty acid content between the

Livno and Travnik areas during the sampling periods, statistically significant differences were found in the content of 17 of the total of the given 24 fatty acids.

The results of this research showed, that a significant influence on the profile of fatty acids of sheep milk from both sampling areas (Livno, Travnik) had the botanical composition of pastures and the period of lactation. A total of 24 fatty acids were determined over three sampling periods (July, August and September). During the sampling period, sheep milk from Livno and Travnik areas contained a higher proportion of SFA compared to unsaturated fatty acids (UFA) and polyunsaturated fatty acids (PUFA). Most SFA in sheep milk from the Livno area showed differences between the sampling periods most likely to be due to differences in the composition of pastures (vegetation) at the time they were used for animal feeding.

Saturated fatty acids from the Livno area were individually statistically significantly related to the sampling period, in order to reduce their content to the end of the lactation period. The content of C4:0 acid in sheep milk samples from the Livno area was significantly lower than the value found in Merino sheep milk by *Mierlita et al. (2011)*. In both sampling areas, the trend of decline in sampling periods was noticed for C4:0, especially for the Travnik area (Tables 1 and 2).

Meals containing a higher amount of sugar cause the formation of C4:0, and for these meals a higher content of C4:0 in milk fat is characteristic. In the area of Livno for C6:0, C8:0 and C10:0 acids, a statistically difference was observed between the sampling period, again with the trend decreasing following the end of lactation, and the established values were lower values mentioned by other authors (*Goudjil et al., 2004; Mierlita et al., 2011*). In raw milk, high concentrations of C4:0, C6:0, C8:0 and C10:0 are not preferred, as it may result in distortion of taste /aroma of milk.

Table 1. Mean values of fatty acids in sheep milk from Livno area

SFA			
Fatty acids (g/100gFA)	I sampling	II sampling	p
C4:0	3.86	3.69	
C6:0	2.08	1.40	***
C8:0	1.64	0.98	***
C10:0	4.29	2.81	***
C12:0	2.66	2.07	***
C14:0	9.55	8.45	***
C15:0	1.18	1.07	***
C16:0	22.30	21.85	
C17:0	0.81	0.82	
C18:0	8.64	9.72	**
C20:0	0.42	0.43	

MUFA			
C14:1cis-9	0.25	0.27	
C16:1cis-9	0.90	1.00	
C18:1cis-9	17.93	22.27	***
C18:1 cis-11	0.89	0.95	
C18:1trans-9	0.28	0.40	
C18:1trans-10	0.50	0.57	
C18:1trans-11	2.87	2.48	
PUFA			
C20:4	0.16	0.17	
C20:5 n-3 (EPA)	0.15	0.12	
C22:6 n-3 (DHA)	0.10	0.09	
C18:2 n-6	2.46	2.70	
C18:3 n-3	2.26	1.34	***
C18:2cis-9, trans-11(CLA)	1,63	1,49	
∑n-3	2.52	1.62	***
∑n-6	2.61	2.91	*
∑SFA	57.29	53.78	**
∑MUFA	23.97	28.09	***
∑PUFA	6.89	6.01	*
∑UFA	31.30	33.86	**
n-6/n-3	1.05	1.92	***
SFA/MUFA	2.36	1.97	***
SFA/PUFA	8.36	8.98	
MUFA/PUFA	3.48	4.63	**
SFA/UFA	1.82	1.61	**
UFA/MUFA	1.29	1.22	***
UFA/PUFA	4.48	5.63	***

Mean values in the same row with different letter codes differ significantly,

*** $p < 0,001$, ** $p < 0,01$; I, II - ; I, II, III—represent sampling periods: July, August and September
SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; UFA – unsaturated fatty acid

Table 2. Mean values of fatty acids in sheep milk for Travnik area

SFA				
Fatty acids (g/100gFA)	I sampling	II sampling	III sampling	p
C4:0	3.43 ^a	3.30 ^a	2.86 ^b	***
C6:0	1.86 ^a	1.77 ^a	1.49 ^b	*
C8:0	1.47	1.32	1.22	
C10:0	3.87	3.60	3.68	
C12:0	2.51	2.24	2.83	
C14:0	9.01 ^a	9.05 ^a	10.19 ^b	*
C15:0	1.21	1.16	1.13	
C16:0	21.62 ^a	22.58 ^a	23.74 ^b	**
C17:0	0.70	0.73	0.66	
C18:0	9.22 ^a	9.37 ^a	7.70 ^b	***
C20:0	0.37	0.41	0.38	
MUFA				
C14:1cis-9	0.55	0.37	0.35	
C16:1cis-9	1.01	1.04	1.16	
C18:1cis-9	20.90	20.77	20.83	
C18:1 cis-11	0.74 ^a	0.71 ^a	0.59 ^b	***
C18:1trans-9	0.26	0.26	0.23	
C18:1trans-10	0.35	0.31	0.26	
C18:1trans-11	3.20 ^a	2.61 ^b	2.55 ^b	**
PUFA				
C20:4	0.23	0.24	0.24	
C20:5 n-3 (EPA)	0.14	0.14	0.15	
C22:6 n-3 (DHA)	0.11 ^a	0.15 ^b	0.18 ^b	**
C18:2 n-6	2.44	2.57	2.19	
C18:3 n-3	1.91 ^b	1.98 ^b	1.64 ^a	**
C18:2cis-9, trans- 11 (CLA)	2.21 ^a	1.69 ^b	2.04 ^a	***
Σn-3	2.08	2.29	2.02	
Σn-6	2.64	2.74	2.55	
ΣSFA	55.93	56.85	56.73	
ΣMUFA	27.39	26.11	27.38	
ΣPUFA	6.71	6.98	6.66	
ΣUFA	33.84	33.25	34.16	
n-6/n-3	1.26 ^{ab}	1.21 ^b	1.31 ^a	*
SFA/MUFA	2.02	2.11	2.01	
SFA/PUFA	8.23	8.30	8.66	
MUFA/PUFA	3.97	3.79	4.27	
SFA/UFA	1.64	1.73	1.64	
UFA/MUFA	1.25	1.26	1.23	
UFA/PUFA	4.97	4.79	5.27	

Mean values in the same row with different letter codes differ significantly, *** p<0,001, ** p<0,01,

* p<0,05; I, II, III—represent sampling periods: July, August and September

SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; UFA – unsaturated fatty acid

In addition to the absolute content of n-3 fatty acids in the meal nothing less is a significant relationship between n-3 and other UFA, which is n-6 fatty acids. Acid EPA, in addition to C18: 3 n-3 and DHA, is the most significant n-3 fatty acid. The acids C18: 2 n-6 and C18: 3 n-3 are the most dominant PUFA in Livno's milk and C18: 2 n-6 and rumenic acid from the Travnik area. The content of C18: 3 n-3 in milk samples from Livno and Travnik ranged from sampling periods, and very high statistically significant differences were found between I and II sampling periods.

The content of C6:0 and C8:0 in sheep milk fat from the Travnik area also had a trend of decreasing values going to the end of the lactation period, with the differences in C6:0 being statistically significant. Saturated fatty acid from the Livno area had a slight decreasing trend in August-II except for C18:0, where a slight increase in the second sampling period was found, while in the examined samples of Travnik milk in the III sampling period there was a decrease in value. Statistically significant differences in the content of fatty acid milk were determined within and between the areas by sampling periods. Depending on the locality and the sampling period statistically significant differences at the level ($p < 0.05$) were observed in MUFA, PUFA, UFA and fatty acid sums ratio (Table 3).

The content of the fatty acids investigated in our research showed a tendency of variation over the months and is characterized by its relatively high content of SFA through the period, so it is possible that the sheep breed may have a greater effect on the SFA concentration than the sampling period. Differences in the content of fatty acids are a possible consequence of the aforementioned differences in nutrition, and probably other factors that affect the fatty acid composition of milk fat. The floral composition of hill pastures is better than the mountainous in terms of botanical composition (*Žan et al., 2006*). During the spring, a more intense growth of biomass is made, compared to the summer when it is influenced by high temperatures and lack of precipitation, the pastures are considerably less expensive (*Ljubicic et al., 2012*).

Table 3. Statistical significance differences in the content of fatty acid sheep milk from Livno and Travnik areas between the sampling period

Fatty acids	L/II	L/III	L/III	II/II	II/III	II/III
C4:0	*	*	*	*	*	*
C6:0					*	*
C8:0					*	
C10:0						
C12:0				*		
C14:0		*	*			
C15:0		*				
C16:0			*		*	*
C17:0						
C18:0						
C20:0		*				*
C14:1 cis-9		*				
C16:1 cis-9						
C18:1 cis-9		*				
C18:1 cis-11						
C18:1 trans-9			*		*	
C18:1 trans-10		*			*	
C18:1 trans-11						
C20:4 n-6		*			*	
C20:5 n-3 (EPA)		*				
C22:6 n-3 (DHA)						
C18:2 n-6		*		*	*	*
C18:3 n-3		*			*	
C18:2 cis-9, trans-11(CLA)						*
∑n-3	*		*	*	*	*
∑n-6						*
∑SFA						*
∑MUFA	*	*	*		*	
∑PUFA				*	*	*
∑UFA	*	*	*			
n-6/n-3	*	*	*	*	*	*
SFA/MUFA	*	*	*		*	
SFA/PUFA						
MUFA/PUFA	*		*	*	*	*
SFA/UFA	*		*			
UFA/MUFA	*		*	*	*	*
UFA/PUFA	*		*	*	*	*

*p<0,05. L –sampling area LIVNO; T – sampling area TRAVNIK. I, II, III – represent sampling periods: July, August and September; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; UFA – unsaturated fatty acid

There are particularly pronounced differences when comparing the values of fatty acids content between different sampling periods of different areas (LI / TI, LI / TII, LI / TIII, LII / TI, LII / TIII). Attention should be paid to the flora of Vlasic Mountain and its age. Vlasic Mountain with its geographic position, terrain configuration and mountain climate significantly influences the composition, layout and dynamics of the appearance of certain plant species in this ecosystem. If the quality of the pasture is observed from this site it can be concluded that the best quality food is given by plant species from the legume group.

The Livno area with its geographical position, terrain configuration and characteristic climate represents the area of a unique flora with a large number of interesting plant species. The botanical composition of fodder plants and their percentage distribution in these localities of the Livno Canton, which can be classified as mountainous lawns in terms of altitude and other climatic edafic conditions, is characterized by the content of hornbeam (*Scabiosa columbaria*, *Knautia arvensis*) and grass (*Nardus stricta*, *Festuca sp.*) indicating a certain acidity of the soil because these plants inhabit the soil of acid reactions (Hrkovic, 2009).

The quantity, composition and characteristics of milk produced, especially sheep held on pasture, in given environmental conditions, depend on the combined effects of seasonal changes in the climate and available food, as well as variations in the metabolic status of sheep resulting from lactation, can explain the established changes in fatty acid composition of milk during this study. In the class of SFA, C4:0, C6:0 and C18:0 in sheep milk from the Travnik area, they were statistically significant for the sampling period, in terms of reducing their content in the month of September-III.

The content of C16:0 in analyzed samples of milk from the Livno area was opposite to the samples from the Travnik area. The content of C16:0 in milk samples from the Livno area was higher in the I sampling period but without statistically significant difference compared to the II period (August), which is consistent with the results of the research (Mihaylova et al., 2005) who point out that the content of C16:0 and C18:0 was the highest in sheep's milk in July. The content of C16:0 in analyzed milk samples from the Travnik area grew by sampling periods and a statistically significant value in comparison to the previous two sampling was recorded in September Table 2. There was no extremely dry period during this research (data from the Federation Hydrometeorological Institute). However, in August-II, fewer precipitation and high temperatures were reported, resulting in less vegetation, and the sheep's meals were solely pond. The annual season as a whole does not act equally to the animal organism, and therefore individual factors (temperature, humidity, air flow and light) need to be observed and their potential impact on production performance. High air temperatures can adversely affect milk fat and milk fat content, which may also affect the fatty acid composition of milk. Leading SFA in most nutrients is C16:0

which, together with C12:0 and C14:0, is considered hypercholesterolemic. In the examined samples of milk from both sampling areas, the dominant fatty acids were C14: 0, C16: 0, C18: 0 and C18: 1 cis-9.

Analysis of the fatty acid composition of sheep milk from the Livno area showed very high statistically significant differences between sampling periods mainly for SFA (C6: 0-C15: 0). The content of SFA in dairy fat from both sampling areas was higher in July-I compared to August-II and September-III, which is likely to be related to nutrition and climatic factors during that period. The content of C12:0 in Livno milk samples was very statistically significantly different between sampling periods (Table 1). *Valvo et al. (2007)* found that the content of C12:0, C14:0 and C16:0 was higher in the milk of sheep which were kept alive than sheep in the pasture, which was the result of a higher proportion of C14: 0 and C16: 0 in the hay and barley compared to pastures of pastures. The most abundant MUFA milk fat in the Livno and Travnik areas was C18:1 cis-9 whose value varied depending on the locality and sampling period, which may be due to the seasonal effect associated with the feeding mode in summer period. *Popović-Vranješ et al. (2010)* found that at the beginning of the past season C18: 1 cis-9 in organic milk gradually increased in August to reach a value higher than the average value found in conventional milk.

In the majority of other MUFA analyzed samples of sheep milk from the Travnik area there was a decrease in the value by sampling periods, ie at the end of lactation. The content of VA in milk samples from the Livno and Travnik areas showed a fall in values according to sampling periods with high statistical significance for the Travnik area. Changes in VA content in analyzed milk fat samples may be the result of changes in the content of C18: 3 n-3 in plants depending on the vegetation phase and differences in the length of grain. Determining the concentration of ARA, EPA and DHA in the field of Livno is not significantly different between the endpoints, while the emphasis on the concentration of DHA highlights the significance of the difference between the two groups. The acid EPA is incapable of partially blocking the conversion of n-6 fatty acids into harmful eicosanoids, thereby decreasing the risk of the cardiovascular lesions (*Popović-Vranješ et al., 2010*).

Both areas contain C18 : 3 n-3 had a trend of falling to the end of the lactation period, and this would be the result of nutrition, that is, the stage of vegetation, because the younger plants are richer in C18: 3 n-3, and its content decreases by decaying the vegetation. Some authors suggest that the increased intake of C18:2 n-6 and feeding on pastures increases the CLA content of milk (*Popović-Vranješ et al., 2010*). The CLA content in both sampling areas has a variation trend over the months of sampling, which may be due to feeding on pastures, especially where vegetation is present in grasslands, as our CLA research shows a downward trend of values going to the end of lactation, and at the end of the drooping period when the nutritional value of the herbicide is reduced.

It is possible to manually manipulate fatty acid milk by the summer feeding on the beans (Purchas et al., 2005). Feeding on pasture increases CLA in milk, especially the presence of grass in the early stage of growth. It should be noted that in the Travnik CLA area was the second most representative of PUFA (immediately after C18: 2 n-6 acids) in sheep's milk. Lower CLA values in Livno's milk dairy can be due to an increased inflow of C18: 1 cis-9 degradation intermediate from buraga, in particular isomer C18: 1 trans-10. The content of these isomers was higher in milk of Livno's sheep, and it was found that they, and without diminishing desaturase activity in milk, could lead to lower CLA values in milk (Marenjak et al., 2005). Once milk is richer with n-3 FA and CLA than cow's milk, and one of the reasons may be that sheep are more often eaten by crap, while cows are more prone to pasture and are more fed with concentrates (Marenjak et al., 2005). Tables 1 and 2 also show the total quantities of SFA, MUFA, PUFA and UFA milk from Livno and Travnik. By examining the samples of sheep milk from the Livno and Travnik areas, statistically significant differences were found in most of the SFA and PUFA acids in the area and between the areas per sampling period. The fatty acids of MUFA in milk fat from the Livno and Travnik areas had statistically significant changes in the value during a different sampling period only in several cases. Despite the differences in the content of certain fatty acids between the sampling periods, the trend remained the same in both areas. The total share of SFA in milk from both areas was greater than the total share of MUFA and PUFA, but without statistical significance for the Travnik area as well as between the areas. Examination of the relationship between SFA / PUFA and statistically significant differences between SFA / MUFA, MUFA / PUFA, UFA / MUFA and UFA / PUFA was found in the samples of milk from the Livno area significant difference. Such SFA values from the Livno area were expected due to the predominantly found values of C14: 0, C16: 0 and C18: 0. Acid PUFAs fulfill many structural and functional roles that are incomparable among fatty acids due to the wide spectrum of biological processes (Andrišić, 2013). The majority of MUFA in the samples from both areas is C18: 1 cis-9 with its value was higher in samples from the Travnik area. UFA / MUFA in milk samples from Livno area was significantly statistically significantly different between sampling periods. No statistically significant difference was found in the samples of milk from the Travnik area of SFA, MUFA, PUFA and UFA, nor between fatty acid sums.

The fatty acid content of sheep's milk in this study showed a tendency of variation, both within and between sampling areas, and characterized by its relatively high content of saturated fatty acid (SFA) during the period of harvest. It is possible that the lactation period had a greater effect on SFA concentration than the breed type because the differences are particularly pronounced when comparing the values of fatty acids content between the different sampling periods within both examined areas.

Conclusion

The fatty acid content of sheep's milk in this study showed a tendency of variation, both within and between the sampling area, and characterized by relatively high content of SFA during the grazing period. It is possible that the lactation period had a greater effect on SFA concentration than the type of strain because the differences are particularly pronounced when comparing the values of fatty acids content between the different sampling periods within both examined areas. Sheep milk samples from both areas of dominant fatty acids were expected to be myristic, palmitic, stearic and oleic. Optimizing the diet and the source of fatty acids in animal foods can improve the fatty acid profile in milk.

The presence of VA in the rumen is a result of incomplete biohydrogenization, and changes in its content in milk fat can be the result of a change in the content of LNA in plants depending on the stage of vegetation and the difference in the length of the grazing period. In the milk samples from both areas, the LNA content had a downward trend towards the end of the lactation period. This can be a consequence of feeding or vegetation, as the content of LNA in plants decreases with the release of vegetation, and / or its more intense metabolism to DHA and EPA, whose share in milk for the Travnik area is growing towards the end of the lactation period. The content of DHA acid in milk from the area of Livno was lower in relation to milk from the area of Travnik, but without statistically significant differences.

Our research shows a trend in the decline in CLA content in milk going towards the end of lactation, and at the end of the gaseous period when the nutritional value of the plant cover decreases.

The content of total n-3 fatty acids in milk from the Livno region had a tendency to decline by the end of the lactation period, and n-6 fatty acids reversed the trend, and these differences between I and II sampling were statistically significant. The highest values of the total n-3 and n-6 fatty acid content for the Travnik area were determined, however, in the II period of sampling, but without statistically significant differences between the sampling period.

By examining the ratio of the sum of different classes of fatty acids in the milk samples from the Livno area, statistically significant differences were found between the sampling period for SFA / MUFA, MUFA / PUFA, UFA / MUFA and UFA / PUFA, with the exception of the SFA / PUFA ratio. In the milk from the Travnik region, the same relationships did not statistically significantly differ from the sampling period, possibly due to the more stable composition of the plant cover. Milk samples from the Travnik area contained more PUFA than milk from the area of Livno and a more favorable SFA / PUFA ratio.

Masno-kiselinski sastav ovčjeg mleka autohtone rase

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Rezime

Istraživanjem je obuhvaćeno ukupno 127 uzoraka mleka ovaca iz dva različita područja (Livno i Travnik), u letnjem periodu hranjenja (juli, avgust i septembar). Životinje su obeležene odgovarajućim brojem ušnih markica, na temelju čega su uvek prikupljeni uzorci od istih životinja kroz različita razdoblja. Masne kiseline u mleku određene su gasnom hromatografijom (GC), te je utvrđena sledeća masno-kiselinska kompozicija: buterna kiselina, kapronska kiselina, kaprilna kiselina, kaprinska kiselina, stearinska kiselina, oleinska kiselina, linoleinska kiselina, arahidonska kiselina, eikozapentaenska kiselina, dokozaheksaenska kiselina, rumenska kiselina. Sadržaj masnih kiselina ovčjeg mleka u ovoj studiji pokazao je tendenciju varijacije, kako unutar tako i između područja uzorkovanja, te je karakterističan po svom relativno visokom sadržaju zasićenih masnih kiselina (SFA) tokom razdoblja žetve.

Ključne riječi: mleko, masno-kiselinski sastav, ovce

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