DOI 10.7251/VETJEN2101121H **UDK 637.12:546.733(497.6)**

Original Scientific Paper

BIOACTIVE ACIDS OF SHEEP`S MILK FROM THE AREA OF LIVNO AND TRAVNIK (VLAŠIĆ)

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Summary

There is no doubt that milk and dairy products have a high nutritional value. However, their current consumption is increasingly dependent on their dietary and health properties. It is noticeable that in recent years, increasing popularity is given to those dairy products that have a positive effect on the human body. The aim of this study was to determine the fatty acid composition of sheep's milk from the area of Livno and Travnik (Vlašić), with special reference to the content of bioactive fatty acids that have a positive effect on human health. The fatty acid composition of the tested milk samples was specific due to the content of fatty acids which have been proven to have an extremely beneficial effect on human health. Concentrations of most bioactive fatty acids varied between areas, and the differences were statistically significant for arachidonic, eicosapentaenoic, docosahexaenoic, and rumenic. Milk samples from the tested areas had an almost ideal ratio of n-6/n-3 fatty acids, which makes them very favorable foods from a health point of view.

Keywords: bioactivity, sheep milk, Livno, Travnik

INTRODUCTION

In recent years, an important sector of the food industry has been promoting the health benefits of food. The use of food for health benefits, and not only for the purpose of nutrition, has opened a completely new field in the production and processing of milk (Hrković-Porobija et al., 2020). Consumption of food, through its biologically active ingredients, is an important factor in neutralizing the effects of harmful substances in the human body. The term bioactive component refers to certain food ingredients, whether they are naturally present or formed during food processing, and have a physiological and biochemical function in the human body (Lisak Jakopović et al., 2018). Bioactive substances are mostly found in plants, and some of them, such as probiotics, conjugated linoleic acid (CLA), n-3 fatty acids and bioactive peptides are found in animal products (milk and milk products). The content of bioactive components in milk can be increased through a traditional feeding system. The opinion of some nutritionists is that fats

contained in milk and dairy products, primarily due to the content of saturated fatty acids (SFA) and trans fatty acids are unfavorable to the health of consumers. On the other hand, according to Elwood et al (2004), regular milk consumption may have a long-term impact on reducing the risk of cardiovascular disease. It is known that the chemicals that we take into our body through food have a strong effect on our psycho-physical health, but at the same time they are important in the treatment of many diseases. Some believe that proper nutrition and choosing the right foods can prevent or alleviate certain diseases such as cancer, dementia, depression, and even schizophrenia.

Milk contains mainly esters of long fatty acids and glycerol, and a certain amount of phospholipids, lecithins and cholesterol. Milk fat contains a large proportion of short-chain fatty acids that oxidize quickly, supplies the body with essential fatty acids and fat-soluble vitamins (vitamins A, D, E, K). β-lactoglobulin and α-lactalbumin are whey proteins that also show anticancer activity by participating in preventing the development and growth of chemically induced tumors (Tudor and Havranek, 2009). The content of CLA isomers in fats of non-ruminants is very low, and the significant presence in fats of ruminant is due to the specific way of metabolizing food lipids in the rumen, where unsaturated fatty acids (UFA) undergo an intensive biohydrogenation process. Acid C18:2 n-6, as one of the main fatty acids in the meal, in this process gives its conjugated isomers, which in part can be absorbed and incorporated into body and milk fat (Crnkić, 2009). Components of the milk fat globule membrane important for health are: cholesterol-lowering factor, cancer cell growth inhibitors, xanthine oxide as an acid-bacterial member, butyrophylline as a possible suppressor of multiple sclerosis, and phospholipids as factors against colon cancer, gastrointestinal pathogens, Alzheimer's illness, depression, and stress (Spitsberg, 2005).

Zemel (2001) described the role of milk and dairy products in lowering blood pressure since calcium reduces levels of vitamin D in the blood and leads to a decrease in intracellular calcium levels in vascular smooth muscle, and thus lowers blood pressure. The aim of this study was to determine the fatty acid composition of sheep's milk from the area of Livno and Travnik (Vlašić), with special reference to the content of bioactive fatty acids that have a positive effect on human health. Although not all pathways of biohydrogenation of fatty acids in rumen are known, as well as the effect of certain nutrients on the composition of milk fat, it was found that certain diets can still affect the content and composition of milk fat. Milk fat molecules can also modulate immunity, reduce low-density lipoprotein, cholesterol, and can act as effective antibacterial agents. Thus, the quality of raw milk can be improved by a certain feeding strategy and thus increase the share of biologically active ingredients in raw milk, indicating that in technological terms such milk can be an excellent raw material for processing and marketing high quality dairy products. Milk should be considered as a complete and complex raw material that contains active substances potentially effective in promoting good health.

MATERIALS AND METHODS

Sheep milk producers from Livno and Travnik (Vlašić) were selected for the study. The analysis were performed on Pramenka Sheep. The animals were marked with the appropriate number of ear tags on the basis of which samples were always taken from the same animals over different time periods. During sampling, sheep feeding was based on grazing. In the Livno area, two milk samplings were performed - July (n = 20) and August (n = 20), while the samples in the third sampling were insufficient in quantity to perform all planned analyzes. In the Travnik area, milk was sampled in three terms - July (n = 25), August (n = 25) and September (n = 25). Milk was sampled in plastic bottles à 50 ml and then deposited at -20°C until the beginning of the analysis. The following fatty acids were determined by gas chromatography (GC) in milk: butyric (C4:0), caproic (C6:0), caprylic (C8:0), capric (C10:0), lauric (C12:0), myristic (C14:0), pentadecane (C15:0), palmitic (16:0), margaric (C17:0), stearic (C18:0), arachic (C20:0), myristoleic (C14:1 cis-9), palmitoleic (C16: 1 cis-9), oleic (C18:1 cis-9), C18: 1 cis-11, elaidic (C18: 1 trans-9), C18:1 trans-10, vaccenic (C18: 1 trans-9) -11), arachidonic (C20: 4c5, c8, c11, c14), eicosapentaenoic acid (C20: 5c5, c8, c11, c14, c17), docosahexaenoic acid (C22: 6 c7, c10, c13, c16, c19), linoleic (C18: 2 n-6), α linolenic (C18: 3 n-3) and rumenic (C18: 2 cis-9 trans-11 CLA).

Samples were transported frozen on dry ice and analyzed at "As Vitas Laboratory", Oslo Innovation Center, Norway. Milk samples were thawed at room temperature and homogenized before analysis. Sample preparation was performed according to the procedure described by Luna et al. (2005), which involves the separation of milk fat by centrifugation and methylation of fatty acids to form fatty acid methyl esters (FAME) which were analyzed on a gas chromatograph. The GC method with high resolution was applied, which enables very good separation of FAME. A 200 mm long Select FAME column and an 18:1 isothermal peak separation were used. The analysis was performed on an Agilent 689N GC instrument with split/splitless injector, 7683B autosempler and flame ionization detector (Agilent Technologies, Palo Alto, CA). Separation was performed using a capillary column of fused silica (Varian Inc.) CP-SELECT CB FOR FAME (200 mm long, 0.25 mm inner diameter and 0.25 μm film thickness). The following temperature program was applied: initial temperature of 70°C was maintained for 4 minutes, then heating at a rate of 20°C per minute to a temperature of 160°C, which is maintained for 80 minutes, and then heating at a rate of 3°C per minute to a temperature of 220°C, which lasts 28 minutes. Hydrogen with a pressure of 314 kPa was used as the carrier gas. The analysis of fatty acids (C4:0-C22:6) was performed by autoinjection of 1 μl of the sample at a split ratio of 70:1, a hydrogen flow of 151 ml/min and a temperature of 280°C. The temperature of the flame ionization detector was 290°C with a flow rate of hydrogen of 40 ml/min, air of 450 ml/min and nitrogen (as make-up gas) of 45 ml/min. The frequency of recording the points on the chromatogram was 10 Hz (reading 10 times per second), and the recording time of one chromatogram was 136 minutes. The results obtained are expressed in grams of individual fatty acids per 100 g of total fatty acids (g/100 g FA).

Statistical analisys was performed using the software package/program SPSS 21.00. The differences were considered statistically significant at the level of $p<0.05$, $p<0.01$ and $p<0.001$. The Mann Whitney test was used to examine differences in milk fatty acid content between individual sampling periods from Livno and Travnik, and the Kruskal-Wallis H test was used to examine differences in bioactive milk fatty acid content between Livno and Travnik areas regardless of sampling period.

RESULTS AND DISSCUSION

The results of this study show that the profile of fatty acids in sheep 's milk from both sampling areas (Livno, Travnik) is significantly affected by nutrition and botanical composition of pastures, as well as lactation period. A total of 24 fatty acids were determined over three sampling time periods (July, August and September). The median values for fatty acid content in sheep's milk from 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. The medians for the values of the majority of SFA in milk from the Livno area (Table 1) were lower in the 2nd compared to the 1st sampling, which was very highly statistically significant in the case of acids C6:0 to C15:0. The median for the C18:0 content in milk was highly significantly higher in 2nd sampling. On the other hand, monounsaturated fatty acids (MUFA) were found in higher amounts in 2nd compared to 1st sampling, and a statistically significant difference was found only for C18:1 cis-9 acid content. The reverse was found for C18:1 trans-11 acid whose median value was numerically lower in 2nd sampling. For polyunsaturated fatty acids (PUFA), there was no clear trend of differences between the two samples, except for C18:3n-3 acid, whose median value was significantly lower in 2nd compared to 1st sampling. The values of the median for majority of fatty acids in the milk of sheep from the Travnik area (Table 2) showed variations between sampling periods. The median values of saturated acids C4:0, C6:0 and C18:0 showed a statistically significant decrease in the 3rd sampling period compared to the first two, while the opposite was found for acids C14:0 and C16:0 (Table 2). In the MUFA class for C18:1 cis11 and C18:1 trans11, a statistically significant trend of decreasing median values by sampling periods was also found (Table 2). In the PUFA class, a statistically highly significant increase in DHA acid was found, and a highly statistically significant decrease in C18:3 n-3 acid. There were also significant differences in CLA, but there was no clear trend. By testing the differences in the content of fatty acids between the area of Livno and Travnik by sampling periods, statistically significant differences in the content of 17 out of a total of 24 determined fatty acids were observed. Comparing the median values of bioactive fatty acids of sheep's milk (Table 4), regardless of the sampling period (all samples collectively), the determined concentration of most fatty acids was higher in sheep's milk from the Travnik area. A statistically significant difference between bioactive fatty acids in sheep's milk from the Livno and Travnik areas was found in C4:0, ARA, EPA, DHA and CLA. The median C4:0 was higher in milk from the Livno area, while the median ARA, EPA, DHA and CLA were higher in milk from the Travnik area.

Table 1 Median value of fatty acid content in sheep milk fat for two sampling periods from the Livno area

	1st sampling	2nd sampling	p
Fatty acids (g/100g FA)	**SFA**		
Butyric C4:0	3.86	3.69	
Caproic C6:0	2.08	1.40	***
Caprylic C8:0	1.64	0.98	***
Capric C10:0	4.29	2.81	***
Lauric C12:0	2.66	2.07	***
Myristic C14:0	9.55	8.45	***
Pentadecane C15:0	1.18	1.07	***
Palmitic C16:0	22.30	21.85	
Margaric C17:0	0.81	0.82	
Stearic C18:0	8.64	9.72	**
Arachic C20:0	0.42	0.43	
	MUFA		
Myristoleic C14:1cis-9	0.25	0.27	
Palmitoleic C16:1cis-9	0.90	1.00	
Oleic C18:1cis-9	17.93	22.27	***
C18:1 cis-11	0.89	0.95	
Elaidic C18:1 trans-9	0.28	0.40	
C18:1 trans-10	0.50	0.57	
Vaccenic C18:1 trans-11	2.87	2.48	
	PUFA		
Arachidonic C20:4 n-6	0.16	0.17	
Eicosapentaenoic C20:5 n-3 (EPA)	0.15	0.12	
Docosahexaenoic C22:6 n-3 (DHA)	0.10	0.09	
linoleic C18:2 n-6	2.46	2.70	
α- linoleic C18:3 n-3	2.26	1.34	***
Rumenic C18:2 cis-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	**
Ratio of the sum of fatty acids			
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	***

***p<0.001, ** p<0.01; *p<0.05; 1st, 2nd sampling – represent sampling periods: July and August

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

Table 2 Median value of fatty acid content in sheep milk fat for three sampling periods from the Travnik area

	1st sampling	2nd sampling	3rd sampling	p
Fatty acids (g/100g FA)		**SFA**		
Butyric C4:0	3.43[a]	3.30[a]	2.86[b]	***
Caproic C6:0	1.86[a]	1.77[a]	1.49[b]	*
Caprylic C8:0	1.47	1.32	1.22	
Capric C10:0	3.87	3.60	3.68	
Lauric C12:0	2.51	2.24	2.83	
Myristic C14:0	9.01[a]	9.05[a]	10.19[b]	*
Pentadecane C15:0	1.21	1.16	1.13	
Palmitic C16:0	21.62[a]	22.58[a]	23.74[b]	**
Margaric C17:0	0.70	0.73	0.66	
Stearic C18:0	9.22[a]	9.37[a]	7.70[b]	***
Arachic C20:0	0.37	0.41	0.38	
		MUFA		
Myristoleic C14:1cis-9	0.55	0.37	0.35	
Palmitoleic C16:1cis-9	1.01	1.04	1.16	
Oleic C18:1cis-9	20.90	20.77	20.83	
C18:1 cis-11	0.74[a]	0.71[a]	0.59[b]	***
Elaidic C18:1 trans-9	0.26	0.26	0.23	
C18:1 trans-10	0.35	0.31	0.26	
Vaccenic C18:1 trans-11	3.20[a]	2.61[b]	2.55[b]	**
		PUFA		
Arachidonic C20:4 n-6	0.23	0.24	0.24	
Eicosapentaenoic C20:5 n-3 (EPA)	0.14	0.14	0.15	
Docosahexaenoic C22:6 n-3 (DHA)	0.11[a]	0.15[b]	0.18[b]	**
linoleic C18:2 n-6	2.44	2.57	2.19	
α- linoleic C18:3 n-3	1.91[b]	1.98[b]	1.64[a]	**
Rumenic C18:2 cis-9, trans-11 (CLA)	2.21[a]	1.69[b]	2.04[a]	***
∑n-3	2.08	2.29	2.02	
∑n-6	2.64	2.74	2.55	
∑SFA	55.93	56.85	56.73	
∑MUFA	27.39	26.11	27.38	
∑PUFA	6.71	6.98	6.66	
∑UFA	33.84	33.25	34.16	
Ratio of the sum of fatty acids				
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	

Median values with different superscripts differ significantly, where***represent $p<0.001$, ** $p<0.01$, * $p<0.05$; 1st, 2nd, 3rd sampling – represent sampling periods: July, August and September; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; UFA – unsaturated fatty acids

Table 3 Statistical significance of differences in milk fatty acid content from the area of Livno and Travnik between sampling periods

Fatty acids	$L1^{st}/T1^{st}$	$L1^{st}/T2^{nd}$	$L1^{st}/T3^{rd}$	$L2^{nd}/T1^{st}$	$L2^{nd}/T2^{nd}$	$L2^{nd}/T3^{rd}$
Butyric C4:0	*	*	*	*	*	*
Caproic C6:0					*	*
Caprylic C8:0					*	
Capric C10:0						
Lauric C12:0				*		
Myristic C14:0		*	*			
Pentadecane C15:0		*				
Palmitic C16:0			*		*	*
Margaric C17:0						
Stearic C18:0						
Arachic C20:0		*				*
Myristoleic C14:1cis-9		*				
Palmitoleic C16:1cis-9						
Oleic C18:1cis-9		*				
C18:1 cis-11						
Elaidic C18:1 trans-9			*		*	
C18:1 trans-10		*			*	
Arachidonic C20:4 n-6						
Eicosapentaenoic C20:5 n-3 (EPA)		*			*	
Docosahexaenoic C22:6 n-3 (DHA)		*				
linoleic C18:2 n-6						
α- linoleic C18:3 n-3		*		*	*	*
Rumenic C18:2 cis-9, trans-11 (CLA)		*			*	
Arachidonic C20:4 n-6						*
∑n-3	*		*	*	*	*
∑n-6						*
∑SFA						*
∑MUFA	*	*	*		*	
∑PUFA				*	*	*
∑UFA	*	*	*			
Ratio of the sum of fatty acids						
n-6/n-3	*	*	*	*	*	*
SFA/MUFA	*	*	*		*	
SFA/PUFA						
MUFA/PUFA	*		*	*	*	*
SFA/UFA	*		*			
UFA/MUFA	*		*	*	*	*
UFA/PUFA	*		*	*	*	*

*p<0.05. L – LIVNO sampling area; T – TRAVNIK sampling area. 1^{st}, 2^{nd}, 3^{rd} – represent sampling periods;
SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; UFA – unsaturated fatty acids

Table 4 The value of the median content of bioactive fatty acids in sheep's milk from the area of Livno and Travnik for all samples collectively

Fatty acids (g/100g FA)	Livno	Travnik	p
Butyric C4:0	3.64	3.24	*
Caproic C6:0	1.75	1.73	
Caprylic C8:0	1.32	1.37	
Capric C10:0	3.69	3.83	
Stearic C18:0	9.02	8.77	
Oleic C18:1cis-9	20.54	21.07	
linoleic C18:2 n-6	2.46	2.36	
Vaccenic C18:1 trans-11	2.73	2.89	
α- linoleic C18:3 n-3	1.83	1.87	
Arachidonic C20:4 n-6	0.21	0.27	*
Eicosapentaenoic C20:5 n-3 (EPA)	0.13	0.15	*
Docosahexaenoic C22:6 n-3 (DHA)	0.11	0.14	*
Rumenic C18:2 cis-9, trans-11 (CLA)	1.66	2.0	*

*p<0.05

During the sampling period, sheep's milk from the Livno and Travnik areas contained a higher proportion of SFA compared to UFA. In most SFAs in sheep milk from the Livno area, the differences found between sampling periods are most likely due to differences in pasture composition at the time they were used for animal feed. Acids from the SFA class from the Livno area were statistically significantly related to the sampling period, respectively, in the direction of reducing their content towards the end of the lactation period (Table 1). The C4:0 acid content in milk samples from the Livno area had similar value as determined by study obtained by Mihaylova et al. (2005), but significantly lower than the value determined for Merino sheep milk by Mierlita et al. (2011). In both sampling areas, a declining trend for C4:0 was determined by sampling periods, with the differences found for the Travnik area being very highly significant (Tables 1 and 2). Meals that contain a higher amount of sugar cause the formation of C4:0, and these meals are characterized by a higher content of C4: 0 in milk fat.

In the area of Livno a statistically very significant difference was found for the values of C6:0, C8:0 and C10:0 acid, respectively, between the sampling period, again with a declining trend towards the end of lactation (Table 1), and the values were lower than the values obtained by other authors (Mierlita et al., 2011; Mihaylova et al., 2005). The C10:0 content in milk from both sampling areas was significantly lower than the values established by other authors (Addis et al., 2005). The content of C6:0 and C8:0 in the milk fat of sheep from the Travnik area also had a declining trend towards the end of the lactation period (Table 2), with the differences in C6:0 being statistically significant.

Depending on the locality and sampling period, statistically significant differences at the level of $p < 0.05$ were observed mostly for MUFA, PUFA, UFA and the ratio of the sum of fatty acids (Table 3). Differences are especially pronounced when comparing the values of fatty acid content between different sampling periods in different areas ($L1^{st}/T1^{st}$, $L1^{st}/T2^{nd}$; $L1^{st}/T3^{rd}$; $L2^{nd}/T1^{st}$, $L2^{nd}/T1^{st}$). Attention should be paid to the

flora of the Vlašić mountain and its age. With its geographical position, terrain configuration and mountain climate, the Vlašić Mountain significantly influences the composition, distribution and dynamics of the appearance of certain plant species in this ecosystem. Given the fact that grazing was a major part of the diet of sheep from both sampling areas in the period of our study, vegetation changes unquestionably affected the fatty acid composition of milk. The Livno area, with its geographical position, terrain configuration and characteristic climate, is an area of unique flora with a large number of interesting plant species. Botanical composition of forage plants and their percentage in these localities of Livno Canton can be, given the altitude and other climatic and edaphic conditions, classified as mountain natural grasslands.

The amount, composition and characteristics of produced milk, especially from sheep kept on pasture in given environmental conditions, depend on the combined effects of seasonal changes in climate and available food, as well as on variations in metabolic status of sheep due to lactation. All of these may explain the identified changes in fat acid composition of milk during this study.

Seasons that do not affect the animal organism as a whole eaquely, should also be observed, and it is necessary to look at individual factors and their possible impact on production performance. High air temperatures can adversely affect milk yield and fat content in milk, which could also affect the fatty acid composition of milk.

The C12:0 content in milk samples from the Livno area differed very highly statistically significantly between the sampling periods (Table 1). In their research, Valvo et al. (2007) found that the contents of C12:0, C14:0 and C16:0 were higher in the milk of sheep kept in stables, unlike sheep on pasture, which is a consequence of a higher share of C14:0 and C16:0 in hay and barley relative to pasture beans.

The most common MUFA in sheep's milk fat from Livno and Travnik is C18:1 cis-9, the value of which varied depending on the location and sampling period (Tables 1 and 2). This could be due to the seasonal effect associated with the feeding pattern in the summer. Popović-Vranješ et al. (2010) found that with the beginning of the grazing period, the share of C18:1 cis-9 in organic milk gradually increased, to reach a value higher than the average value determined in conventional milk in August. MUFA and PUFA acids show numerous positive effects, but it is important to note that they are very prone to oxidation, both inside and outside the body, resulting in the formation of highly reactive free radicals (Kravić, 2010; Marenjak et al., 2006).

In addition to the absolute content of n-3 fatty acids in the meal, the relationship between n-3 and other types of UFA, which are n-6 fatty acids, is no less important. In the analyzed milk samples from both areas, the content of C18:3 n-3 had a declining trend towards the end of the lactation period (Tables 1 and 2), which may be due to the vegetation stage, because younger plants are richer in C18:3 n-3, and its content decreases with the aging of vegetation. Acid C18:2 n-6 is metabolized in ARA, and C18:3 n-3 in DHA or EPA. This transformation is possible in the human body as well as in most animals, which has been proven by the use of denatured C18:3 n-3 (Tota and Milin, 2000). Increasing the intake of C18:2 n-6 in sheep during the grazing period is of

particular importance because it creates the conditions for increasing the CLA content in milk fat. Some authors indicate that increased C18:2 n-6 intake and grazing feed also increases the CLA content in milk (Popović-Vranješ et al., 2010).

The content of CLA in both sampling areas has a trend of variation by months of sampling, which may be due to feeding on pastures, especially in which phase of vegetation grasses are present, because our study shows a declining trend for CLA towards the end of lactation and the end of grazing period, when the nutritional value of plants decrease. Pasture feeding increases CLA in milk, especially the presence of grasses in the early stages of growth. Lower CLA values in sheep's milk from the Livno area may be due to the increased inflow of C18:1 cis-9 degradation intermediates from the rumen, especially the C18:1 trans-10 isomer. Tables 1 and 2 also show the total amounts of SFA, MUFA, PUFA and UFA milk of sheep from the area of Livno and Travnik. Statistically significant differences were found in most of the SFA and PUFA acids within the area and between areas by sampling periods (Table 1). Despite the differences in the content of individual fatty acids between the sampling periods, the trend remained the same in both areas. The cumulative share of SFA in sheep milk from both areas was higher compared to the cumulative share of MUFA and PUFA, but without statistical significance for the Travnik area, as well as between areas (Tables 1 and 3).

Examining the ratio of the amounts of fatty acids in milk samples from the Livno area, very highly statistically significant differences were found between the sampling periods for SFA/MUFA, MUFA/PUFA, UFA/MUFA and UFA/PUFA (Table 3), but not for SFA/PUFA (Table 1). PUFA acids fulfill many structural and functional roles that are incomparable among fatty acids due to the wide range of biological processes in which they participate (Andrišić, 2013). CLA has attracted considerable attention since it was found that they inhibit the synthesis of mammary tumor cells (Chinnadurai et al., 2008). This composition of bioactive fatty acids in sheep's milk from two areas can be particularly affected by climatic factors and soil composition because they determine the composition of plant communities on pastures used for sheep feeding.

CONCLUSION

Variation in fatty acid content both within and between sampling areas was found in this study, with relatively high SFA content. Also, sheep's milk from the Livno and Travnik areas contained a higher proportion of SFA compared to UFA. As expected, myristic, palmitic, stearic and oleic acids were the dominant fatty acids in both examined areas. Comparing the content of bioactive fatty acids of sheep's milk from the area of Livno and Travnik, a statistically significant difference was found in C4:0, ARA, EPA, DHA and CLA. Comparing the median values of bioactive fatty acids, regardless of the sampling period, the determined concentration of most fatty acids was higher in the milk of sheep from the Travnik area.

The content of total n-3 fatty acids in milk from the Livno area tended to decrease towards the end of the lactation period, and n-6 fatty acids reversed, and these differences between 1st and 2nd sampling were statistically significant. The highest values of the

content of total n-3 and n-6 fatty acids for the area of Travnik were determined in the 2nd sampling period, but without statistical significance of the differences between the sampling periods. Examining the ratio of sums of different classes of fatty acids in milk samples from the Livno area, statistically significant differences were found between sampling periods for SFA/MUFA, MUFA/PUFA, UFA/MUFA and UFA PUFA, with the exception of SFA/PUFA ratio. In milk from the Travnik area, the same ratios did not differ statistically significantly between sampling periods, possibly due to a more stable plant composition. Milk samples from the Travnik area contained more PUFA compared to milk from the Livno area and a more favorable SFA/PUFA ratio.

Conflict of interest statement: The authors declare that there is no conflict of interest.

REFERENCES

Addis M., Cabiddu L., Pinna G., Decandia M., Piredda G., Pirisi A., Molle G. (2005): Milk and Cheese Fatty Acid Composition in Sheep Fed Mediterranean Forages with Reference to Conjugated Linoleic Acid cis-9, trans-11. *J. Dairy Sci.*, 88:3443-3454.

Hrković-Porobija A., Rustempašić A., Velić L., Batinić V. (2020): Masno-kiselinski sastav tokom laktacije i zdravstveni značaj ovčijeg mlijeka. Radovi Poljoprivredno-prehrambenog fakulteta Univerziteta u Sarajevu, LXV, 70:99.

Elwood P. C., Pickering J. E., Hughes J., Fehily A. M., Ness A. R. (2004): Milk drinking, ischaemic heart disease and ischaemic stroke II. Evidence from cohort studies. *Eur. J. Clin. Nutr.,* 58(5):718-724.

Flis Z., Molik E. (2021): Importance of Bioactive Substances in Sheep's Milk in Human Health. *Int. J. Mol. Sci*. 22, 4364.

Lisak Jakopović L. K., Barukčić I., Božanić R. (2018): Bioaktivne komponente mlijeka. 43. hrvatski simpozij mljekarskih stručnjaka s međunarodnim sudjelovanjem, 36-37.

Ljubičić I., Britvec M., Mioč B., Prpić Z., Pavić V., Vnučec I. (2012): Florni sastav ovčarskih pašnjaka otoka Paga. *Mljekarstvo*, 62(4):269-277.

Luna P., Juarez M., De La Fuente M. A. (2005): Validation of a rapid milk fat separation method to determine the fatty acid profile by gas chromatography. *J. Dairy Sci.*, 88(10):3377-3381.

Marenjak T. S., Poljičak-Milas N. (2005): Utjecaj hranidbe krava na sastav bioaktivnih masnih kiselina u mlijeku. *Krmiva*, 47(5):245-252.

Marenjak T. S., Poljičak-Milas N., Delaš I. (2006): Biološki aktivne tvari u kravljem mlijeku i njihov učinak na zdravlje. *Mljekarstvo*, 56(2):119-137.

Mihaylova G., Jahre G., Odjakova T., Kafedjiev V. (2005): Fatty acid profile of milk from sheep raised on mountain pastures. *Biotechnology in Animal Husbandry*, 21(5-6):93-96.

Mierlita D., Daraban S., Lup F. (2011): Effects of breed on milk fatty acid profile in dairy ewes, with particular reference to cis-9, trans-11 conjugated linoleic acid. *South Afrikan J. Anim. Sci.*, 41(3):223-231.

Pascal G. (1996): Les apports quotidiens recommandés en lipides et en acides gras. *OCL*, 3:205-210.

Popović-Vranješ A., Krajinović M., Kecman J., Trivunović S., Pejanović R., Krajinović G., Mačak G. (2010): Usporedba sastava masnih kiselina konvencionalnog i organskog mlijeka. *Mljekarstvo*, 60(1):59-66.

Purchas R. W., Knight T. W., Busboom J. R. (2005): The effect of production system and age on concentrations of fatty acids in intramuscular fat of longissimus and triceps brachii muscles of Angus-cross heifers. *Meat Science*, 70(4):597-603.

Spitsberg, V. L. (2005): Bovine milk fat globule membrane as a potential nutraceutical. *J Dairy Sci*., 88(7):2289-2294.

Tudor M., Havranek J. (2009): Nutritivna i zdravstvena vrijednost fermentiranih mlijeka. *Hrvatski časopis za prehrambenu tehnologiju, biotehnologiju i nutricionizam*, 4(3-4):85-91.

Tota M., Milin Č. (2000): Metabolizam polienskih kiselina. *Biochemia Medica*, 10(1-2):11-20.

Valvo M. A., Bella M., Scerra M., Biondi L. (2007): Effects of ewe feeling system (gross vs concentrate) on milk fatty acid composition. *Options Mediterraneennes, series A*, 74:227-231.

Zemel, M. B. (2001): Calcium Modulation of hypertension and obesity: Mechanisms an implications. *J. Am. Coll. Nutr.,* 20(5):428-435.

Paper received: 03.07.2021.
Paper accepted: 13.10.2021.