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INFLUENCE OF VEGETATION ON THE FATTY ACID COMPOSITION OF SHEEP'S MILK FROM THE LIVNO AREA

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Abstract

Sheep milk is interesting as a food source, because it contain a significant amounts of ω-3 and ω-6 fatty acids in milk fat, high content of dry substances, and a large amount of minerals and vitamins. The aim of this study was to investigate effects of vegetation to fatty acid composition of sheep's milk, used for production of indigenous Livno cheese.

Livno area with its geographic location, terrain configuration and climate characteristic, represents area of unique flora with numerous interesting plant species. Utilizing summer pasture, it is possible to use natural way in manipulation of milk fatty acids. A total of 40 sheep's were used to investigate the effects of different milking and sampling period on milk fatty acids. The research was conducted during July and August 2008, at Livno area. Fatty acids in sheep milk were determined by gas chromatography in the laboratory Vitas As Oslo Innovation Centre, Norway.

A total of 24 fatty acids were determined over two sampling periods (July and August). During the sampling period, sheep milk from Livno areas contained a higher proportion of saturated fatty acids (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.

Composition of fatty acids in sheep's milk in this study had tendency of fluctuation and was characterized by relatively high content of SFA during pasture period.

***Key words**: Pramenka breed, Milk, Fatty acid.*

1. Introduction

Milk has a high nutritional and biological value and it is a significant part in the human diet. Sheep milk is interesting as a food source, because it contain a significant amounts of ω-3 and ω-6 fatty acids in milk fat, as well as other less common, such as linoleic acid isomers. Sheep's milk is widely produced in semi-arid countries and is mainly used for milk consumption and the production of a wide range of cheeses, fermented milk products and to a lesser extent, milk powder. Sheep's milk is characterized by a high content of dry substances and a large amount of minerals and vitamins.

Milk of sheep naturally grazed at permanent pasture areas usually is showing higher content of PUFA and MUFA in contrast with intensive or semi-intensive sheep breeds reported in the literature (Ptaček et al., [2]).

Utilising of summer pasture, it is possible to use natural way in manipulation of fatty acids content in milk. However, knowledge about effects of feeding type (grass, grains) to fatty acids in milk are scant. Pasture have positive influence to milk fat and composition of fatty acids, compared to grains. Altitude also have signifficant effect to fatty acids composition of milk, which is related to ingestion of more non-legume flowering plants in high pastures. Grazing positively affects the fatty acids (FA) composition in sheep milk fat with increased availability of PUFA in the milk. Changes of the FA profile in sheep milk due to pasture altitude, were related to variation in FA concentration in the pasture and the botanical composition of the pasture location. (Civdini et al., [1]).

Some authors suggest that the high concentration of PUFA in milk fat from cows grazing at high altitudes compared with lowlands, may be due to a number of factors associated with high altitude grazing including: lower temperatures, greater walking distance and/ or a decrease in fat intake (Cividini *et al.*, [1]). The addition of fresh grass, to dairy animal diets enhances the proportion of unsaturated fatty acids in milk fat compared to SFAs and increases the amount of conjugated linoleic acid (Djordjevic *et al.*, [3]).

Livno area with its geographic location, terrain configuration and characteristic climate, represents area of unique flora with numerous interesanting plant species. Botanic composition of fodder and their percentage ration in areas of Livno canton, considering high altitude and other climatic factors, could be classified as mountain natural lawn, characterised with content of flowering plants (*Scabiosa columbaria, Knautia arvensis*) and grass (*Nardus stricta, Festuca* sp.), which indicates a certain acidity of soil, because mentioned plants are residents of acid soils.

Having all of this in mid, the aim of this study was to investigate effects of vegetation to fatty acid composition of sheep's milk, used for production of indigenous Livno cheese.

2. Materials and Methods

A total of 40 sheep were used to investigate the effects of different sampling period on milk fatty acids (FA) profiles. The research was conducted during July and August, at Livno area (village Guber at 724 meters above sea level 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 and August-II). In the area of Livno, milk samples were taken twice - July (n = 20) and August (n = 20). 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.* [4], 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: C4:0, C6:0, C8:0, C10:0, C18:0, C18:1 cis-9, C18:3 n-3, C20:4 n-6, ARA, C20:5 n-3, EPA, C22:6 n-3, DHA, C18:2 cis 9, trans-11, CLA.

Statistical analysis was performed using the software package/SPSS 21.00. Nonparametric statistics were used for processing Wilcoxon test (distribution free tests) (for the area of Livno) were used. The differences were considered statistically significant at $p < 0.001$.

3. Results and Discussion

The mean values of fatty acid in milk of sheep breed in the area of Livno expressed in grams of each fatty acid per 100 g of total fatty acid (g/100g FA) are shown in Table 1, as well as the statistical significance of differences between sampling periods. The results of this research showed a significant influence on the profile of fatty acids in sheep's milk from Livno areas.

A total of 24 fatty acids were determined over two sampling periods (July and August). During the sampling period, sheep milk from Livno 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 it was used for animal grazing.

The literature indicates that grazing small ruminants, when adequately managed, contributes to grassland biodiversity maintenance. On the other hand, milk and cheese from grazing animals show higher nutritional and aromatic quality than those from stall-fed animals (Claps *et al.*, [5]). Grazing behavior is another key factor in specific landscape and pasture biodiversity. Grazing behavior has important contribution beside animal nutrition only, it affects the specific characteristics, features and quality of milk (Bonnanome *et al.*, [13]; Claps *et al.*, [6]). During sampling period, sheep's milk in our study had bigger ratio of SFA related to UFA. Milk fat have complex composition of fatty acids and some of important profiles of fatty acids in milk lipids are high ratio of C4:0 and other short chain and medium long chain fatty acids and low ratio of PUFA (Barbir *et al.*, [9]). In the most of SFA in sheep's milk, found differences between periods of sampling most likely are result of pasture composition during time it was used for grazing.

Table 1. Median values of fatty acid content in sheep milk fat for two samples from the Livno area

Parameters	I sampling	II sampling	p
Fatty acids (g/100 g FA)	**SFA**		
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	
C18:1 trans-9	0.28	0.40	
C18:1 trans-10	0.50	0.57	
C18:1 trans-11	2.87	2.48	
	PUFA		
C20:4 n-6	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	***
C18:2 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			
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	***

Legend: Mean values in the same row with different letter codes differ significantly; *** $p < 0.001$, ** $p < 0.01$; I, II - ; I, II, - represent sampling periods: July and August, SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids; UFA - unsaturated fatty acid.

Acids from SFA class individually have been statistically important related for sampling period in a way of reducing their content at the end of lactation period (Tab. 1). Then, for C6:0, C8:0 and C10:0 acids, statistically high differences were found between periods of sampling and again it was at the end of lactation period (Table 1), while found values were lower in a comparation with results mentioned by other authors (Mihaylova *et al.*, [12]; Mierlita *et al.*, [11]).

Different values of fatty acids in sheep's milk could be consequence of different management for feeding. Various feed like pasture, concentrates and different packed food used in rations, have effect to ratio of milk fatty acids (Tsiplakou *et al.*, [15]). After observation of pasture quality in Livno fields it could be ascertain that species from the group of legumes were most quality feed and vegetation changes in pasture had unquestionably effect to fatty acids content in milk.

SFA acids had mild trend of reducing in august, except C18:0 in which we've found mild increase (Table 1). Amount, composition and characteristics of produced milk, especially ewes kept in grazing conditions and other zoohigienic factors, depend of combined seasonal changes in climatic factors and available food, as well as variations of metabolic status of ewes which appear during late lactation, which could explain found changes in fatty acids content in milk during study.

Drough periods were not recorded, but in august lesser rainfalls were noted which resulted in more scarce vegetation, while feed source for ewes was only the pasture. Analysis of fatty acids composition in sheep's milk have shown high statistically important differences between periods of sampling and it was mostly in SFA. Valvo et al. [7], in their study had found that content of C12:0, C14:0 and C16:0 was bigger in milk of sheep kept in barn unlike grazing sheep, which is result of higher ration in C14:0 and C16:0 in hay and barley unlike legumes from pasture. Surroundings and pasture with their parameters have effect to fatty acids composition, volatile ingridients and proteolytic activity in milk. (Shingfield *et al.*, [8]; Hrkovic-Porobija *et al.*, [14]).

The most represented MUFA is C18:1 cis-9 whose value have fluctuated in according to period of sampling, which can be consequence of seasonal effect related to feed management during summer period. Popović-Vranješ et al. [10]) have found that with begining of grazing period, ratio of C18:1 cis-9 in organic milk had gradually increased and in august had reached values higher then average value found in conventional milk. Concentrations of ARA, EPA and DHA did not have statistical differences during sampling periods (Table 1), while EPA have ability to partially block conversion of n-6 fatty acids in harmful eicosanoids, which reduced risk of cardiovascular disorders (Popović-Vranješ *et al.*, [10]). Beside absolute content of n-3 fatty acids in rations, no less significant is relation between n-3 and other types of UFA, like n-6 fatty acids. Acid EPA, beside C18:3 n-3 and DHA is one of the most significant n-3 fatty acid.

Acids C18:2 n-6 and C18:3 n-3 are most dominant PUFA in milk. Content of C18:2 n-6 had trend of increase toward second period of sampling (Tab. 1). In analyzed milk samples, content of C18:3 n-3 had trend of decrease toward end of lactation period, which can be effect of feed, respectively stage of vegetation, because younger plants have more C18:3 n-3 and it decrease in older plants. More ingestion of C18:2 n-6 in sheep during grazing have important significance by creating conditions for increase of CLA content (isomer cis-9 trans-11-CLA) in milk fat, which is partly created by biohydrogenisation of C18:2 n-6 acid in rumen. Some authors point to the fact that with more ingestion of C18:2 n-6 and grazing, content of CLA in milk is increased (Popović-Vranješ *et al.*, [10]). Content of CLA had trend of variations according to months of sampling, which could be effect of feed in pastures, especially stage of vegetation of grass and other plants. This is because our study of CLA have shown trend of decreased values toward the end of lactation, while it is also the end of grazing period, because nutritional value of plants is decreasing. During summer grazing it is possible in natural way to manipulate with fatty acids content.

4. Conclusions

- Composition of fatty acids in sheep's milk in this study had tendency of fluctuation and was characterized by relatively high content of SFA during pasture period.
- It is possible that period of lactation had higher impact to concentration of SFA then type of pasture, because differences are more expressed, when values of fatty acids content are compared between sampling periods within same area. In milk samples as expected, dominant fatty acids were myristic, palmitic, stearin and oleic.

5. References

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