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THE EFFECT OF ALTITUDE ON THE FATTY-ACID PROFILE OF SHEEP MILK FROM BOSNIA AND HERZEGOVINA

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Abstract

Sheep breeding on the mountain pastures of Bosnia and Herzegovina is a tradition inherited from the ancestors. Indigenous sheep breeds are widespread and well-adapted to harsh mountain farming conditions. Each year, after weaning, the sheep start grazing in the lowlands, then gradually moving to mountain pastures. Grazing positively affects the composition of fatty acids in sheep milk, with increased availability of polyunsaturated fatty acids in grass, and subsequently in milk. Consequently, this work aimed to study the profile of fatty acids in sheep milk during grazing in two geographical areas (Livanjsko polje and Vlašić Mountain).

Milk was sampled from a total of 115 Pramenka sheep at two different pasture locations that differed in altitude: the location of pastures of Livanjsko Polje at an altitude of 700 - 750 m.a.s.l., mountain pastures of the Vlašić Mountain at an altitude of 1260 to 1280 m.a.s.l. Sheep milk samples were gathered during the grazing season from June to September and were analyzed by gas chromatography in the "As Vitas" laboratory of the Oslo Innovation Centre, Norway.

The content of fatty acids in sheep's milk in this study showed a tendency to vary, with a relatively high content of saturated fatty acids. Milk samples from the Vlašić Mountain contained more polyunsaturated fatty acids than milk from the Livno area and a more favorable ratio of saturated to polyunsaturated fatty acids. Milk samples from both study areas contained an almost ideal ratio of n-6/n-3 fatty acids. The concentration of most bioactive fatty acids was higher in sheep's milk from the Vlašić Mountain area.

Therefore, we can conclude that the altitude can influence the fatty acid profile of sheep milk.

***Key words**: Sheep, Milk, Fatty acid profile, Altitude.*

1. Introduction

The fatty acid (FA) profile plays a crucial role in determining the quality of milk and dairy products because it includes compounds considered potentially beneficial for human health, such as polyunsaturated FAs (PUFAs) belonging to the omega-3 and omega-6 families (PUFA n3, and PUFA n6), as well as conjugated linoleic acid (CLA), while also including others that are potentially harmful, such as certain saturated FAs (SFAs) (Kravić [9]).

The interest in improving the FA profile of dairy foods is evidenced by recent studies on the inclusion of this trait as a breeding goal in sheep breeding (Correddu *et al.*, [2]). Sheep's milk is an important source of bioactive substances with health-promoting functions for the body. The valuable composition of sheep's milk is due to its high content of fatty acids, immunoglobulins, proteins, hormones, vitamins, and minerals. Many biopeptides found in milk have antibacterial, antiviral, and anti-inflammatory properties. The nutritional quality of animal foods has become an important issue in the last few decades, and diet plays a major role in modulating the fatty acid composition of ruminant milk. Recently, yogurts with the addition of omega-3 fatty acids (O-3FAs) have been gaining popularity.

Sheep's milk contains short- and medium-chain fatty acids, representing approximately 11% of the fraction, which are extremely important for a healthy human diet. Sheep's milk has higher concentrations of butyric acid (C4:0), omega-3 fatty acids, and conjugated linoleic acid (CLA) compared to milk from other ruminants (Fils and Molik, [5]). Altitude directly influences the availability and botanical composition of the pasture, as well as indirectly affecting the amount of concentrate supplemented in the diet. Therefore, the altitude at which farms are located may provide indirect information about the type of feeding system through the analysis of milk fat (Mangia *et al.*, [3]). According to Collomb *et al.*, [4], different botanical forages in highlands have higher PUFA content, implying increased PUFA content in milk. By utilizing summer pasture, it is possible to naturally manipulate the fatty acid content in milk. However, knowledge about the effects of feeding types (grass, grains) on fatty acids in milk is limited. Pasture has a positive influence on milk fat and the composition of fatty acids compared to grains. Altitude also has a significant effect on the fatty acid composition of milk, which is related to the ingestion of more non-legume flowering plants in high pastures. Grazing positively affects the fatty acid composition in sheep milk fat by increasing the availability of PUFA in the milk. Changes in the FA profile in sheep milk due to pasture altitude are related to variations in FA concentration in the pasture and the botanical composition of the pasture location (Cividini *et al.*, [6]); Hrković-Porobija *et al.*, [7]).

This study aimed to determine the influence of altitude on the fatty acid composition of sheep's milk from the Livanjsko polje area and the Vlašić (Travnik) mountain.

2. Materials and Methods

The objective of this study was to investigate the impact of the geographical area, specifically the central and southwestern parts of Bosnia and Herzegovina, on the fatty acid profile of sheep milk. The study involved 115 sheep from two locations: Livanjsko Polje and Vlasic Mountain. The study included collecting fresh sheep milk samples during morning milking sessions conducted manually on three different occasions (July, August, and September). Milk was sampled in plastic bottles of 50 mL each and then transported in a frozen state using dry ice *via* express mail to the As Vitas Laboratory at Oslo Innovation Centre in Norway for the analysis of fatty acid composition. The fatty acids, including butyric acid (C4:0), caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), C14:1c9, C15:0, palmitic acid (C16:0), C16:1c9, C17:0, stearic acid (C18:0), C18:1 t9, C18:1 t10, C18:1 t11, C18:1 c9, C18:1 c11, gamma-linolenic acid (C18:2 n-6), alpha-linolenic acid (C18:3 n-3), C18:2 c9t11 (CLA), arachidic acid (C20:0), C20:4 c5, c8, c11, c14 (ARA), eicosapentaenoic acid (C20:5 c5, c8, c11, c14, c17 (EPA), and docosahexaenoic acid (C22:6 c7, c10, c13, c16, c19 (DHA), were determined in the milk using gas chromatography (GC).

Before analysis, the milk samples were thawed at room temperature and homogenized. Sample preparation was conducted following the procedure described in the study by Luna *et al.*, [8]). which involved separating the milk fat through centrifugation and methylating the fatty acids to obtain fatty acid methyl esters (FAME), which were then analyzed using gas chromatography (GC). The data were statistically processed using the SPSS 21.00 software package/program. Differences were considered statistically significant at the levels of $p < 0.05$, $p < 0.01$, and $p < 0.001$.

3. Results and Discussion

The composition of fatty acids (FA) in dairy products has gained significant interest among consumers due to its nutritional and health implications. Specific fatty acids found in dairy products can impact human health and play a crucial role in preventing metabolic diseases. This growing interest is evident in recent studies that have explored the potential of enhancing the fatty acid profile of milk in sheep and goats through breeding programs (Nudda *et al.*, [11]). A total of 24 fatty acids were determined across three sampling periods. The median values for the fatty acid content in sheep's milk are expressed in grams of each fatty acid per 100 grams of total fatty acids (g/100 g FA) and are presented in Tables 1 and 2.

In recent years, there has been significant scientific interest in the health benefits associated with the consumption of animal products, particularly milk and meat from ruminants. These products are known to be rich sources of polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA). Specifically, the n-3 and n-6 PUFA have been shown to have positive effects on human health, including protection against coronary and cardiovascular diseases, as well as prevention of cancer and other diseases. Some researchers suggest that maintaining a low n-6/n-3 ratio (1 - 4 : 1) may lead to better health outcomes, as it allows for the optimal conversion of α-linoleic acid to eicosapentaenoic acid, which is beneficial for human health (Massouras *et al.*, [10]).

Table 1. Median values of the fatty acid content in sheep's milk fat for two samplings from the area of Livanjsko Polje

Fatty acid (g/100g FA)	I sample	II sample	p
	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	
Σn-3	2.52	1.62	***
Σn-6	2.61	2.91	*
ΣSFA	57.29	53.78	**
ΣMUFA	23.97	28.09	***
ΣPUFA	6.89	6.01	*
ΣUFA	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.

Table 2. Median values of the fatty acid content in sheep's milk fat for two samplings from the Vlašić Mountain area

Fatty acid (g/100g FA)	I sample	II sample	III sample	p
		SFA		
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		
C14:1 cis-9	0.55	0.37	0.35	
C16:1 cis-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]	***
C18:1 trans-9	0.26	0.26	0.23	
C18:1 trans-10	0.35	0.31	0.26	
C18:1 trans-11	3.20[a]	2.61[b]	2.55[b]	**
		PUFA		
C20:4 n-6	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]	**
C18:2 cis-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	
Ratio				
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	

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.

The research conducted on both sampling areas has shown that the botanical composition of the pastures and the lactation period had a significant effect on the fatty acid profile of sheep's milk. Median values for most saturated fatty acids (SFA) (Tab. 1) were lower in the second sampling period (II) compared to the first sampling period (I). On the other hand, monounsaturated fatty acids (MUFA) were found in higher amounts in the second sampling period (II) compared to the first sampling period (I), and a statistically significant difference was observed only for the content of C18:1 cis-9 acid.

In terms of polyunsaturated fatty acids (PUFA), there was no clear trend in differences between the two sampling periods, except for C18:3n-3 acid, where the median value was significantly lower in the second sampling period (II) compared to the first sampling period (I). Median values for most fatty acids in sheep's milk from the Vlašić Mountain area (Table 2) showed variations between the sampling periods. Significant differences in the content of 17 out of 24 fatty acids were observed between the Livanjsko Polje and Vlašić Mountain (Travnik) areas in different sampling periods. Statistically significant differences were found between the fatty acid composition in sheep's milk from the Livanjsko polje and Vlašić Mountain areas for C4:0, C20:4 n-6, ARA, EPA, DHA, and CLA, with higher median values for ARA, EPA, DHA, and CLA in milk from the Vlašić Mountain area (Table 3).

Table 3. Statistical significance of differences in the fatty acid content of milk from the Livanjsko Polje and Vlašić Mountain between sampling periods

Fatty acid g/100g FA	Livanjsko Polje	Vlašić Mountain	p
C4:0	3.64	3.24	*
C20:4 n-6	0.21	0.27	*
C20:5 n-3 (EPA)	0.13	0.15	*
C22:6 n-3 (DHA)	0.11	0.14	*
C18:2 cis-9, trans-11 (CLA)	1.66	2.0	*

In both sampling areas, regardless of the altitude, the milk samples contained a higher proportion of saturated fatty acids (SFA) compared to unsaturated fatty acids (UFA). The dominant fatty acids in the milk samples from both areas were myristic acid, palmitic acid, stearic acid, and oleic acid, as expected. The content of oleic acid, which has high biological and nutritional value, was higher in the milk samples from both areas compared to the values reported by other researchers in their studies. Our research shows a trend of decreasing CLA content in milk towards the end of lactation and the end of the grazing period when the nutritional value of the vegetation decreases. The content of total n-3 fatty acids in milk from the Livanjsko polje area tended to decrease towards the end of the lactation period, while n-6 fatty acids showed the opposite trend, and these differences between the first and second samplings were statistically significant. The highest values of total n-3 and n-6 fatty acid content for the Vlašić Mountain area were found in the second sampling period but without statistically significant differences between the sampling periods. When examining the ratios of different classes of fatty acids in milk samples from the Livanjsko Polje area, statistically significant differences were found between the sampling periods for SFA/MUFA, MUFA/PUFA, UFA/MUFA, and UFA/PUFA ratios, except for the SFA/PUFA ratio. In milk from the Vlašić Mountain area, these ratios did not significantly differ between the sampling periods, possibly due to a more stable composition of the vegetation. Milk samples from the Vlašić Mountain area contained higher PUFA content compared to milk from the Livanjsko Polje area, and a more favorable SFA/PUFA ratio. The concentration of most bioactive fatty acids was higher in sheep's milk from the Travnik area, and the differences were statistically significant for ARA, EPA, DHA, and CLA. Milk from sheep in the Livno area contained significantly higher levels of only butyric acid.

One of the current research directions in modern animal production is the investigation of CLA content in animal products and the possibilities of increasing it, due to the biological activities of CLA, which include anticarcinogenic, antiatherogenic, and antidiabetic effects. In milk samples from the Travnik area, CLA was the second most abundant PUFA. Lower levels of CLA in sheep's milk from the Livno area may be due to the increased influx of byproducts of oleic acid degradation from the rumen, especially the C18:1 trans-10 isomer. The content of these isomers was higher in the milk from sheep in the Livno area, and it has been found that even without impaired desaturase activity in the mammary gland, they can lead to lower CLA values in milk. Statistically significant differences at a significance level of $p < 0.05$ were observed in the content of MUFA, PUFA, UFA, and the ratio of total fatty acids in milk, depending on the location and sampling period. Attention should be paid to the flora of the Vlašić Mountain and its age. The Vlašić Mountain, with its geographical position, terrain configuration, and mountain climate, significantly influences the composition, distribution, and dynamics of certain plant species in this ecosystem. Considering that grazing was the main part of the diet for sheep in both sampling areas during our research period, vegetative changes undoubtedly affected the fatty acid composition of the milk. Livno area, with its geographical position, terrain configuration, and distinctive climate, represents an area with unique flora and a large number of interesting plant species.

The botanical composition of forage plants and their proportional representation in these locations in the Livno Canton, which can be classified as natural mountain grasslands based on altitude and other climatic and edaphic conditions play a significant role in the observed variations. Comparing the median values of fatty acids in sheep's milk (Table 3), regardless of the sampling period (all samples combined), higher concentrations of most fatty acids were found in milk from the Vlašić Mountain area. Statistically significant differences in fatty acid composition were observed between sheep's milk from the Livnjsko Polje and Vlašić Mountain areas for C4:0, C20:4 n-6, ARA, EPA, DHA, and CLA. The median value of C4:0 was higher in milk from the Livnjsko Polje area, while the median values of ARA, EPA, DHA, and CLA were higher in milk from the Vlašić mountain area. Collomb *et al.*, [4], emphasize the influence of the botanical composition of grass on milk composition in lowlands (altitude 600 - 650 m), mountains (900 - 1200 m), and hills (1275 - 2120 m). Lowland grasslands consist of grasses and legumes, and their botanical diversity is significantly lower compared to mountain pastures. As the altitude increases, the proportion of grasses decreases, and the number of species, especially Compositae, Rosaceae, and Plantaginacea, increases.

In addition to the absolute content of n-3 fatty acids in the diet, the ratio between n-3 and another type of UFA, namely n-6 fatty acids, is equally significant. In both sampling areas, the content of C18:3 n-3 showed a decreasing trend towards the end of the lactation period (Tables 1 and 2), which could be attributed to the stage of vegetation. Younger plants tend to be richer in C18:3 n-3, and their content decreases as vegetation progresses.

The content of CLA in both sampling areas exhibited monthly variations, which could be influenced by the grazing feed, particularly the stage of vegetation of the present grasses. Our research indicates a declining trend of CLA values towards the end of lactation and the end of the grazing period when the nutritional value of the plant cover decreases. Grazing on pastures increases CLA in milk, especially when grasses are abundant in their early growth stage. Tables 1 and 2 also present the total quantities of SFA, MUFA, PUFA, and UFA in sheep's milk from both sampling areas. Statistically significant differences were observed mainly in SFA and PUFA acids within and between the areas across the sampling periods (Table 1). Despite variations in the content of individual fatty acids between sampling periods, the overall trend remained the same in both areas.

By examining the ratios of total fatty acids in the milk samples (Table 2), highly 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 numerous structural and functional roles that are incomparable among fatty acids due to their involvement in a wide range of biological processes (Andrišić, [1]). The composition of fatty acids in sheep's milk from the two areas can be influenced by altitude, as well as climatic factors, and soil composition, as they determine the composition of plant communities in the pastures used for sheep grazing. Cividini *et al*., [6], emphasize the effect of mountain grazing and altitude on the FA profile of sheep's milk. Diet plays a major role in modulating the fatty acid composition of ruminant milk. It is well known that the intake of fresh forages has a positive influence on polyunsaturated fatty acids (PUFA), particularly CLA and omega-3, compared to diets based on dry forage and concentrates. Altitude directly influences grass availability and the botanical composition of the pasture and indirectly affects the amount of concentrate supplemented in the diet. Therefore, the altitude at which farms are located can provide indirect information about the type of feeding system through the analysis of milk fat.

4. Conclusions

- The content of fatty acids in sheep's milk in this study showed a tendency to vary, with a relatively high content of saturated fatty acids. Milk samples from the Vlašić Mountain contained more polyunsaturated fatty acids than milk from the Livno area and a more favorable ratio of saturated to polyunsaturated fatty acids.
- Milk samples from both study areas contained an almost ideal ratio of n-6/n-3 fatty acids. The concentration of most bioactive fatty acids was higher in sheep's milk from the Vlašić Mountain area.
- The composition of fatty acids in milk fat can vary significantly between lowland and highland regions due to differences in botanical composition. The plants on which the animals graze can be considered as a factor that naturally modifies the composition of milk fat.

5. References

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