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THE INFLUENCE OF OIL ADDITION ON THE FATTY ACID COMPOSITION OF SHEEP'S MILK DURING LACTATION

SUMMARY

The aim of this study was to determine the impact of plant sources of fat in food for sheep on the fatty acid composition of milk samples obtained from 210 sheep (herd of pure Pramenka and a herd of crossbred sheep) in the area of the Una Sana Canton. The studies were conducted in three periods: winter, spring and summer, and the herd is divided into experimental and control groups of sheep. Extruded flax seed has been added to nutrition in experimental group of sheep in every period of research in the amount of 3.5%. The total content of saturated fatty acids was the highest during the summer with the experimental group (70.75%), which plant sources of fat were added to. The most common saturated fatty acids in the analyzed samples of milk were: palmitic, myristic, stearic and lauric, whose values varied depending on the treatment of nutrition and research period. The differences found in the content of saturated fatty acids between the period of investigation of milk sample of experimental and the control group showed statistically very highly significant effect ($p<0.001$). The content of unsaturated fatty acids was the highest during the summer in the control group of sheep (32.00%). Of monounsaturated fatty acids mostly consisted of oleic during the summer period (25.60%) in the milk of the control group. Of polyunsaturated fatty acids, linoleic acid (3.6%) in the milk of control and α-linolenic (1.90%) of milk experimental group of sheep were the most prevalent in the winter. The lowest ratio of omega-6/omega-3 fatty acids in sheep's milk was achieved in the period of feeding sheep with old grass (July), 1.46% in the milk of the experimental group of sheep that were fed with concentrates in which omega-3 preparations were added.

Keywords: sheep's milk, fatty acids, SAFA, MUFA, PUFA

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INTRODUCTION

Sheep farming is an important branch of livestock production, especially in hill-mountain areas that are rich with important natural resources. Based on production scope, sheep's milk does not have the same value as cow's milk, but based on its composition and technological properties, it has considerably better quality and is a better raw material for processing. In EU countries, sheep's milk production is significantly better organized compared to other countries of the world thanks to the market that recognized quality of sheep's milk products (Bulletin of the IDF No. 391/2004). Over the last few years, fatty acids content and quality of human nutrition have become the main nutrition topic. Evidences for this can be found in more recent French nutrition guidelines that point out to the recommended input of some fatty acids in diversification of nutrition (Anses, 2011). Dairy industry needs to confront with 2 main issues regarding these new recommendations: (1) identification of fatty acids content to the consumer's request and (2) the way of a precise notation of fatty acids content. In correlation to the first issue, conducted researches have shown that modification of nutrition (Chilliard *et al*., 2007; Raynal-Ljutovac *et al*., 2008; Coppa *et al*., 2013) and genetic selection (Arnould & Soyeurt, 2009; Schennink *et al*., 2009; Stoop *et al*., 2009) can be effective at change of composition of fatty acids found in milk. Many researches warned about possibility of modelling fatty acids' content of milk fat in order to increase content of desired n-3 unsaturated fatty acids and to decrease content of saturated fatty acids with adequate nutrition. The primary goal and task of this paper were, based on theoretical and experimental studies, to make an assessment about influence of nutrition that is enriched with omega-3 preparations (extruded flax seed 3.5%) and decrease of disproportion in relation between omega-6 and omega-3 fatty acids in sheep's milk. Researches have shown that fatty acids of milk fat make milk especially important for consumers, due to its positive effects on human health (Babayan & Rosenau, 1991; Haenlein, 2002; Sretenović *et al*., 2009). However, only a small number of studies was conducted on fatty acids in sheep's milk, majority of studies were based on researches of fatty acids in cow's milk. Majority of studies is directed in the first place on influence of nutrition on profile of fatty acids of milk fat (Addis *et al*., 2005; Mele *et al*., 2007; Gomez-Cortes *et al.*, 2008; De La Fuente *et al.*, 2009; Tyagi *et al*., 2010). Few factors affect total profile of fatty acids in milk, but it seems that nutrition is the most important one. De La Fuente *et al*., (2009) found factors that are indirectly closely related to animal feed (e.g. combined effect of feed and the season). In that sense, it has been shown that during feeding, FA content of milk can be efficiently changed in order to obtain profile of FA closest to the ideal one: increased share of MUFA and PUFA, and decreased per cent of SAFA (Cabiddu *et al*., 2005; Gómez-Cortés *et al*., 2008). Concentration of PUFAs in the milk can be increased by nutrition (addition of linseed oil) that is enriched with α-linolenic acid, even if a part of them is hydrated in rumen (Addis *et al*., 2005).

MATERIAL AND METHODS

Two herds of sheep were taken for observations (a herd of pure Pramenka and a herd of crossbred sheep) in the area of the Una Sana Canton. The herds were placed with breeders in the area of Gata, Vrsta and Rujnica, Bihać Municipality. In herds, groups of 35 animals as experimental sheep and 35 control sheep, kept under the same conditions, were formed. Besides regular nutrition, concentrate mixtures in daily amount of 300 g per head were added to the sheep. The experimental group of sheep consisted of selected heads of Pramenka and crossbred sheep, which consumed a concentrate mixture with omega-3 added to their regular meal. The control group of sheep also consisted of selected heads of Pramenka and crossbred sheep, which, in addition to the regular meal (hay or pasture), consumed a concentrate mixture without the addition of omega-3 preparations. The experimental research was done during three different sheep feeding periods, as follows:

•First phase of the experiment - winter feeding period (February-March), Gata (35+35);

•Second phase of the experiment - grazing period, young grass (June), Vrsta (35+35);

•Third phase of the experiment - grazing period, old grass (July), Rujnica (35+35).

Concentrate mixture with addition of omega-3 preparation was added to the experimental group (Sg) of sheep. In addition to regular meal, concentrate mixture without omega-3 preparation is added to the control group (Cg) of sheep. Concentrated mixtures for this purpose were prepared according to the given recipe in the animal feed industry "JATA EMONA" Ljubljana (Slovenia). The chemical composition of the concentrate mixtures is shown in Table 1. and the composition of fatty acids in Table 2. Experimental researches were done during three different periods. The first period was winter, the second one spring, and the third one summer. During each period, research was carried out on 70 sheep (35 experimental and 35 control sheep). A total of 210 sheep were fed (105 experimental and 105 control herd).

Table 1. Chemical composition of concentrate mixtures added to experimental and control groups of sheep

Parameters	**Concentrate mixture with addition of omega-3 preparation**	**Concentrate mixture without addition of omega-3 preparation**
Dry matter (%)	88.9	88.9
Moisture (%)	11.1	11.1
Protein (%)	15.7	14.8
Fibers	7.4	7.3
Fat (%)	3.4	1.5
Ash (%)	8.6	11.5
Groups of n-3 fatty acids	**26.4**	**3.9**

In all research periods, a concentrate mixture with identical nutritional composition was fed to the sheep and the only difference was in the mixture for the experimental groups of sheep, to which omega-3 preparations were added.

Table 2. Fatty acid composition of the concentrate mixtures that were added to experimental and control groups of sheep

Fatty acids	Concentrate mixture with addition of omega-3 preparation	Concentrate mixture without addition of omega-3 preparation
C 10:0	0.1	0.1
C 12:0	0.0	0.0
C 14:0	0.1	0.2
C 15:0	0.1	0.1
C 16:0	11.2	15.3
C 17:0	0.1	0.1
C 18:0	2.7	2.1
C 20:0	0.2	0.3
C 22:0	0.2	0.2
C 23:0	0.1	0.1
C 24:0	0.2	0.2
C 16:1 c+t	0.2	0.3
C 18:1 c+t	22.1	24.3
C 20:1 c+t	0.3	0.4
C 18:2 c+t, n-6	35.9	52.2
C 20:2 n-6	0.1	0.1
C 18:3 c, n-3	26.4	3.9
Total area	100	100
Σ n-3	26.4	3.9
Σ n-6	35.9	52.3
n-6/n-3	1.4	13.3
MUFA	22.7	25.0
PUFA	62.4	56.2
SAFA	15.0	18.7
MUFA+PUFA	85.0	81.3
MUFA+PUFA/SAFA	5.7	4.3

Note: UFA - Unsaturated fatty acids; SAFA - Saturated fatty acids; MUFA - Monounsaturated fatty acids; PUFA - Polyunsaturated fatty acids.

Sampling time period

During the first phase of the experiment - the period of winter feeding that lasted two months (February and March, 2015), in addition to hay, the sheep of the experimental group were given a mixture with omega-3 preparations, and the sheep of the control group were given a mixture without omega-3 preparation. During the first phase of the experiment, samples were taken four times, in accordance with the instructions of the Rulebook on Raw Milk (Official Gazette of Bosnia and Herzegovina No. 21/11) and the Rulebook on Amendments to the Rulebook on Raw Milk (Official Gazette of Bosnia and Herzegovina No. 62/14) into dedicated sampling vessels. Samples of raw sheep's milk were taken every fifteen days from each head individually, for a total of thirty samples in both groups (control and experimental group). At the end of the first phase of the experiment, the fatty acid composition was determined on a collective sample obtained from milking. The second phase of the experiment was conducted in June. This is the period when the sheep were grazing and young grass was used as the basic food, which the sheep grazed on the pastures. Along with grazing, a concentrate mixture was added to the sheep nutrition: the experimental group was given a mixture with the addition of omega-3 preparations and the control group was given a concentrate without omega-3 preparations. The mixtures were given to the sheep in the morning before grazing. Samples of raw sheep milk were taken twice (every fifteen days), also thirty samples from both each group of sheep (experimental and control group), whereby the analysis of the fatty acid composition was performed on the collective samples obtained from milking. During the third phase of the experiment - the period of grazing with old grass (month of July), with the addition of concentrate mixtures, identical to the second phase. Samples of raw sheep milk were taken twice from thirty sheep from both groups of sheep (experimental and control) and the fatty acid composition was determined on collective samples obtained from milking.

Methods

Determination of fatty acids in sheep's milk has been done using gas chromatography (GC) with flame ionization detector (FID), according to ISO/TS 17764-2:2002. Preparation of fatty acid methyl esters (FAME) was done by in situ transesterification (ISTE) method by methanol without previous extraction, which is based on fatty acids hydrolysis in lipids and origination of fatty acid methyl esters. Separation of fatty acid methyl esters (FAME) is determined with usage of capillary gas chromatography.

The analysis was done on the device of Agilent Tehnologies 6890 N, Serial No. CN10351006. Capillary column of Supelco Omegawax 320 (dimensions 30 m, of the inner diameter 0.32 mm and thickness of stationary phase film of 0.25 µm), stock No. 24152, serial No. 53624-04-A was used. As a gas carrier, helium with pureness of 99,999% was used, and flow of 0.3 ml/min, make-up gas N2-25 ml/min, H2 - 30 ml/min, synthetic air (21% O2) 400 ml/min. FAMEs were identified by comparing time of keeping peaks in chromatogram with known fatty acids in standard solutions (Nu-Check Prep. Inc. Sigma). Weight percentage (ut. %) is calculated by using corresponding factor (Rf.) that is established based on quantitative standards.

Statistical analyses

For testing the influence of factors (term and treatment) on observed traits, variance analysis (ANOVA) was used. Among post-hoc tests for detection of differences between modalities, Tukey test was used. To represent the mutual connection (correlation) of the fatty acid composition in sheep's milk, Principal Component Analysis (PCA) was used, which was carried out on the basis of the correlation matrix. All statistical analyzes in this paper were performed in the computer program "R v.3.2.1" (R Development Core Team, 2015).

RESULTS AND DISCUSSION

The values of the analyzed fatty acids (g/100g of total fatty acid methyl esters) of sheep's milk fat from sheep monitored during the three diet periods (winter, spring, summer) of two groups of sheep (experimental - Sg and control group - Cg) are shown in table 3. By an analysis of the variance (ANOVA), it was established that there was statistically very significant influence of the experimental factor (period) on almost all the examined fatty acids of the sheep's milk ($p<0.001$), except for those fatty acids that had trace values or were not identified. Factor of treatment showed a very high statistically significant effect ($p<0.001$) on most of the tested fatty acids of the sheep milk. In addition, statistically very significant influence of factor interaction on the majority of the tested fatty acids was established. The most abundant SAFA in sheep's milk samples: palmitic, myristic and stearic, while the lauric was present in slightly lower values. The most abundant saturated fatty acid in sheep's milk samples was palmitic, which had a value in the winter diet period 32.6%, while in the milk of the control group, this value was significantly lower (27.2%). In the second study period (spring), palmitic acid value was 28.35% in the milk of the experimental group and in the control it increased to 35.7%, which was the highest established value of this saturated fatty acid during the study. In the third period (summer) in the milk of experimental group, there was a slight increase in palmitic acid (29.7%) compared to the milk of the control group (28.95%). The established differences in palmitic acid content between the research period, the milk of both the experimental and control group showed a very high statistically significant effect ($p<0.001$). The determined content of myristic acid was 12.5% in the milk of the experimental group of sheep in the period of winter nutrition, and in the control 12.85%. In the second period of research (spring), the values of myristic acid were in the milk of the experimental group 11.85% and in the milk of the control group 12.1% respectively. In the third period, the values were the highest, with the milk of the experimental group the determined value of 13.3% and in the milk of the control group 12.95%. The third in terms of presence is stearic one whose values of the experimental group were the largest in the second period of research (spring) 14.3% while in the milk in the control group it was lower - 10.05%. In the third period (summer) stearic acid was found in Sg - 12.9% and in Cg - 12.8%. In the first period (winter), stearic acid had the lowest established value (Sg: 10.7%, Cg: 9.6%).

Table 3. Profile of fatty acids in sheep's milk during research periods

Fatty acids (g/100 g FAME)[4]	**Winter [1]**		**Spring[2]**		**Summer [3]**		**Significance level (*p*)**		
	Sg	**Cg**	**Sg**	**Cg**	**Sg**	**Cg**	**period**	**treatment**	**interaction**
C12:0	3.7	6.0	3.70	6.00	4.20	3.65	***	***	***
C 14:0	12.50	12.85	11.85	12.10	13.30	12.95	***	***	***
C 16:0	32.6	27.2	28.35	35.70	29.70	28.95	***	***	***
C 18:0	10.7	9.6	14.30	10.05	12.90	12.80	***	***	***
C 20:0	0.25	0.3	0.40	0.40	0.30	0.35	*	ns	ns
C 22:0	0.1	0.1	0.20	0.20	0.20	0.20	***	ns	ns
C 24:0	0.1	0	0.1	0.1	0.1	0.1	ns	ns	ns
Σ Saturated	**68.50**	**68.45**	**68.25**	**69.35**	**70.75**	**67.65**	***	***	***
C 14:1	0.70	0.30	0.20	0.30	0.20	0.10	***	***	***
C 16:1 c+t	1.60	1.70	1.30	2.30	1.20	1.20	***	***	***
C 18:1 c+t	22.75	22.70	24.0	22.0	22.50	25.60	***	***	***
C 19:1 c+t	0.20	0.0	0.0	0.0	0.0	0.0	ns	ns	ns
C 20:1 c+t	0.20	0.0	0.0	0.0	0.20	0.0	ns	ns	ns
C 18:2 c+t, n-6	3.01	3.60	3.20	3.20	2.80	2.60	***	***	***
C 18:3 c, n-6	0.0	0.10	0.10	0.10	0.0	0.10	ns	ns	ns
C 20:3 n-6	0.10	0.0	0.0	0.0	0.0	0.0	ns	ns	ns
C 20:4 n-6	0.20	0.2	0.20	0.10	0.20	0.20	***	***	***
C 18:3 c, n-3	1.90	1.70	1.70	1.60	1.55	1.50	***	***	*
C 20:3 n-3	0.10	0.0	0.0	0.0	0.0	0.0	ns	ns	ns
C 20:5 n-3	0.10	0.20	0.20	0.20	0.20	0.10	***	ns	***
C 22:5 n-3	0.30	0.30	0.20	0.20	0.20	0.20	***	ns	ns
C 22:6 n-3	0.10	0.10	0.10	0.10	0.0	0.10	ns	ns	ns
Σ Unsaturated	**31.85**	**31.50**	**31.70**	**30.65**	**29.15**	**32.0**	***	***	***
Σ MUFA	**26.15**	**25.40**	**25.90**	**25.10**	**24.10**	**27.20**	**	***	***
Σ PUFA	**5.70**	**6.10**	**5.80**	**5.55**	**5.05**	**4.80**	***	**ns**	***
SAFA/UFA	2.14	2.17	2.15	2.26	2.11	3.00	***	***	***
Σ n-6	3.40	3.90	3.50	3.40	3.00	2.90	***	***	***
Σ n-3	2.30	2.20	2.30	2.15	2.05	1.90	***	**	ns
n-6/n-3	**1.48**	**1.77**	**1.52**	**1.58**	**1.46**	**1.53**	**	***	**
MUFA/SAFA	0.38	0.37	0.38	0.35	0.34	0.40	***	***	***
PUFA/MUFA	0.22	0.24	0.22	0.22	0.21	0.18	***	**	***
PUFA/SAFA	0.08	0.09	0.08	0.08	0.07	0.07	***	ns	***

*Note: p - significance level: *p<0.05; **p<0.01; *** p<0.001; ns - no statistical significance; Sg - Experimental group; Cg - Control group; 1,2,3- research periods: 1(winter); 2(spring); 3(summer); 4FAME - fat acid methyl esters; UFA - Unsaturated fatty acids; SAFA - Saturated fatty acids; MUFA - Monounsaturated fatty acids; PUFA - Polyunsaturated fatty acids.*

The total average value of SAFA in the first research period (winter) in experimental milk was 68.5% and in the control one 68.45%. In the second period (spring), the total average value of SAFA in the milk of the experimental group was 68.25% and in the milk of control group 69.35%. The highest total SAFA value was determined in the third period (summer) Sg: 70.75% and Cg: 67.65%. A very high statistically significant effect (p<0.001) was shown by both factors on the total SAFA content as well as on their mutual interaction. The total saturated fatty acids in sheep milk are presented graphically (Fig 1).

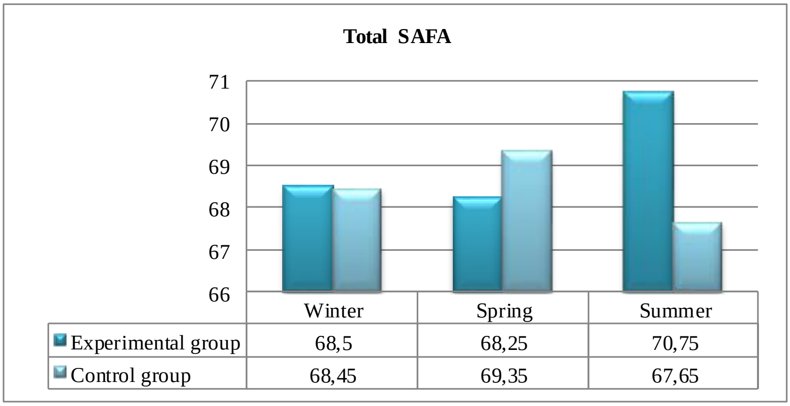

Figure 1. Total SAFA (%) in sheep's milk

Gómez-Cortés *et al*., (2009) in their studies added extruded flax seed to sheep concentrates and determined palmitic acid values of 21.02%, which is significantly lower than our results. The same authors established the values of myristic of 8.11%. which is not in accordance with our results, while the value of stearin of 12.46% was in correlation with our results in the third period of research. Similar results were found in earlier studies (De La Fuente *et al*., 2009) that established statistically high significance (p<0.001) in different periods of fatty acid research of sheep's milk, which is in correlation with our results. The most abundant unsaturated fatty acid in sheep's milk was oleic (MUFA). In the milk samples, its determined value in the first study period (winter) was 22.75% in the milk of the experimental group (Sg), and in the milk of the control group (Cg) 22.70%. In the second period (spring) in the milk of experimental group, its value increased to 24.0%. and in the milk of the control group it was 22.0%. In the third study period (summer), the value of oleic acid in the milk of the experimental group was 22.5% and in the milk of the control group it had the highest established value of 25.6%. At the beginning of the grazing period (spring and summer), the proportion of oleic acid in milk was gradually increasing. The influence of grazing on the increase in oleic acid content are indicated in research by Salamon *et al*., (2006) and Baltusnikienė *et al*., (2008). The established value of linoleic acid in the first peroid of research in the milk of the experimental group was 3.1% and was lower than the milk of the control group 3.6%. In the second period, the values were the same - 3.2%, while in the third period in the group of experimental groups the value decreased to 2.8%, and in the milk of the control group to 2.60%. The next significant PUFA was α-linolenic its values were decreasing from the first to the last research period and in both types of milk samples. Thus, the value of α-linolenic acid in the first period in milk of the experimental group was 1.90%, and in the milk of the control one 1.70%. In the second period, the milk of the experimental group had the value of 1.70% and milk of the control one 1.60%, so that in the third period of research its value was the lowest and amounted to the first 1.55%, and in the second 1.50%. Statistically very significant influence ($p<0.001$) on the content of unsaturated fatty acids was shown by the factor of term and factor of treatment, as well as on their mutual interaction. Linseed oil is a good source of α-linolenic acid whose content is of 62.2 ± 1.24 g/100 g in the fatty acids (Li *et al*., 2012). The mentioned authors added linseed oil to the feed for goats and the research was being carried out for eight weeks. As a result, the content of stearic, oleic and α-linolenic acid has been increased, which is in correlation with our research. The average value of MUFA in the first study period (winter) in the milk of the experimental group was 26.15% and in the milk of the control one 25.40%. In the second period (spring), the total average value of MUFA was 25.90% in the milk of the experimental group, and 25.1% in the milk of the control one, while the third period (summer) the milk of the experimental group was 24.10% MUFA and the milk of the control group 27.20%. The significance level ($p<0.01$) was shown by the factor of the term to the total MUFA content, while the factor of nutrition and interaction among factors showed a very high statistically significant effect ($p<0.001$). The total MUFA in sheep's milk was presented graphically (Fig 2).

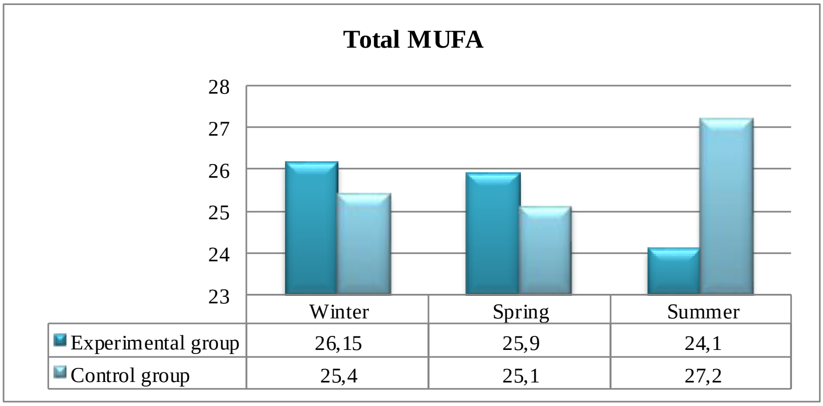

Figure 2. Total MUFA (%) in sheep's milk

Vargas-Bello-Pérez *et al*., (2014) along with the extruded linseed in ewes feed tested the content of fatty acids in sheep's milk. In their research, the value of this group of fatty acids was consistent with our results (26.95% and 28.81%). The average value of PUFA in the first study period was in the milk of the experimental group 5.7%, and in the control group milk, slightly higher - 6.1%.

PUFA values decreased during the study, and in the second period, in the milk of experimental group were 5.8% and in the control one 5.55%. In the third period, the determined values were at least 4.8%. The nutritional treatment did not show statistical significance (ns) to PUFA content in research periods. Total PUFA in sheep's milk was presented graphically (Fig 3). In the studies conducted by Vargas-Bello-Pérez *et al*., (2014) values of total PUFAs (5.24% and 5.77%) were correlated with our research.

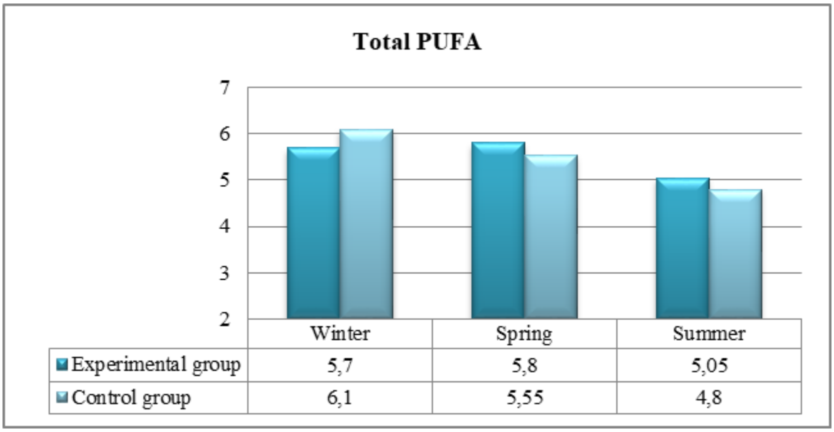

Figure 3. Total PUFAs (%) in sheep's milk

Omega-6 fatty acids found in sheep's milk samples are: linoleic, γ-linolenic and arachidonic. Their total value in the first period of research in the milk of the experimental group of sheep was 3.40%, and in the milk of the control group 3.9%. In the second period, the milk of the experimental group had 3.50% and that of the control group 3.40% of total omega-6 fatty acids. The third period had the lowest omega-6 values, 3.0% in the milk of the experimental group, and 2.90% in the milk of the control group. Omega-3 fatty acids found in milk samples are: α-linolenic, eicosapentaenoic (EPA), docosapentaenoic (DPA) and docosahexaenoic (DHA). In the first period of the research, the total value of omega-3 fatty acids determined in the milk of the experimental group was 2.30%, and in the milk of the control group 2.20%. In the second period, the determined value in the milk of the experimental group was 2.30%, and in the milk of the control group, 2.15%, while in the third period, the lowest value was determined and was 2.05% in the milk of the experimental group, and 1.90% in the milk of the control group. In all research periods, the milk of the experimental group of sheep had higher values of omega-3 fatty acids. In our research, the ratio of omega-6/omega-3 fatty acids is correlated with numerous studies by authors from the Nordic countries. The established ratio of omega-6/omega-3 fatty acids in the milk of the experimental group of all research periods had lower values than the milk of the control group. The ratio of omega-6/omega-3 fatty acids in the first period of the research in the milk of the experimental group was 1.48% and in the milk of the control group 1.77%. In the second period of research, the value of the ratio of omega-6/omega-3 fatty acids in the milk of the experimental group was 1.52%, and in the milk of the control group, 1.58%. In the third research period (old grass), the lowest ratio of omega-6/omega-3 fatty acids were determined and was 1.46% in the milk of the experimental group and 1.53% in the milk of the control group (Fig 4).

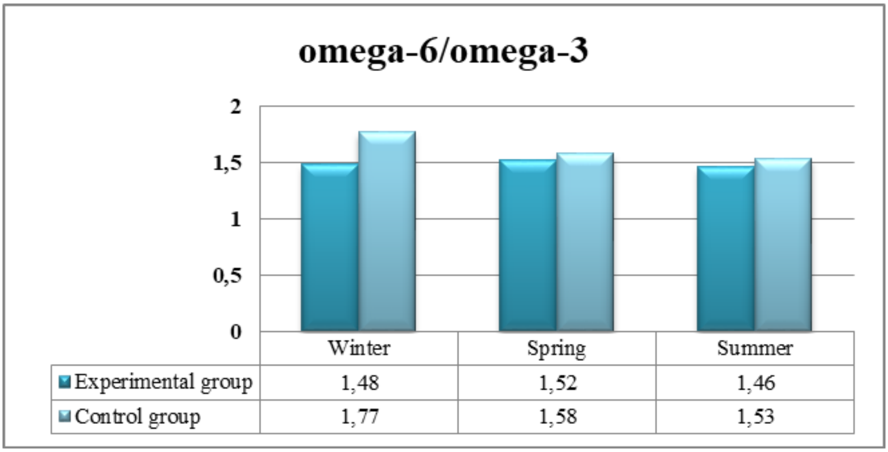

Figure 4. Graphic representation of the ratio of omega-6/omega-3 fatty acids in sheep's milk

Cattaneo *et al.*, (2006) found an increase in omega-3 unsaturated fatty acids in milk, such as EPA, DHA and C18:3, when feeding goats with meals supplemented with fish oil. According to Mele et al., (2008) and Chilliard *et al.*, (2003), with supplements of protected soybean oil in goat rations, an increase in the proportion of α-linolenic acid (C18:3), followed by an increase in oleic (C18:1) and stearic (C18:0), most likely due to incomplete protection of that oil. When adding unprotected oils to goat meals, the content of stearic (C18:0) and oleic (C18:1) increased due to the bio hydrogenation of polyunsaturated fatty acids in the rumen. In milk, the ratio between omega-6 and omega-3 fatty acids is lower and more favorable compared to other products, except for fish and seafood (Haug et al., 2007). Comparing the ratio of omega-6 to omega-3 fatty acids in milk from Nordic countries, Thorsdottir *et al.*, (2004) reported in their research that the lowest ratio in Iceland was 2.1:1 compared to 4.7:1 in the milk of the other Nordic countries. Furthermore, they indicate that the higher supply of omega-3 fatty acids through milk (thus reducing the mentioned ratio) in Iceland, compared to other Nordic countries, can explain the lower presence of type 2 diabetes and mortality due to CHD (Coronary Heart Disease). With a proper feeding regime, milk and ruminant meat can be the main source of omega-3 fatty acids in the human diet, as is the case in France (Haug *et al.*, 2007). It is believed that the diet should be rich in oleic acid, with a smaller ratio between omega-6 and omega-3 fatty acids, close to 1-2:1. Milk fat, and therefore milk, fits this description better than any other type of food. In addition to the ratio of omega-

6/omega-3 fatty acids in sheep's milk, the following ratios were determined: MUFA/SAFA, PUFA/MUFA and PUFA/SAFA, which are presented in Fig 5.

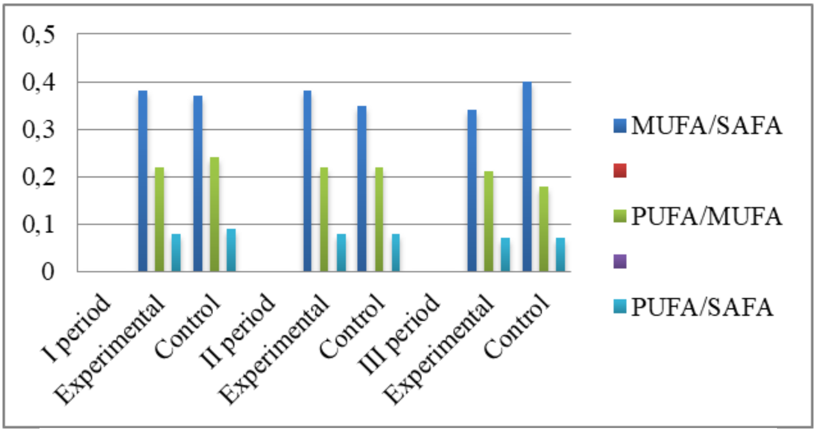

Figure 5. Ratios of certain groups of fatty acids in sheep's milk

The highest ratios of the examined groups of fatty acids were MUFA/SAFA values in the milk of the experimental group did not deviate much compared to the milk of the control group, only in the third period the milk of the control group had a slightly higher value. The determined values ranged from 0.34% to 0.40%. Lower values were found in PUFA/MUFA ratios (from 0.18% to 0.24%). The lowest values (from 0.07% to 0.09%) were found in the ratios of PUFA/SAFA fatty acid groups. These relationships did not show significant deviations between the research periods and in the third research period the values were the lowest.

Correlation PCA - Principal Component Analysis (sheep's milk, the most abundant fatty acids)

Principal Component Analysis (PCA - Principal Component Analysis) was used to represent the mutual relationship (correlation) of fatty acids in sheep's milk. The analysis was carried out on the basis of a correlation matrix, which included the mean values of fatty acids that are most abundant in sheep's milk during the three research periods, in the experimental and control herds. The most represented fatty acids in sheep's milk in the three investigated periods were given by three components. The first three main components, obtained from the analysis of the seven fatty acids most represented in sheep's milk, contained 84.25% of the total variance of the experiment, and the first two 67.57%. Grouping of three research periods (control and experimental group) against the first two main components (PC1 and PC2), calculated via the correlation matrix based on the seven monitored fatty acids (left) and grouping of the seven monitored fatty acids against the first two main components (PC1 and PC2) (right).

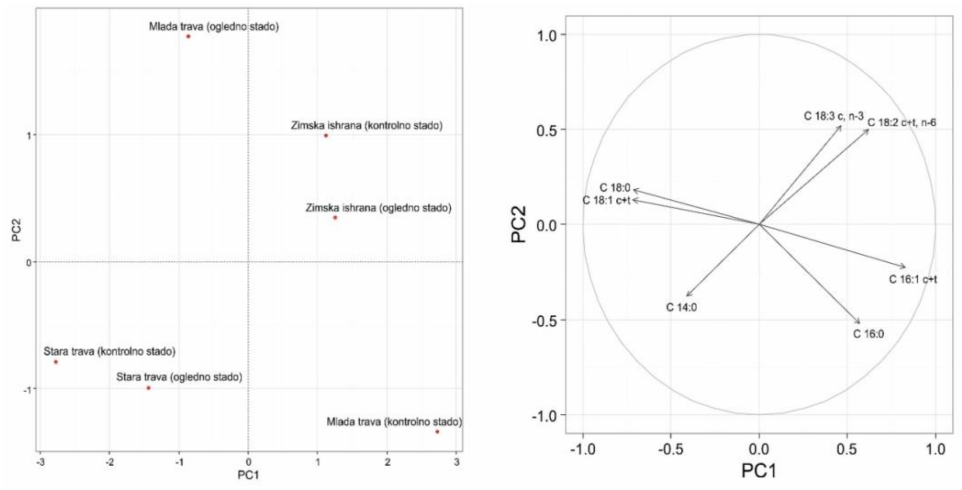

Figure 6. Biplot of the grouping of three research periods (control and experimental herd) against the first two principal components (PC1 and PC2)

Figure 6 shows the most abundant fatty acids in sheep's milk. With PCA analysis, we determined the correlations between the main fatty acids in the milk samples. There are strong positive correlations between α-linolenic and linoleic acids as well as between stearic and oleic fatty acids. Negative correlations were observed between myristic and linoleic acids. In research by De La Fuente *et al*., (2009) presented correlations between ten fatty acids, out of 36 total that were identified. Correlations were positive between fatty acids within each group and negative between each other, which is consistent with our results. These values can be interpreted biologically (Bobe *et al*., 1999; Chilliard *et al*., 2003; Bauman *et al*., 2006) and explain the similarity of their origin, i.e. synthesis in the mammary gland of sheep. Correlation coefficients were higher between fatty acids with a similar number of carbon atoms.

CONCLUSIONS

The highest total value of SAFA is established in the third period (summer) in milk of experimental group (Sg) of sheep (70.75%). Very high, statistically significant influence ($p<0.001$) were shown by both factors on content of total SAFAs. On the content of total MUFAs, factor of nutrition treatment showed very high statistically significant influence ($p<0.001$). By observing average value in all periods of research, conclusion is that in the first period, in milk of experimental group (Sg). PUFA value was 5.7%, PUFA values were decreasing in the course of the research, so in the second period in milk of experimental group (Sg) this value was 5.8%, while in the third period, the established values were the lowest - 4.8%. The highest values of PUFAs were detected in the spring period. The determined value of linoleic acid (PUFA, omega-6) in the first research period in the experimental group was 3.1%. In the second period of the research, the values were the same in the milk of the experimental and control groups and amounted to 3.2%, while in the third period in the milk of the experimental group of sheep, this value decreased to 2.8%. The content of α-linolenic acid (PUFA, omega-3) in all research periods decreased from the first to the last research period in the milk of the experimental and control groups of sheep. Based on the conducted research, it can be concluded that the hypothesis has been confirmed, which states that the addition of omega-3 preparations (extruded flax 3.5%) in the diet of sheep will have a lower ratio of omega-6/omega-3 fatty acids in sheep's milk.

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