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Fat dispersion and fatty acid profile, including health indicators in goat milk from different flora composition of grazing sites

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The aim of the study was to evaluate the chemical composition, cholesterol content, fat dispersion and fatty acid profile, including health indicators, in milk of goats grazing in mountainous and upland areas hence, subject to different flora composition of their grazing sites. The research included 273 goats raised in four farms, including two located in mountain areas and two in upland areas in Poland. The study material consisted of 480 milk samples, including 338 samples from the mountain areas and 142 from the upland ones. The goats raised in the mountain regions produced milk with a more favourable chemical composition, i.e. higher content of fat, protein, including casein, and dry matter. Milk obtained from the mountain regions had significantly ($p<0.01$) lower content of hypercholesterolaemic saturated fatty acids, and atherogenic and thrombogenic indices. The flora of the pastures situated in the mountains contributed to a favourable increase in the content of polyunsaturated fatty acids, especially trans-vaccenic acid and the sum of conjugated linoleic acid (CLA) isomers, in the milk. Furthermore, more favourable polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA) ratio, content of n-6 and n-3 fatty acids, odd-chain fatty acids (OCFAs) and branched-chain fatty acids (BCFAs) in the milk obtained from the mountain areas were due to a greater botanical biodiversity of pastures. These fatty acid groups in milk were strongly correlated with species richness of flora in pastures and with meadow and grassland species. Concluding, milk obtained from goats maintained in mountain areas characterized by the greater botanical biodiversity is a richer source of basic nutrients and biologically active compounds present in the fat fraction with regard to raw material from uplands.

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In recent years goat milk has become increasingly important in the human diet, alongside cow milk. The growing interest in goat milk is reflected in scientific research focusing on the factors determining its chemical composition, and in particular the concentrations of bioactive substances benefiting the human body, as well as its suitability for processing. In terms of nutrition, information on the content of fatty acids (especially those with health-promoting effects) in a specified quantity of a given food product is becoming increasingly important to the consumer. Goat milk has higher content of short- and medium-chain saturated fatty acids, including at least double the content of C6-C10, in comparison to cow milk [Strzałkowska *et al.* 2012]. These acids affect the organoleptic, sensory and health-promoting properties of goat milk and products based on it [Rutkowska *et al.* 2015, Milewski *et al.* 2018]. Polyunsaturated fatty acids (PUFA), such as linoleic acid (n-6) and linolenic acid (n-3), exert most beneficial effects on the consumers' health. Conjugated linoleic acid (CLA) has been shown to have anti-carcinogenic effects, vaccenic acid can reduce tumor growth and the risk of cardiovascular diseases (CVD), while linolenic acid (ALA) has demonstrated protective effects against inflammation and neurological disorders, and CVD. However, it is important to maintain a proper balance between n-6 and n-3 fatty acids, within the limits of 4-5:1, in the diet. The exceeding supply of n-6 fatty acids and very high n-6/n-3 ratio lead to development of many diseases, including cardiovascular, cancer, immune disorders and autoimmune. Also important in the milk fat are odd-chain (OCFAs) and branched-chain (BCFAs) fatty acids of varying length (C3-C27). The health-promoting effects of these compounds were indicated in scientific reports [Ran-Ressler *et al.* 2011, Rutkowska *et al.* 2015, Pfeuffer and Jaudszus 2016]. The interest in OCFAs pentadecanoic and heptadecanoic acids grew as an inverse association between OCFA concentration (15:0, 17:0, or both combined) in plasma phospholipids or red blood cells (RBCs) and the risk of cardiovascular disease (CVD) had been found [Pfeuffer and Jaudszus 2016]. However, BCFAs have been reported to reduce the occurrence of necrotizing enterocolitis in a mouse model and shift microbiota toward organisms that use BCFAs in their membranes [Ran-Ressler *et al.* 2011].

The available scientific literature contains numerous studies confirming the influence of the diet of goats on the fatty acid profile of their milk [Morand-Fehr *et al.* 2007, Chion *et al.* 2010]. The authors demonstrated that pasture feeding increases the proportion of beneficial monounsaturated and polyunsaturated fatty acids, including CLA, while decreasing that of saturated fatty acids [Rutkowska *et al.* 2012, Barłowska *et al.* 2016].

Another important factor influencing milk quality is the region where the animals are pastured and the associated composition of the feed [Collomb *et al.* 2002a, Roda *et al.* 2015]. Collomb *et al.* [2002a] reported differences in the fatty acid profile of milk from cows grazing at different elevations above sea level (lowlands, uplands and mountain areas), which they linked to differences in the botanical composition

of the pasture sward. They found that the predominant plant species in mountain and upland areas were negatively correlated with the content of saturated fatty acids in milk and positively correlated with polyunsaturated acids, including CLA, and monounsaturated *trans* C18:1 acids. In the study of Roda *et al.* [2015] in milk from cows pastured in Alpine region (June and July at 400-700 m and at 1,400-2,250 m of altitude), the percentage of oleic, vaccenic, rumenic and α-linolenic acids increased as a function of the altitude. The available literature lacks studies evaluating the relationship between the flora composition of grazing sites for goats and the fatty acid profile of the milk they produce.

Therefore, the aim of the study was to evaluate the chemical composition, fat dispersion, and fatty acid profile, including health promoting compounds (e.g., CLA, desirable fatty acids, hypercholesterolaemic saturated fatty acids, atherogenic and thrombogenic indices), of the milk of goats grazing in mountainous and upland areas, taking into account the flora composition of their grazing sites.

Material and methods

Research material

The subject of the study was 273 goats raised on four farms – two located in mountain areas and two in upland areas, in Poland. The goats were upgrades by the Saanen breed.

Since the main feed for the goats on the farms during the period from spring to autumn was pasture forage, a floristic evaluation of the grazing sites was conducted at the beginning of July 2015. The evaluation was carried out on the four farms mentioned above, i.e. two in mountainous locations (500-1,000 m a.s.l.) – farms A and B, and two in upland regions (300-500 m a.s.l.) – farms C and D.

Farm A kept 110 goats and had approximately 16 hectares of permanent grassland for grazing. The number of goats kept on farm B was 264 and the pasture area was about 50 ha. There were 47 goats on farm C, with about 4 ha of permanent grassland for grazing. Farm D kept 79 goats, which used about 7 ha of grassland.

A total of 133 species of vascular plants, belonging to 33 families, were recorded in the study areas. Four families represented in the highest numbers were *Poaceae* – 23 species, *Asteraceae* – 19 species, *Fabaceae* – 16 species and *Lamiaceae* – 8 species [Wysocki and Sikorski 2002]. Almost two-fold higher richness of species was noted on farms located in the mountain areas. The presence of more species of meadow plants and herbs were observed.

In the summer season the diet was based on pasture forage *ad libitum* (grazing from about 7:30 a.m. to 6:30 p.m.). The feed ration was supplemented with on-farm concentrate in the form of cereal meal (wheat and oats, 1:1) in the amount of approx. 0.3 kg, which was administered during milking at 7:00 a.m. and 7:00 p.m. During the winter season, the goats were fed hay (approx. 1.5 kg) and haylage (approx. 2.0

kg), and during milking they received on-farm concentrate in the form of cereal meal (wheat and oats) in the amount of approx. 0.3 kg. The goats had permanent access to drinking water and licks. The nutritional values of feed used in goats' nutrition are shown in Table 1.

Table 1. Nutritional value of fodder used in feeding of goats (in 1 kg of dry matter)

Specification	Dry matter (%)	Total protein (g)	Crude fiber (g)	UFL[1]	PDIN[2] (g)	PDIE[3] (g)
Pasture forage	17.77	123.73	270.23	0.98	78.14	91.00
Haylage	54.97	161.63	173.63	0.97	101.43	94.28
Hay	89.57	93.10	332.01	0.72	57.49	72.63
Wheat and oat ground grain	87.62	126.31	74.85	1.15	85.35	101.73

[1]UFL – feed unit for lactation
[2]PDIN – protein truly digestible in the small intestine when N limits microbial protein synthesis.
[3]PDIE – protein truly digestible in the small intestine when energy limits microbial protein synthesis.

The study material consisted of 480 milk samples, including 338 samples from the mountain areas and 142 from the upland ones taken from goats in their 2nd lactation. Milk samples (in the amount of 350 mL each) were taken proportionally from the morning and evening milkings at the beginning of July (90-120 days of lactation). Next, they were transported under refrigeration conditions to the laboratory. Daily milk yield of goats was determined (in mL) by measuring all the milk collected from the morning and evening milkings.

Laboratory analyses

The following parameters were determined in the milk samples: content of protein, fat, lactose, and dry matter, using Infrared Milk Analyzer (Bentley Instruments Inc., Chaska, MN); somatic cell count (SCC) – in 1,000/mL using a Bentley Somacount 150 (Bentley Instruments Inc.); milk fat dispersion (mean surface area of fat globules in the field of vision, mean circumference of fat globules and mean diameter) determined on photographs from two fields of vision of microscope slides (1,000 x magnification) stained with Sudan III, using Motic Images Plus 2.0. software.

Cholesterol content and fatty acid content were determined in a representative number of milk samples (in total 182, in that 90 samples from the mountain areas and 92 from the upland areas). Cholesterol content were analysed according to the method developed by the National Research Institute of Animal Production in Balice, Poland, (described in detail below) with own modifications. The procedure for cholesterol determination in the milk was as follows: 35 mL of a mixture of chloroform and methanol was added to 5 mL of milk. After centrifugation, 4 mL of the mixture was taken from the bottom layer and placed in Supelco vials and evaporated under a stream of nitrogen. The resulting evaporation residue was saponified in sealed vials with 3 mL of 0.5 N NaOH at 80°C (modification – NaOH was used instead of KOH). After cooling,

3 mL of hexane was added and the fat was extracted in sealed vials. Then 1 mL of the hexane layer was collected and evaporated at 65°C under a stream of nitrogen and 4 mL of glacial acetic acid was added to the residue. From this, 3.5 mL of the mixture was collected and 2.5 mL of a colour solution was added, and this was mixed and cooled. The measurements were made on a Carry 300 spectrometer (Varian) at 570 nm. Fatty acid content was determined, following fat extraction by a modification of the Röse-Gottlieb method [AOAC 2000], after which direct conversion to fatty acid methyl esters (FAME) was performed by transmethylation of the fat sample using a mixture of concentrated H_2SO_4 (95%) and methanol according to AOCS Official Method Ce 2-66 [2000]. FAMEs were separated by gas chromatography (GC) according to PN-EN ISO 5508 [1996] and PN-EN ISO 12966-1: 2015-01 [2015] using a Varian GC 3900 chromatograph with a flame ionization detector (FID) (WalnutCreek, CA, USA). FAMEs were separated in a CP 7420 capillary column (Agilent Technologies, USA), 100 m in length, inner diameter 0.25 mm, film thickness 0.25 μm. The analysis was carried out in increasing temperature conditions. The initial temperature of the oven was 50°C and the final temperature was 250°C. The temperature of the injector and the detector was 270°C, the carrier gas (hydrogen) flow rate 2 mL/min, the size of the injected samples 1 μL, and the split ratio 1:50. Fatty acids were identified by comparing the retention times of individual FAMEs with the retention times of fatty acid methyl ester standards (Supelco 37 Component FAME Mix CRM 47885, CLA methyl ester O5632 – Sigma-Aldrich, Branched Chain FAME Mixture BR2, BR3, BR4 – Larodan AB) and literature data. Results for individual fatty acid methyl ester (FAME) were expressed as percentages of the sum of total FAMEs identified (g/100 g FAME), using Star GC Workstation Version 5.5. software (Varian Inc., Walnut Creek, USA). The FAME results were converted to fatty acid (FA), using correction factors for individual fatty acids and the FAME-to-FA conversion factor known as the Sheppard factor [Greenfield and Southgate 2003]. On this basis the following groups of fatty acids, their ratios and indices were calculated:

– SCSFA – short- and medium-chain saturated fatty acids,
– LCSFA – long-chain saturated fatty acids,
– OCFA – odd-chain fatty acids,
– BCFA – branched-chain fatty acids,
– SFA – saturated fatty acids,
– MUFA – monounsaturated fatty acids,
– PUFA – polyunsaturated fatty acids,
– PUFA/SFA and MUFA/SFA ratios,
– DFA – desirable fatty acids = (MUFA + PUFA + C18:0) – according to Medeiros *et al.* [2014],
– HSFA – hypercholesterolaemic saturated fatty acids = (C12:0 + C14:0 + C16:0) – according to Renna *et al.* [2012],
– AI – atherogenic index = (C12:0 + 4 × C14:0 + C16:0) ÷ (MUFA + PUFA),
– TI – thrombogenic index = (C14:0 + C16:0 + C18:0) ÷ (0.5 × MUFA + 0.5 ×

n6 + 3 × n3 + n3 ÷ n6) according to Ulbricht and Southgate (1991) qtd in: [Renna *et al.* 2012].

Statistical analysis

Statistical analysis of the results was performed with the StatSoft Inc. Statistica 13.1 software [2016] using one-way analysis of variance. The SCC data were transformed into $\log_{10}$ SCC for each sample before statistical analysis. The statistical analysis included the flora composition category effect using the following linear model:

$$y_i = \mu + A_i + e_i$$

where:

y_i – dependent variable;

μ – overall mean;

A_i – fixed flora composition category effect (i = 1, 2);

e_i – random residual.

Results and discussion

Chemical composition of milk

Analysis of the proximate chemical composition of the milk revealed that the milk from goats raised in mountain areas had significantly ($p<0.01$) higher dry matter content (by 0.89 percentage points), including protein (by 0.17 pp) and fat (by 0.62 pp)

Table 2. Chemical composition, fat dispersion and cholesterol content in goat milk with regard to flora composition characteristic for upland and mountain areas (mean and SD)

Item	Flora composition			
	upland		mountain	
	mean	SD	mean	SD
Number of milk samples	142		338	
Daily yield (L)	1.73	0.75	1.67	0.75
Fat (%)	3.29^{A}	0.74	3.91^{B}	0.94
Protein (%)	2.95^{A}	0.47	3.12^{B}	0.66
Lactose (%)	4.58	0.22	4.60	0.30
Dry matter (%)	11.40^{A}	1.48	12.29^{B}	1.57
Log 10 SCC	2.79^{A}	3.00	3.27^{B}	3.26
Surface area of fat globules in field of view (μm^2)	4.63^{A}	1.69	5.25^{B}	2.55
Circumference of fat globules in field of view (μm)	6.68	1.14	6.9	1.33
Mean diameter of fat globules in field of view (μm)	2.12	0.36	2.20	0.42
Number of milk samples	90		92	
Cholesterol (mg/100 mL)	16.21	4.34	15.5	3.83

^{AB}Means bearing different superscripts differ significantly at $p<0.01$.

than that of the goats kept in upland areas. The milk of the goats raised in upland areas had a 3-fold lower somatic cell count ($p<0.01$). There were no significant differences between the daily yields of goats in these areas (Tab. 2).

The results obtained by Žan *et al.* [2006] differ from those presented in our study. The authors' comparison of Saanen goats grazed in uplands and Alpine grazed in mountains revealed lower protein, fat, lactose and dry matter content in individuals grazing in the mountains as compared to those grazed in upland areas. It should be noted that the authors were comparing two different breeds raised in different production regions, so the differences in the content of basic milk components may have also been influenced by the breed and possible interaction of breed*flora composition. Steinshamn *et al.* [2014] found that goats grazing in uncultivated areas produced less milk (26%) but with higher fat and dry matter content than animals raised on cultivated pastures. In general, pasture grazing (as compared to a diet based on hay) increased the concentration of fat, protein and dry matter and reduced the content of urea and free fatty acids. Inglingstad *et al.* [2014] reported that pasture grazing resulted in an increase of protein content, including casein.

Milk fat dispersion and cholesterol content

The fat in milk is present in the form of dispersed fat globules of varying size. Their size, and the associated degree of dispersion, affect the digestibility of the fat and determine the final quality of the products. Milk fat globules are surrounded by a biological membrane 10-50 nm thick. One of the components of the membrane is cholesterol [Lopez *et al.* 2011]. In our study, a significant ($p<0.01$) correlation was observed between higher cholesterol content and greater milk fat dispersion, i.e. fat globules with a smaller mean diameter and circumference. According to Kovács *et al.* [2004], increase of the cholesterol-to-fat ratio in dairy products results from a high proportion of small fat globules, which in total have a larger membrane surface area and thus higher cholesterol content. This opinion is shared by Barłowska *et al.* [2011].

Analysis of the data in Table 2 did not reveal any effect of the production region on the fat dispersion and cholesterol content in the milk, as confirmed by two-way analysis of variance.

Fatty acid profile

Milk produced in mountain areas had significantly ($p<0.01$) lower (by 2.7%) concentration of saturated fatty acids (SFA) than milk produced in the uplands, including 2.3% lower content of long-chain fatty acids (LCSFA) ($p<0.05$), and in particular C16:0 ($p<0.01$) – Table 3. At the same time it had a significantly ($p<0.01$) higher concentration of polyunsaturated fatty acids, by about 30%, including CLA (by about 36%). Milk from mountain areas had also a significantly ($p<0.05$) higher concentration of fatty acids with an odd number of carbon atoms (1.99% vs. 1.85%). Goats grazed in mountain areas produced milk with a significantly higher total content of *trans*-C18:1 acids, including 1.5 times more *trans*-vaccenic acid. This milk had

also significantly ($p<0.01$) higher content of linoleic and α-linolenic acids, EPA and DHA. Moreover, the milk from mountainous regions was found to contain more odd-chain fatty acids (OCFAs) and branched-chain fatty acids (BCFAs) – Table 3. In the case of OCFA, significant differences were found for C13:0, C17:0 and C23:0, and in the case of BCFA, for *iso* C13:0, *iso* C16:0, *iso* C17:0 and *anteiso* C17:0. Adamska

Table 3. Fatty acid profile of goat milk with regard to flora composition characteristic for upland and mountain areas, g/100 g of fatty acids (mean and SD)

Item	Flora composition			
	upland		mountain	
	mean	SD	mean	SD
Number of milk samples	90		92	
C 4:0	2.35	0.33	2.39	0.31
C 6:0	2.53	0.41	2.46	0.30
C 8:0	2.78	0.68	2.64	0.50
C 10:0	9.81^{b}	2.71	9.07^{a}	2.06
C 12:0	4.05	1.29	3.94	1.25
∑ SCSFA	21.52	4.70	20.50	3.74
C 14:0	10.17	1.77	10.09	2.08
C 16:0	27.99^{B}	4.48	25.48^{A}	3.09
C 18:0	9.88^{a}	4.27	11.30^{b}	3.93
C 20:0	0.21	0.07	0.24	0.09
C 22:0	0.07^{A}	0.03	0.09^{B}	0.03
C 24:0	0.05	0.02	0.04	0.02
∑ LCSFA	48.34^{b}	3.07	47.21^{a}	3.45
C 11:0	0.08	0.04	0.08	0.06
C 13:0	0.08^{A}	0.02	0.09^{B}	0.03
C 15:0	0.89	0.16	0.93	0.28
C 17:0	0.74^{A}	0.17	0.82^{B}	0.22
C 21:0	0.03	0.01	0.04	0.02
C 23:0	0.05^{b}	0.02	0.04^{a}	0.02
∑ OCFA	1.85^{a}	0.28	1.99^{b}	0.52
iso C 13:0	0.04^{b}	0.02	0.03^{a}	0.01
anteiso C 13:0	0.03	0.01	0.03	0.01
iso C 14:0	0.10	0.03	0.11	0.04
iso C 15:0	0.20	0.05	0.21	0.07
anteiso C 15:0	0.47	0.10	0.47	0.13
iso C 16:0	0.27^{a}	0.09	0.30^{b}	0.10
iso C 17:0	0.36^{a}	0.08	0.39^{b}	0.10
anteiso C 17:0	0.63^{B}	0.15	0.57^{A}	0.15
iso C 18:0	0.05	0.02	0.05	0.02
∑ BCFA	2.13	0.37	2.14	0.50
∑ SFA	73.84^{B}	4.64	71.85^{A}	4.42
C 10:1	0.24	0.09	0.21	0.09
C 14:1 *c*9	0.14^{b}	0.09	0.11^{a}	0.06
C 16:1 *c*13	0.08^{A}	0.05	0.13^{B}	0.10
C 16:1 *c*7 n-9	0.24	0.07	0.23	0.05
C 16:1 *t*9	0.02^{A}	0.01	0.03^{B}	0.01
C 16:1 c9 n-7	0.55^{B}	0.21	0.43^{A}	0.11
C 17:1 *c*9	0.22	0.06	0.21	0.07
C 18:1 *t*6/7	0.14^{A}	0.05	0.18^{B}	0.09

Table 3. Continued

Item	Flora composition			
	upland		mountain	
	mean	SD	mean	SD
C 18:1 *t*9	0.15^{A}	0.04	0.19^{B}	0.07
C 18:1 *t*10	0.16^{A}	0.06	0.26^{B}	0.16
C 18:1 *t*11	0.83^{A}	0.60	1.34^{B}	1.24
C 18:1 *c*9 n-9	19.24	4.60	19.08	4.05
C 18:1 *t*15	0.16^{A}	0.10	0.22^{B}	0.10
C 18:1 *c*11	0.44^{A}	0.14	0.49^{B}	0.10
C 18:1 *c*12	0.10^{A}	0.03	0.13^{B}	0.04
C 18:1 *c*13	0.05^{A}	0.02	0.06^{B}	0.03
C 18:1 *t*16	0.25^{A}	0.10	0.35^{B}	0.12
C 20:1 *c*11 n-9	0.05	0.02	0.05	0.02
C 20:1 n-7	0.03	0.01	0.04	0.01
C 20:1 *t*	0.04^{a}	0.02	0.05^{b}	0.03
∑ C18:1 *trans*	1.69^{A}	0.86	2.54^{B}	1.51
∑ MUFA	23.09	4.67	23.77	4.12
C 18:2 *t*9 *t*12	0.16^{A}	0.07	0.22^{B}	0.09
C 18:2 *c*9 *t*12	0.20^{A}	0.04	0.24^{B}	0.04
C 18:2 *c*9 *c*12 n-6	1.40^{A}	0.38	1.91^{B}	0.46
C 18:3 *c*6,9,12 n-6	0.04	0.02	0.04	0.02
C 18:3 *c*9,12,15 n-3	0.50^{A}	0.32	0.88^{B}	0.33
CLA *c*9*t*11 + *t*9*c*11	0.35^{A}	0.22	0.55^{B}	0.46
CLA *t*11 *c*13	0.04	0.01	0.06	0.01
CLA *t*10 *c*12	0.06	0.02	0.04	0.01
CLA *t*11 *c*15	0.04	0.02	0.04	0.03
∑ CLA	0.39^{A}	0.24	0.61^{B}	0.50
C 20:2 n-6	0.06	0.02	0.07	0.03
C 20:3 *c*8,11,14 n-6	0.04^{B}	0.02	0.03^{A}	0.01
C 20:4 n-6	0.12^{b}	0.03	0.11^{a}	0.04
C 20:5 n-3	0.05^{A}	0.02	0.08^{B}	0.03
C 22:5 n-3	0.09^{A}	0.03	0.15^{B}	0.06
C 22:6 n-3	0.04^{A}	0.02	0.05^{B}	0.03
∑ PUFA	3.07^{A}	0.64	4.39^{B}	1.24

$^{aA,\cdots}$Means bearing different superscripts differ significantly: small letters – $p<0.05$; capitals – $p<0.01$.

and Rutkowska [2014] showed that the content of odd- and branched-chain fatty acids is higher in organic milk and in milk produced with a large share of pasture grazing than in an indoor, TMR system.

The almost 2-fold higher levels of n-3 acids in the milk produced in the mountains (1.17 vs. 0.67 g/100 g FA) are also worth noting. More favourable ($p<0.01$) n-6/n-3 and PUFA/SFA ratios and atherogenic and thrombogenic indices, as well as significantly ($p<0.01$) lower content of hypercholesterolaemic fatty acids, were noted in the milk of goats raised in the mountain regions (Table 4). The scientific literature contains few studies on the effect of the production area (different elevations above sea level) on the fatty acid profile in milk. Žan *et al.* [2006] reported reverse correlations for fatty acid content in mg/100 g milk to those obtained in the present study (i.e. they found higher values in the milk from uplands than from mountains). The differences between that study and our own are most likely due to differences in fat content in the

Table 4. Indices characterizing fatty acid profile of goat milk with regard to flora composition characteristic for upland and mountain areas, g/100 g of fatty acids (mean and SD)

Item	Flora composition			
	upland		mountain	
	mean	SD	mean	SD
Number of milk samples	90		92	
Atherogenic index (AI)	2.92^{B}	0.85	2.60^{A}	0.81
Thrombogenic index (TI)	3.36^{B}	0.70	2.82^{A}	0.52
HSFA	42.21^{B}	6.50	39.51^{A}	5.18
n-3	0.67^{A}	0.36	1.17^{B}	0.42
n-6	1.65^{A}	0.39	2.15^{B}	0.51
n-6/n-3	3.16^{B}	1.71	2.09^{A}	0.87
PUFA/SFA	0.04^{A}	0.01	0.06^{B}	0.02
MUFA/SFA	0.32	0.09	0.34	0.08

^{AB}Means bearing different superscripts differ significantly at $p<0.01$.

milk. The authors reported higher fat content in milk produced in upland areas than in the mountains (3.77% vs. 3.36%). In our study, fat content was higher in the milk produced in the mountains than in the uplands (3.91% vs. 3.29%). Also Bergamaschi *et al.* [2016] noted a marked increase in milk fat content ($p<0.001$) after the cows were moved to the summer Alpine pasture – temporary highland farm (1,860 m above sea level), concomitant with a substantial reduction in milk yield, resulting in a much smaller decrease in daily milk fat yield ($p<0.001$). This is related to the species richness of the flora and probably a greater degree of feed conversion. Results obtained by Chion *et al.* [2010] confirm the key role of fresh forage in the diet of cows raised in mountain environment for production of milk and cheese with a more favourable fatty acid profile and better potential health benefits than conventionally produced lowland milk. It seems likely that this is also the case with goat milk. Morand-Fehr *et al.* [2007], citing information presented verbally by Pizzillo *et al.* [2005], who conducted research on milk quality of goats grazed on pastures in plains, uplands and mountains, reported a higher share of PUFA in milk from the mountains. Furthermore, Chilliard and Ferlay [2004] suggested that the botanical composition of mountain meadows in particular appears to contribute favourably to higher CLA content in milk. Collomb *et al.* [2002b], in a study on the fatty acid composition of cow milk from lowlands, uplands and mountains, showed that milk from the mountains had the highest content of unsaturated fatty acids, including PUFA and CLA, as well as n-3 acids. Lowland milk, on the other hand, had the highest content of saturated fatty acids. Bugaud *et al.* [2001] found higher content of monounsaturated fatty acids in the milk of cows grazed in mountain pastures. According to those authors, this is explained by the environmental conditions specific to these areas, which are more difficult for the animals (e.g. lower temperatures and more movement over mountainous terrain). This can affect lipid mobilization and increase the proportion of C18:1.

To conclude, the goats raised in the mountain regions produced milk of more favourable chemical composition, i.e. higher content of fat, protein, including casein, and dry matter, but also a higher SCC. Differences in SCC could be a result of difficult environmental conditions prevailing in the mountains. Milk obtained from goats maintained in the mountains had significantly lower content of saturated fatty acids and higher content of polyunsaturated fatty acids, including CLA. Furthermore, significantly lower atherogenic and thrombogenic indices and lower content of hypercholesterolaemic saturated fatty acids were observed in the milk obtained from this area, as well as more favourable n-6/n-3 and PUFA/SFA ratios. More favourable fatty acid profile in milk obtained from mountain areas was due to a greater botanical biodiversity of the pastures. Grazing of goats on natural mountain pastures, with greater floristic richness, and in particular the presence of more species of meadow plants and herbs, contributed to an increase in the proportion of beneficial fatty acids in the milk. A strong correlation was observed between flora species richness, including meadow and grassland species, and the content of fatty acids benefiting human health, e.g. PUFA, including CLA, and branched-chain fatty acids (BCFA).

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