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COMPARISON OF THE QUALITY OF MOUNTAIN SHEEP MILK OBTAINED FROM ANIMALS KEPT ON A NATURAL AND ORGANIC MOUNTAIN PASTURE*

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Abstract

The purpose of this study was to evaluate the quality of mountain sheep milk obtained from animals kept on mountain pastures: natural and organic. The experimental procedure was conducted under production conditions, during annual grazing of mountain sheep on two mountain pastures in the region of the Polish Carpathians: on organic pasture certified for organic farming and natural mountain pasture belonging to the National Park. This study showed greater plant species diversity in the organic mountain pasture, but its yielding was lower than that of the natural pasture. The sward of the natural pasture was characterised by lower dry matter and fibre content but contained more total protein. The type of pasture did not affect the basic composition of the milk of mountain sheep, while differences were found in terms of acidity, alcohol number, fatty acid profile of fat and milk protein fractions. Milk obtained from sheep grazed on organic pasture was characterised by a higher proportion of medium chain fatty acids, as well as polyunsaturated fatty acids, and also essential unsaturated fatty acids, both linoleic acid, α-linolenic acid, γ-linolenic acid and arachidonic acid. In milk originating from the natural mountain pasture, a higher proportion of monounsaturated fatty acids, docosahexaenoic acid and the CLA c9-t11 isomer was found. The composition of milk protein fractions varied according to the location of grazing: milk from sheep grazing on natural pasture had a higher content of whey fractions, important for consumer health, milk from organic pasture had a higher content of κ-casein, of greatest importance in dairy processing technology. The floral composition of the pasture had an influence on the quality of sheep milk in relation to the fatty acid profile of fat and protein fractions of milk, which is important in terms of both the health-promoting and technological aspects of the raw material and may affect the quality of products made from it.

Key words: mountain pasture, milk quality, mountain sheep

Going back to traditional systems of livestock feeding on pastures is both economically rational and a source of cheap and healthy fodder. Pasture forage is the main and cheapest way to improve the quality of milk, which, obtained from animals kept in such a system, has a more favourable chemical composition from the point of view of human health, and contains more polyunsaturated fatty acids, fat-soluble vitamins, including antioxidants, sterols, or more whey proteins (Cabiddu et al., 2005; Barłowska et al., 2012; Martini et al., 2021). This, combined with higher taste qualities, makes dairy products made using milk from animals kept on pastures more attractive to modern consumers, who are able to pay a higher price for it.

A small area of the Polish Carpathians is used for grazing mountain sheep, whose method of using and managing their milk has remained unchanged for centuries; still, this is in line with pastoral tradition. Collective, large-scale sheep grazing on mountain pastures lasts from May to September, after the lambs are weaned. The sheep are milked in pastures, and the milk obtained from manual milking is used to produce regional cheeses according to a recipe that has been handed down for centuries (Kawęcka et al., 2020). Three of them (bryndza podhalańska, oscypek, redykołka) have been granted the status of the EU Protected Designation of Origin (PDO). The lay of the land, soil and climatic conditions have led to the development of a specific vegetation cover in mountain and foothill areas; these areas are mainly meadows and pastures where pastoral activity has been conducted for centuries. The grasslands in this area have several particularly principal functions, including production, protection of the soil, plants, and animals, as well as providing aesthetics and enriching the mountain landscape and, above all, providing valuable fodder for ruminant livestock and free-living animals. For centuries, the Carpathian grasslands were grazed by large herds of animals, which had a huge influence on the shape and

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landscape of the Polish Carpathians and, until the 20th century, formed the basis of the economy in the area. The crisis of sheep farming, the decline in the sheep population and the abandonment of grazing led to unfavourable changes in the pastureland's appearance and its botanical composition. The emergence of vegetation typical for woodland communities impoverished grassland ecosystems, reduced their species diversity, and naturally valuable species were displaced by invasive ones (Drożdż and Twardy, 2004). The return of sheep to their historical range, the reintroduction of grazing in the mountains, in areas of natural value in national parks and on mountain pastures contributed to social and cultural aspects, aimed at maintaining the cultural heritage of the region and the sustainable development of rural areas, and at the same time influenced the mountain landscape, the botanical composition of pastures, and owing to organic fertilisation, by enriching the soil with nutrients, had a positive effect on the yield and condition of the grassland sward.

Milk and milk products from pastured animals contain a number of bioactive compounds that are important for human health, such as fatty acids, vitamins and sterols of animal and plant origin (Plaza et al., 2021; Kholif and Olafadehan, 2022). Multiple studies on the nutritional quality of milk have demonstrated the benefits of grazing cows, goats, and sheep. The results of studies carried out, among others, on dairy cows using pasture indicate better technological parameters of the milk obtained from them and a higher content of components beneficial to human health such as whey proteins, polyunsaturated fatty acids including CLA (conjugated linoleic acid) and fat-soluble vitamins (Barłowska et al., 2012). Variation in the botanical composition of grassland feed may also be relevant. Collomb et al. (2002) observed a relationship between the botanical composition of the pasture and the proportion of individual fatty acid groups in cows' milk fat, while Bugaud et al. (2001) found a higher content of monounsaturated fatty acids in the milk of cows grazing on mountain pastures. According to Mel'uchová et al. (2008), seasonal changes in the content of n-3 EFAs (essential fatty acids) and CLA in sheep milk fat are mainly related to changes in α-linolenic acid content in grass fats, seasonal changes in botanical composition and phenological stage of grasses. Morand-Fehr et al. (2007) analysing the influence of animal housing systems and associated feeding on the composition and quality of sheep and goat milk, found that animals kept in systems based on natural pastures produce milk rich in fatty acids and vitamins.

The purpose of this study was to evaluate the quality of mountain sheep milk obtained from animals kept on mountain pastures: natural and organic mountain pasture.

Material and methods

The experimental procedures and animal care conditions followed the European guidelines for the care and use of animals in research (Directive 2010/63/EU). With regard to this study, no special permission was needed. The experimental procedure was conducted under production conditions, during annual grazing of mountain sheep on two mountain pastures in the region of the Polish Carpathians (the Pieniny range): the Dursztyn pasture (740 m above sea level) and the Majerz pasture (689 m above sea level). In the Majerz pasture belonging to the Pieniny National Park, sheep grazing and cheese production is carried out by a farmer whose farm is certified for organic farming and whose sheep density is 0.5 LU (livestock unit) per ha (organic mountain pasture – OMP). The pasture in Dursztyn is a natural mountain pasture (NMP), with a stocking rate of 1 LU/ha.

For the purpose of this study, milk was obtained from two herds of 100 ewes of purebred Polish Mountain Sheep (PMS). Traditional, seasonal, collective grazing of mountain sheep takes place in the mountain pastures, after weaning the lambs. It is a large-scale grazing area in which the animals spend all day in pastureland and are kept in enclosures at night and during milking. Mountain sheep are milked manually in the morning and in the evening. The ewes included in this study were aged 3 to 7 years, all lambs were weaned at 60 days of age. The bulk milk samples from collected during evening milking, three times during the grazing season: in May, July, and September (in the middle of each month), six in each period. There was milk from 10 ewes in each sample, the samples were immediately transported to the laboratory and analysed.

The following parameters were determined in the milk under study: chemical composition of milk according to AOAC (2019), time of renneted milk coagulation by Schern method (moment of appearance of first casein flakes), alcohol number. Density was determined using a lactodensimeter. Acidity was determined by means of the titration method according to PN-86/A-86122; pH assessment was carried out with the use of digital pH meter CP-411 Elmetron (Poland) equipped with ERH-111 electrode by Hydromet (Poland). The profile of fatty acids in milk was determined by gas chromatography (P 015 version 1, 22.05.03, WKT method), using a VARIAN 3400 gas chromatograph (Varian, Walnut Creek, CA, USA) with an Rtx 2330 column (dimensions 105 m × 0. 32 mm × 0.2 μ; column operating temperature: initial 60°C for 10 min; temperature rise up to 120°C (20°C/min); up to 240°C (3°C/min), analysis time: 60 min, dispenser temperature: 250°C; detector: 250°C; carrier gas: helium, 3 mL/min.; injection of 1.0 mcl). Samples were extracted with the chloro-form/methanol mixture (2:1, v/v). Fatty acids were saponified (0.5 N NaOH in methanol, 80°C) followed by esterification with boron trifluoride/methanol. The separation of protein fractions was performed according to the SDS-PAGE gel electrophoresis method. The separation was performed using a Mini-Protean 3 Cell device (Bio-Rad) and was subjected to quantitative analysis with the application of a software for the analysis of electrophoretic gels, Gelscan version 2.0 (Poland).

Table 1. Botanical composition of pastures according to the extended Braun-Blanquet scale

Species	Natural mountain pasture (NMP)	Organic mountain pasture (OMP)
	*The degree of coverage of the species	
Grass		
Agrostis capillaris	2a	+
Alopecurus pratensis	–	+
Anthoxanthum odoratum	–	2m
Cynosurus cristatus	3	2b
Dactylis glomerata	1	1
Deschampsia caespitosa	2m	2m
Festuca pratensis	2a	2a
Festuca rubra	3	2b
Lolium perenne	2b	2a
Poa pratensis	2a	2a
Poa trivialis	-	1
Trisetum flavescens	2a	2m
Fabaceae		
Lathyrus pratensis	–	+
Lotus corniculatus	+	1
Trifolium pratense	1	2a
Trifolium repens	2a	2a
Dicotyledons		
Achillea millefolium	1	1
Agrimonia eupatoria	1	-
Alchemilla sp.	1	1
Bellis perennis	–	+
Cardamine pratensis	–	+
Carum carvi	+	1
Centaurea jacea	–	+
Cerastium holosteoides	+	+
Cirsium eriophorum	–	r
Dactylorhiza maculata	–	+
Geum rivale	–	+
Juncus effusus	+	+
Leontodon autumnalis	1	1
Lychnis flos-cuculi	–	+
Lysimachia nummularia	–	+
Pimpinella saxifraga	–	+
Plantago lanceolata	+	1
Plantago major	1	1
Primula elatior	–	+
Ranunculus acris	+	1
Ranunculus auricomus	–	+
Ranunculus repens	+	-
Rhinanthus minor	–	+
Stachys germanica	–	+
Stellaria graminea	–	+
Taraxacum officinale	1	1
Veronica chamaedrys	+	+

*The degree of coverage of the species according to the Braun-Blanquet scale (extended).
r – Single specimens.
+ – Sparse occurrence with coverage does not exceed 1%.
1 – Coverage between 1 and 5%.
2m – The species covers about 5% of the photo area.
2a – The species covers 5–12.5% of the photo area.
2b –The species covers 12.5–25% of the photo area.
3 – The species covers 25–50% of the photo area.
4 – The species covers 50–75% of the photo area.
5 – The species covers 75–100% of the photo area.

Before commencement of grazing, a phytosociological inventory was made using the Braun-Blanquet method. During the vegetation period, assessment of pasture yield was performed four times using the Różycki method. Moreover, during the evaluation green growth samples were taken for chemical analyses in which dry matter, crude ash, total protein, crude fat, crude fibre, and its fractions (NDF, ADF and ADL) were determined according to the procedure described by AOAC (2019). Fatty acid content was determined using an INGOS LCP 5020 liquid chromatograph, 8 × 250 mm steel column packed with OSTION LG-KS 0800 H+ from Tessek, mobile phase: 5 mM H_2SO_4.

The average botanical composition of the examined pastures is presented in Table 1. Based on phytosociological photographs taken before the commencement of grazing, it can be concluded that both pastures were dominated by plants belonging to the Poaceae family. On the natural mountain pasture the occurrence of Lolio-Cynosuretum R. Tx. 1937 was confirmed. In the phytosociological pictures 6 to 9 species of grasses, 2–3 species of bean plants and 10–13 species of other dicotyledonous plants were found. The species which achieved the highest coverage in the photos were: *Cynosurus cristatus* L., *Agrostis capillaris* L., *Lolium perenne* L. and *Trisetum flavescens* (L.) P. Beauv. Among the dicotyledonous plants, the following were found: *Alchemilla* sp., *Achillea millefolium* L., *Plantago* sp. *Leontodon* sp., *Agrimonia eupatoria* L., *Ranunculus acris* L. Between 25 and 40 plant species were found in the ecologically valuable pasture. The species achieving the highest coverage in the photographs were: *Festuca rubra* L., *Trisetum flavescens* L., *Poa pratensis* L., *Trifolium repens* L., *Dactylis glomerata* L., *Festuca pratensis* L., *Cynosurus cristatus* L., *Ranunculus acris* L., *Trifolium pratense* L., *Carum carvi* L., *Plantago lanceolata* L., *Plantago major* L., *Leontodon autumnalis* L., *Lotus corniculatus* L. There were also such species as: *Alchemilla* sp., *Achillea millefolium L.*, *Cirsium eriophorum* (L.) Scop, *Geum rivale, Dactylorhiza maculata* (L.), *Stachys germanica.* In the examined period the yield of the natural mountain pasture averaged 7.45 $t \cdot ha^{-1}$ and the yield of the organic pasture sward was 5.90 $t \cdot ha^{-1}$.

The sward of the natural mountain pasture was characterised by a lower content of dry matter, contained less fibre (21.93 v. 23.79%) and more total protein (19.52 v. 18.38%); no statistically significant differences were found in the content of EFAs in the sward of the tested pastures (Table 2). Linoleic acid C18:2 n-6 and α-linolenic acid C18:3 n-3 had the highest percentage share in total fatty acids, constituting 13.09 and 35.62%, respectively, of the sward in the mountain pasture and 13.82 and 36.39% of the sward in the organic pasture.

The results were statistically analysed with the use of Dell Statistica package, v. 13.1 and one-way ANOVA ('analysis of variance'). The mathematical model included fixed effect due to type of pasture (natural and organic mountain pasture) on the composition of mountain sheep milk. All values are expressed as means and standard deviation. Multiple mean comparisons were

made using Duncan's multiple range test. Testing was carried out at the significance level P≤0.05 and P≤0.01.

Results

Table 3 shows the chemical composition of milk obtained from mountain sheep. The influence of pasture type on the basic composition was not found; milk from mountain sheep had an average content of 22.22% solids, 8.59% fat, 4.25% protein, lactose was 3.31% and minerals 0.67%. Statistical differences occurred in relation to milk pH and alcohol number: lower pH and higher alcohol number value were found in milk collected in natural mountain pasture.

Table 2. Chemical composition (%) and fatty acid (% of total fatty acids) profile of forage

Item	NMP		OMP	
	$\bar{x}$	SD	$\bar{x}$	SD
Chemical composition				
dry matter	21.59 a	0.64	22.89 b	0.75
ash	7.36	0.27	7.42	0.25
crude fat	2.43 a	0.15	3.08 b	0.11
crude protein	19.52 b	0.58	18.38 a	0.69
crude fibre	21.93 a	0.53	23.79 b	0.64
NDF	52.45	10.22	53.79	10.23
ADF	28.11 a	0.12	29.59 b	0.17
ADL	3.52 a	0.11	4.87 b	0.19
Fatty acids				
C12	0.93	0.08	0.87	0.07
C14	1.8	0.06	2.07	0.05
C16	24.15	0.07	23.96	0.06
C16:1 n-7	2.1	0.14	2.21	0.07
C18	3.36	0.09	2.96	0.06
C18:1 n-9	12.98	0.17	12.21	0.46
C18:2 n-6	13.09	0.34	13.82	0.22
C18:3 n-3	35.62	0.56	36.39	0.31
C18:3 n-6	0.12	0.03	0.13	0.04
C20	0.12	0.02	0.11	0.01
C20:1 n-9	0.16	0.04	0.11	0.02
C20:2 n-6	0.14	0.02	0.13	0.03
C20:5	0.15	0.03	0.14	0.03

Explanations: NMP – natural mountain pasture, OMP – organic mountain pasture; $\bar{x}$ – mean value; SD – standard deviation; NDF – neutral detergent fibre; ADF – acid detergent fibre; ADL – acid detergent lignin; a, b – P≤0.05 – means in rows and denoted using different letters differ statistically significantly.

Table 3. Chemical composition (%), physical and technological traits of sheep's milk

Item	NMP		OMP	
	$\bar{x}$	SD	$\bar{x}$	SD
Solids	21.19	6.93	22.53	7.03
Fat	9.06	1.23	8.12	1.90
SNF	8.19	1.57	8.20	1.72
Protein	4.28	0.82	4.13	0.85
Lactose	3.24	0.62	3.38	0.96
Minerals	0.67	0.13	0.67	0.14
Density (g/cm^3)	1.03	0.01	1.03	0.01
Acidity (°SH)	9.62	0.01	9.61	0.01
pH	6.45 A	0.05	6.61 B	0.01
Rennet coagulation time (min.)	2.51	0.05	2.49	0.05
Alcohol number (cm^3 96% C_2H_5OH)	3.73 B	0.12	2.35 A	0.05

Explanations: NMP – natural mountain pasture, OMP – organic mountain pasture; $\bar{x}$ – mean value; SD – standard deviation; A, B – P≤0.01 – means in rows and denoted using different letters differ statistically significantly.

Grazing site had a significant effect on the fatty acid profile of milk fat (Table 4). Differences were found in the content of most FA. A higher proportion of medium-chain fatty acids, containing 8–12 carbon atoms, such as caprylic (C8), capric (C10) and lauric (C12) was found in the milk of sheep grazing on organic mountain pasture. Analysing these relationships between acid groups, similar relationships were found for SFAs (saturated fatty acids) and PUFAs (polyunsaturated fatty acids). However, in milk from sheep grazing on mountain pasture, monounsaturated fatty acids (MUFAs) predominated. More essential fatty acids (EFAs), both linoleic acid LA (C18:2 n-6), α-linolenic acid ALA (C18:3 n-3), γ-linolenic acid GLA (C18:3 n-6) as well as arachidonic acid AA (C20:4 n-6) were found in milk from the organic pasture, while docosahexaenoic acid DHA (C22:6 n-3) and the CLA c9-t11 isomer were found in milk from the mountain pasture. Higher ratios of UFA/SFA, DFA/OFA, MUFA/SFA, PUFA/SFA acids and a lower PUFA 6/3 ratio were found in milk from sheep grazing on NMP.

Table 4. Fatty acid profile of milk (%)

Item	NMP		OMP	
	$\bar{x}$	SD	$\bar{x}$	SD
C8	1.833 A	0.013	2.381 B	0.003
C10	4.905 A	0.053	6.469 B	0.048
C12	3.385 A	0.036	4.1204 B	0.042
C14	12.645 A	0.089	13.650 A	0.111
C16	26.401 b	0.001	26.128 a	0.028
C16-1	1.465	0.039	1.339	0.072
C18	11.486 B	0.134	10.332 A	0.107
C18-1	29.461 B	0.113	26.822 A	0.033
C18-2	1.451 A	0.027	1.688 B	0.097
Gamma18-3	0.014 A	0.001	0.022 B	0.001
C20	0.220 a	0.004	0.245 b	0.006
C18-3	1.966 a	0.016	2.117 b	0.027
*CLA c9-t11	3.834 b	0.019	3.667 a	0.023
*CLA c9-c11	0.217 a	0.003	0.254 b	0.009
*CLA t9-t11	0.044	0.004	0.039	0.004
C22	0.103	0.003	0.101	0.676
C20-4	0.189	0.005	0.256	0.031
C22-1	0.012 a	0.001	0.013 b	0.985
EPA	0.146	0.005	0.151	0.195
DHA	0.215	0.003	0.197	0.665
SFA	60.980 A	0.110	63.429 B	0.338
UFA	39.019	0.111	36.571	0.338
MUFA	30.938	0.039	28.175	0.436
PUFA	8.081 a	0.071	8.395 b	0.199
PUFA-6	1.654 A	0.091	1.967 B	0.164
PUFA-3	2.328 a	0.029	2.466 b	0.938
DFA	40.470	0.208	38.259	0.243
OFA	49.494	0.218	53.096	0.832
UFA/SFA	0.639 B	0.002	0.576 A	0.386
DFA/OFA	0.817 B	0.006	0.720 A	0.622
MUFA/SFA	0.507 B	0.001	0.444 A	0.843
PUFA/SFA	0.132	0.001	0.132	0.082
PUFA 6/3	0.711 A	0.046	0.798 B	0.352

Explanations: NMP – natural mountain pasture, OMP – organic mountain pasture; $\bar{x}$ – mean value; SD – standard deviation; SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, UFA – unsaturated fatty acids, DFA – hypocholesterolemic fatty acids, OFA – hypercholesterolemic fatty acids, EPA – eicosapentaenoic acid, DHA – docosahexaenoic acid; a, b – $P \leq 0.05$, A, B – $P \leq 0.01$ – means in rows and denoted using different letters differ statistically significantly.

Table 5 shows the fractions of milk proteins. Casein proteins constituted 74.59–78.41% of all proteins. The proportion of protein fractions was found to vary according to location of sheep grazing: the proportion of α-casein was higher in milk from NMP sheep, while the other fractions of this protein had a higher proportion in milk from OMP pasture. Whey proteins accounted for 21.56–25.8% of all proteins, and the differences in content concerned α-lactalbumin and serum albumin, the proportion of which was higher in milk protein from natural mountain pasture.

Table 5. Fractions of milk protein (% of total protein)

Item	NMP		OMP	
	$\bar{x}$	SD	$\bar{x}$	SD
α-casein	39.49 B	1.37	37.01 A	0.44
β-casein	30.19 A	2.63	34.28 B	1.56
κ-casein	4.91 A	0.89	7.13 B	0.03
β-lactoglobulin	12.17	2.16	11.10	0.05
α-lactalbumin	4.79 b	0.55	4.01 a	0.11
Immunoglobulin	0.76	0.15	0.63	0.07
Serum albumin	3.84 B	0.51	2.58 A	0.53
Lactoferrin	3.28	0.61	2.25	1.07
Peptides	0.95	0.45	0.99	0.33

Explanations: NMP – natural mountain pasture, OMP – organic mountain pasture; $\bar{x}$ – mean value; SD – standard deviation; a, b – $P \leq 0.05$, A, B – $P \leq 0.01$ – means in rows and denoted using different letters differ statistically significantly.

Discussion

The floristic diversity of meadows and pastures depends on many natural factors such as climate, water conditions, soil, landforms, and human activities, i.e., fertilisation, care, use, intensification of production (Klaus et al., 2013; Jankowska-Huflejt, 2016). The diversity of grassland ecosystems and thus of different pasture types affects the chemical composition of the grass sward. Grassland make a valuable source of fodder rich in nutrients, mainly protein, carbohydrates, macro- and microelements and vitamins. The pasture sward may contain about sixty species of herbs, rich in specific active substances improving the palatability and efficiency of forage utilisation. The performed phytosociological pictures showed that the grass sward of the ecological pasture contained more species than the natural pasture, especially herbs.

A study carried out in the Carpathian mountain pastures, where grazing of Polish mountain sheep took place, showed that the most common plants in the pasture sward were bentgrass (*Agrostis capillaris* L.), perennial ryegrass (*Lolium perenne* L.) white clover (*Trifolium repens* L.), plantain (*Plantago lanceolata* L.) (Kawęcka et al., 2020). Sheep selectively take up plants, preferring meadow fescue (*Festuca pratensis* L.), white (giant) bentgrass (*Agrostis gigantea* Roth), meadow timothy (*Phleum pratense* L.), perennial ryegrass (*Lolium perenne* L.), cocksfoot (*Dactylis glomerata* L.), as well as white clover (*Trifolium repens* L.), plantain (*Plantago lanceolata* L.), caraway (*Carum carvi* L.), dandelion (*Taraxacum officinale* F.H. Wiggers coll.) and yarrow (*Achillea millefolium* L.) (Kawęcka et al., 2017).

From a nutritional point of view, the content of dry matter, fibre and total protein in the pasture sward is especially important; too elevated level of fibre in the forage causes a rapid feeling of satiety in the animals, but due to the low concentration of energy it does not cover the demand for nutrients (Allen, 2000). Comparing the quality of green fodder and hay, Peeters (2008) found that a poor-quality, low digestibility hay has high fibre (crude fibre – CF – about 35–40% of DM) and low protein content (crude protein – CP – about 6.25% of DM) while a highly digestible young grass is fibre-poor (CF about 15–20% of DM) and protein-rich (CP about 18–22% of DM). Based on the chemical analyses, it can be concluded that the pasture pasture sward of both sites in question met the nutritional requirements of grazing animals.

The chemical composition and physical characteristics of sheep milk are highly variable and are determined by a variety of genetic, environmental, and physiological factors. In addition, the technology used to keep the animals, especially the type of feed used to feed ruminants, has a significant impact on the composition of the milk. Sheep milk may contain from 15.42 to 20.9% solids, from 4.6 to 9.05% fat and from 4.75 to 7.2% protein (Bonczar et al., 2009). According to Kawęcka et al. (2020), milk from three mountain sheep breeds (Podhale Zackel, Polish Mountain Sheep and Coloured Mountain Sheep) had an average content of 19.68% solids, 8.48% fat, 6.63% protein and 4.15% lactose. There was no effect of grazing place on the basic composition of the milk of Polish Mountain Sheep in our study, nor on the density, titratable acidity, and coagulation time of the milk, which were within the range reported for sheep milk by the authors mentioned earlier. The other technological parameters determining the suitability of milk for processing, i.e., pH and alcohol number, indicating its thermal stability, despite differences resulting from the place of collection, were also contained within the standards.

Musiał et al. (2018) analysed parameters of milk from Polish Olkuska sheep grazed on a sward of xerothermic grassland and on intensive grassland. The grass sward of intensive grassland, came from sowing a mixture that included fourteen plant species dominated by *Lolium multiflorum* L. Xerothermic grasses were characterised by the presence of vegetation from *Festuco-Brometea* class, which is a community of low flowering grasslands composed of eminently calcareous and thermophilous plants found on shallow limestone soils. The most important species for this family, such as: *Aster amellus* L., *Cirsium pannonicum* L. and *Linum hirsutum* L. are rare compo-

nents of the Polish flora, which have been included in the species protection. Other species, whose share was up to 5%, and which improved the utility value of the habitat were mainly bean plants. Analysis of sheep milk when grazed on grasslands of *Festuco-Brometea* class, showed a higher solids content in milk (17.7%), compared to conventional feeding (11.7%). Fat and protein contents were not significantly different among groups. Studies on other ruminants report a relationship between grazing location and milk composition. Steinshamn et al. (2014) compared the effect of grazing on mid-forest pasture or cultivated pasture on goat milk production and its quality. Goats kept on forest pasture gave less milk but with solids and fat content. According to Barłowska et al. (2012) the highest content of basic components was found in milk obtained from cows in the Beskid Niski region, which the authors explain by both the breed of cows and the greater diversity of botanical composition of pastures.

Feeding fresh green fodder has a particularly positive effect on the fatty acid profile of milk, which has been confirmed by many studies (Atti et al., 2006; Zervas and Tsiplakou, 2011). When pasture green fodder is provided, the content of α-linolenic acid, CLA and t-vaccenic acid (C18:1 11trans; TVA) increases in milk; the ratio of n-6/n-3 acids changes (Gabryszuk et al., 2013). Indeed, pasture is an excellent source of ALA and is one of the most effective feeds to alter the FA composition of milk (Nudda et al., 2003; Gómez-Cortés et al., 2009). In pasture green fodder, 70–90% of the fatty acids are unsaturated ones, with linoleic and linolenic acids having the highest proportion (Dewhurst et al., 2001). It was found that a higher proportion of grasses in the green growth of the natural pasture positively affected the content of unsaturated fatty acids, especially MUFAs (monounsaturated fatty acids) and the content of docosahexaenoic acid DHA (C22:6 n-3) along with the CLA isomer c9-t11. On the other hand, a higher proportion of dicotyledon species in the green growth of the organic pasture, such as *Carum carvi*, *Plantago lanceolata* L., *Plantago major* L.*, Leontodon autumnalis*, *Lotus corniculatus* and *Trifolium pratense* positively affected the content of polyunsaturated fatty acids (C18:2 n-6, C18:3 n-3, C18:3 n-6 and C20:4 n-6). The distinct effect of pasture on milk fat ALA, VA and CLA content was related to the high content of ALA in green pastures, which undergoes partial biohydrogenation to VA in the rumen and is subsequently secreted into milk and partially converted to c9-t11 CLA in glandular tissue by the action of stearoyl-CoA desaturase.

Pasture plant species can have a clear effect on the FA profile of milk. A study by Collomb et al. (2002) on grazing cows on different pasture types showed that the proportion of plants of the *Poaceae* family in mountain and upland pastures correlated positively with the concentration of saturated fatty acids, but negatively with all other fatty acid groups detected in milk fat. The contribution of plant species such as: *Leontodon hispidus*, *Plantago alpina*, *Aposeris foetida*, *Lotus corniculatus* (and *alpina*) and *Deschampsia cespitosa* correlated negatively with the concentration of saturated fatty acids, while the occurrence of *Leontodon hispidus*, *Lotus corniculatus* (and *alpina*) and *Trifolium pratense* correlated positively with the concentration of polyunsaturated fatty acids and with the concentration of CLA and monounsaturated trans C18:1 fatty acids. Additionally, the polyunsaturated fatty acid content was positively affected by *Carum carvi* and *Aposeris foetida* and the CLA concentration by *Plantago alpina* and *Prunella vulgaris* (Collomb et al., 2002). Steinshamn and Thuen (2008), on the other hand, indicate that the fatty acid profile of milk is particularly favourably impacted by red clover, compared to both grasses and white clover.

Feeding of pasture sward with a high proportion of legume plants increased CLA and ALA concentrations and reduced saturated fatty acid content compared to ryegrass pastures (Addis et al., 2005). Morand-Fehr et al. (2007) analysing the influence of animal housing systems and associated feeding on the composition and quality of sheep and goat milk, found that milk from animals kept in systems based on natural pastures is enriched in fat and micronutrients that are beneficial for human health (fatty acids, vitamins) and in volatile components (aroma, terpenes). Comparing natural pastures on the plains, hills, and mountains, they found that milk yields on the latter were slightly lower, but fat and protein contents and the percentage of PUFAs were higher. Grass originating from natural pasture in the early growth phases has a beneficial effect on the PUFA and CLA content of milk. Similarly, Steinshamn et al. (2014) found, in the milk fat of goats grazed on forest pasture, a lower proportion of medium chain fatty acids: C10:0 – C14:0 and C18:2c9t11, but a higher proportion of C18:0, C18:2c912 and C20:0 than on intensive pasture.

Diversity in FA profile in milk of Bovec sheep was found by Cividini et al. (2019) during grazing in four geographical areas in the Alps that differ in altitude, ranging from lowland to high altitude. According to the authors, grazing positively affected the fatty acid composition of sheep milk fat with increased availability of polyunsaturated fatty acids in grass and subsequently in milk. The authors found decreases in saturated fatty acid concentrations in milk with changes in pasture height, while MUFA, PUFA and n-3 PUFA concentrations in milk increased significantly with pasture height. The results confirmed influence of pasture location in the alpine region on the FA profile of sheep milk and its altitude-related changes, which were due to the variability of FA concentration in pasture sward and botanical composition, dependent on its location. The aforementioned Musiał et al. (2018) found a higher proportion of omega-3 fatty acids in the milk of sheep grazing on a naturally valuable xerothermic plant community. In a study by Biondi et al. (2008), it was found that pasture feeding can be a simple, natural method to influence the nutritional properties of sheep milk, and that the change in feeding regime had a particular effect on medium- and long-chain fatty

acids, with differences in fatty acid content already occurring between the second and third day after switching to pasture (Biondi et al., 2008). Linoleic and vaccenic acid contents decrease with plant vegetation (Cabiddu et al., 2005) and the seasonal fluctuations of CLA content in sheep milk are related to seasonal fluctuations of α-linoleic acid content in grass lipids (Meľuchová et al., 2008).

The fractional composition of proteins constitutes an important parameter of milk, both from a technological and human health point of view. Casein proteins are the main group of milk proteins (80%); among them the κ-casein fraction is the most important for cheese production and is responsible for initiating the milk coagulation process. In the health-promoting aspect, the content of functional whey proteins is particularly important, which shows multidirectional effects on the human body, preventing occurrence of many modern-age diseases (Król et al., 2011). They account for 20–25% of milk proteins, of which approximately 75% are albumins, i.e., α-lactalbumin (α-LA), β-lactoglobulin (β-LG) and serum albumin. According to Barłowska et al. (2012), in case of whey proteins, it was found that their content was higher in milk obtained from cows in mountainous regions, which the authors explain by both inter-breed differences and the botanical composition of the basic feed, which was green fodder from permanent pasture. Botanical and weight analyses of collected samples of pasture vegetation showed that in the Bieszczady and Beskid Niski regions there was a significantly higher proportion of herbs and weeds, compared to the lowland region. We found differentiation in the composition of protein fractions in milk depending on the place of grazing: in milk from sheep kept in natural pasture, the content of whey fractions was higher, whereas milk from ecological pasture had higher content of total caseins including κ-casein, of the greatest importance in dairy processing technology.

Conclusion

This study showed greater plant species diversity in the organic mountain pasture, but its yielding was lower than that of the natural pasture. The sward of the natural pasture was characterised by lower dry matter and fibre content but contained more total protein; no statistically significant differences were found in the content of essential fatty acids in the sward of the pastures examined. The type of pasture did not affect the basic composition of the milk of mountain sheep, while differences were found in terms of acidity, alcohol number, fatty acid profile of fat and milk protein fractions. Milk obtained from sheep grazed on organic pasture was characterised by a higher proportion of medium chain fatty acids, and a higher content of saturated fatty acids, as well as polyunsaturated fatty acids, and also essential unsaturated fatty acids, both linoleic acid (C18:2 n-6), α-linolenic acid (C18:3 n-3), γ-linolenic acid (C18:3 n-6) as well as arachidonic acid (C20:4 n-6). However, in milk originating from the natural mountain pasture, a higher proportion of MUFAs (monounsaturated fatty acids), docosahexaenoic acid (C22:6 n-3) and the CLA c9-t11 isomer was found. The composition of milk protein fractions varied according to the location of grazing: milk from sheep grazing on natural pasture had a higher content of whey fractions, important for consumer health, milk from ecological pasture had a higher content of κ-casein, of greatest importance in dairy processing technology.

To sum up, it should be stated that the floral composition of the pasture had an influence on the quality of sheep milk in relation to the fatty acid profile of fat and protein fractions of milk, which is important in terms of both the health-promoting and technological aspects of the raw material and may affect the quality of products made from it.

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