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RESEARCH ARTICLE

Mountain grasslands diversity and the impact on cow's milk fatty acid composition

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ABSTRACT

Milk from pasture fed cows is widely recognized not only as a very valuable source of some nutrients, but also as a functional food. The aim of this study was to determine the content of fatty acids (saturated, monounsaturated and polyunsaturated) and their relationship in the milk fat of cows that were grazed on mountain pastures, during three phenological phases (early vegetative – early June, late vegetative – mid July, and reproductive – late August). The research was conducted on *Bromus erectus* Huds. semi-natural dry grassland in the Durmitor mountain, northern part of Montenegro. During the experimental period, changes in the chemical composition of the biomass from this grassland were determined. The most significant changes were an increase in the content of DM, crude cellulose and the decrease in the content of crude protein. The share of plants of excellent, very good and good quality was highest in the second phase, medium quality plants in the third phase, poor quality plants in the first phase, while the worthless and toxic in the first phase. A significant influence of the phenological phase on the milk fat content was found especially in the third phase, while milk protein, lactose and solids-nonfat content did not differ significantly. The content of the observed parameters in milk was high in all three phases, especially in the third phase, when was the highest content of fat (4.35%) and protein (3.39%) in milk. The content of saturated fatty acids (SFA) was the highest in the second phenological phase (85.16%), while the content of monounsaturated fatty acids (MUFA) decreased through the phenological phases. The polyunsaturated fatty acids (PUFA) content was the highest in the first phenological phase (3.02%), and latter it decreased significantly.

Key words: Botanical diversity, cow's milk, fatty acids, grassland, phenological phase.

INTRODUCTION

The importance of extensive pastures today is reflected in the provision of many ecosystem services. Permanent grassland covers about 34% of the used agricultural area of the European Union (Schils et al., 2022), while in Montenegro semi-natural meadows and pastures constitute 95% of the total utilized agricultural land (Monstat, 2023).

Grazing can significantly contribute to increasing the diversity of grasslands. In heavily modified agricultural landscapes, grazing lands may be the only areas that can provide essential resources for native grassland species (Tonn et al., 2022). Natural pastures in Montenegro have been little studied and cover poorer land, inadequate for intensive exploitation (Radonjic et al., 2019). Low yield and unfavorable floristic composition characterize these pastures. Livestock production in Montenegro, especially milk production is largely based on the use of pastures (Dubljević et al., 2020).

Due to the richness and diversity of plants, the mountain area offers a valuable potential of natural grasslands that are exploited by cattle through grazing, providing quality food. The milk produced by animals using the mountain pastures is enriched in fats and microcomponents, which are beneficial for human health such as fatty acids, vitamins, etc. (Liu at el., 2020). Various natural factors have an impact on the changes in floristic composition and differences in yield and quality characteristics of grass biomass in different phenological phases. The quality and nutritional value of pasture and hay depends of species composition and the quantitative ratio of the individual biological groups (Tenikecier and Ates, 2018). Bozhanska (2022) found correlations between the chemical composition of the grassland biomass and the participation of more important plant species, grasses and legumes. Differences in the chemical composition of biomass are also present among different types of lawns, which causes differences in nutritional value. The highest biodiversity is found in communities with extensive grazing of ruminant where no agrotechnical procedures are carried out (Chabuz et al., 2019).

Stoycheva and Vasileva (2021) state the influence of the type of grassland and altitude on the nutritional value and biomass yield of natural grasslands. The nutritional value and digestibility of animal fodder significantly depends of biochemical characteristics of perennial meadow grasses.

Cow’s milk is the most-consumed milk worldwide as a convenient source of nutrition, because of its widespread availability and large production volumes. Protein, fat, lactose, and minerals are the four major components in all milks, irrespective of the species. The milk composition is continuously changing depending on breeding, nutrition, herd management, lactation stage and season. Cow milk contains about 87% water, 4.6% lactose, 3.4% protein, 4.2% fat, 0.8% minerals and 0.1% vitamins (Park et al., 2016).

Milk and dairy products from cows on pasture-based farms predominantly consuming fresh grazed grass (typically classified as “grass-fed” milk) have been previously shown to possess a different nutrient profile, with potential nutritional benefits, compared with conventional milk derived from total mixed ration (Calvache et al., 2024). Conjugated linoleic acid (CLA) which is synthesized by ruminal microbiota from vaccenic acid (*trans*-11 octadecenoic acid) is the most active antioxidant in milk fat. Despite the above, the health-promoting properties of milk fat have been discredited in the scientific community due to the high content of saturated fatty acids (FAs) and cholesterol (Chabuz et al., 2019). The CLA content of milk ranges from 2-37 mg g^{-1} fat, and is determined by cattle breed, diet, age of the animal, season (CLA content is higher in May, June and July). Fatty acid content also has economic importance due it is an important factor in the technological quality of raw milk. In this sense, making targeted modifications to the FA profile has the potential to significantly contribute to the production of dairy products with higher added value (Hanuš et al., 2018).

Diet composition is the main factor that can cause shifts in the microbial diversity of the rumen with subsequent changes in milk FAs. This is because individual categories of bacteria have different lipid metabolism and thus produce higher proportions of specific FAs (Conte et al., 2018).

Management of animal nutrition during lactation is considered to be critical for producing a high-quality FA profile in milk fat, more than any other factors such as cow breed and genotype, age, health, and aspects of lactation (Samková et al., 2018). Morales-Almaráz et al. (2018) show that increasing the proportion of fresh (pasture) or preserved forage (generally fiber) improves the milk FA profile by increasing unsaturated FA and rumenic acid (RA; C18:2 c9, t11; isomer of conjugated linoleic acid (CLA) content in milk fat.

Many studies have proved the significant impact of the phenological phase on milk FA content. The milk FA profile may be influenced by grazing management and phenological phase of pasture. The quantity and quality of available forages, and hence the quality of dairy products, are influenced by seasonal variations and any changes that occur in pastures may also have an influence (Coppa et al., 2015).

The aim of this research was to evaluate changes in the composition of pasture and milk on mountain pastures in three phenological phases, and to continue the researches of pastures in Montenegro which have just been conceived.

MATERIALS AND METHODS

Study site

The research was performed in the dry semi-natural pasture in northern part of Montenegro, Durmitor mountain 1600-1700 m a.s.l. (43°09′11.43″ N, 19°10′25,9″ E), during 2017 (from early June to late August). The area of Durmitor has strong climatic diversity, which is influenced by the orography of this mountain. This causes the retention of cold air in bays and valleys, precipitation on the windward sides, changes in the direction of air currents, air waves on the windward sides, creating conditions for the development of local disasters, etc.

The climate is characterized by short and fresh summers, long, cold and snowy winters, a large amount of precipitation and high cloudiness (Burić and Micev, 2008).

The soil of the Durmitor area is different in certain morphological units. The valley extensions are made of fluvioglacial sediments. The lake surface is covered with moraine sediments, from which limestone heads rise. Deposits are made of sand, silt, boulders and blocks. The slopes of the Durmitor ridge are composed of limestone, they are rocky and only in the crevices is red rock found (Milojević, 1955).

The research included 20 multiparous middle lactation cows, crossbreeds (Simmental, Holstein) which were fed exclusively on pasture during the observed period. An extensive livestock system, which is mostly reflected in poorer nutrition in the winter based on hay obtained from natural meadows and only grazing during summer, is typical for this area. The first milk and grass sampling was done 10 d after the cows started with grazing in order to adapt to a new feed. The interval between phases was 45 d. Milk samples were taken three times during the grazing season in order to examine the influence of the maturity stage of plants on the amount of milk, parameters of the physical and chemical composition, number of somatic cells and content of fatty acids (FA) in the milk. Floristic composition of the pasture was determined at the same time as milk and pasture samples were taken. The first phenological phase represents the early vegetatitive, the third phase is fully reproductive phase, already mature pasture, while the second phase is the middle between the two, late vegetative phase.

Botanical assessment

Braun-Blanquet methodology (Braun-Blanquet, 1964) was used for floristic composition and structure of plant comunities determination. The releves on pasture were taken inside the three plots (1 - 43°09’39.84” N, 19°09’48.01” E; 2 - 43°09’11.43” N, 19°10’25,9” E; 3 - 43°09’79.52” N, 19°10’52,49” E) size 10 × 10 m^2, in three different aspects: Early June (early vegetation phase), mid of July (late vegetation phase), late August (full reproductive phase). Identification of plant material was performed with standard keys for plant identification (Tutin et al., 1964-1993). Herbarium material is deposited in the herbarium collection at the University of Montenegro (TGU), Podgorica. Certain proportion of functional groups, such as grasses (Poaceae), legumes (Fabaceae) and other herbs was determined for the needs of pasture quality determination. In the analysis of the quality of the tested pastures, modified quality indices for individual plant species were used, so that the descriptive quality assessment was translated into numerical values and modified according to Peeters and Dajić (2006).

Grassland biomass chemical composition analysis

Forage sample collection was done according to procedure AS-1064 (2012), and chemical analyses were conducted according to AOAC (2000). Samples were taken from eight locations at random. The following parameters of pasture were determined: Moisture content by the gravimetric method; crude protein content based on total N content by Kjeldahl method and multiplication by factor 6.25; total cellulose content of the fiber extraction device according to the manufacturer’s procedure (Fibretherm; Gerhardt, Königswinter, Germany) and in accordance with the appropriate method according to Henneberg and Stohmann (1859), total fat content in accordance with the Soxhlet method; total ash content by the gravimetric method. Parameters of the grass chemical composition were analyzed in the Laboratory for Animal Nutrition of the Biotechnical Faculty in Podgorica.

Milk chemical composition

Two samples were taken from each cow, according to ISO 707:2008/IDF 50:2008 (ISO, 2008), one for basic chemical analyses and the other for determining the content of FAs (2×50 mL). Hand portable refrigerators were used for transporting samples from the farm to the laboratory reception, which prevented changes in the quality of the milk samples. The analyses were performed using standard and instrumental methods in the laboratories, the Dairy Laboratory at the Biotechnical Faculty. Analysis of milk was performed using Combi Foss 5000 instrument (Foss Electric A/S, Hillerød, Denmark), consisted of MilkoScan 4000 and Fossomatic 5000 basic. MilkoScan operates on the principle of infrared spectrophotometry (ISO 9622:2000 (IDF 141:2000)) (ISO, 2000).

Determination of fatty acids

The FA content of the milk was determined by gas chromatography. Milk samples were stored in freezers at a temperature of -40 °C. Determination of the FA content was carried out at the Center for Ecotoxicological Research in Podgorica, using the gas chromatograph with mass detector (GCMS) (GCMS-QP2010 Plus; Shimadzu, Kyoto, Japan), on column SP-2560 fused silica capillary column 100 m, 0.25 mm ID, 0.20 µm film. The results are expressed in % as a proportion of individual FAmethyl esters in the total FA content. Methyl esters were analyzed according to ISO Standard 15885 (ISO, 2002). The content of saturated, monounsaturated and polyunsaturated FA (C 4:0 to C 24:1) was determined.

Statistical analysis

Statistical analysis included the calculation of basic statistical parameters. The statistical significance of results was tested by ANOVA. The LSD test was performed for testing the influence of phenological phase on grassland biomass chemical composition and cow milk characteristics. For statistical analysis of data program Statistica 10 (StatSoft, Tulsa, Oklahoma, USA) was used.

RESULTS

Vegetation of this pasture is represented by a community with *Bromus erectus* (Table 1) which consists of 70 species, with 61 genera and 25 families. The taxonomic spectrum of families is dominated by Poaceae (11 species), Asteraceae (9 species), Fabaceae (6 species), Rosaceae (4 species), Plantaginaceae (5 species) and Lamiaceae (4 species).

The dominant species were: *Agrostis capillaris*, *Alchemilla xanthochlora*, *Bromus erectus*, *Festuca pratensis*, *Festuca rupicola*, *Koeleria splendens*, *Lathyrus pratensis*, *Linaria vulgaris*, *Plantago lanceolata*, *Poa alpina*, *Potentilla crantzii*, *Scabiosa columbaria*, *Succisa pratensis*, *Thymus longicaulis*, *Trifolium repens*, *Trisetum flavescens*, *Verbascum nigrum*, *Veronica chamaedrys* and *Viola tricolor*.

Examination of the quality of this community showed that the group of species of excellent, very good and good quality is represented by 11.4%, the group of plants of medium quality by 10.0%, and the group of poor quality by 20.0%. Worthless plants made up as much as 40.0%, and harmful (toxic) 18.5% (Figure 1).

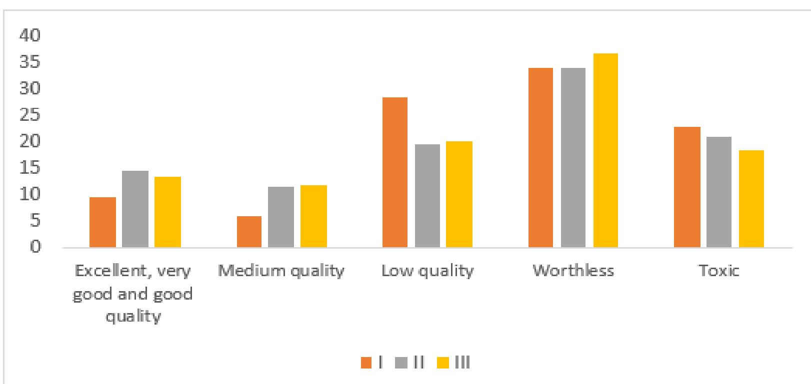

Figure 1. Participation (%) of plant quality groups in three phenological phases. I: Early vegetative phase; II: late vegetative phase; III: reproductive phase.

The floristic composition varied significantly through aspects. Some plants were present in the reproductive phase in the pasture during all three sampling phases (e.g., *Carex caryophyllea*, *Festuca bosniaca*, *Festuca rupicola*, *Bromus erectus*, *Galium anisophyllon*, *Phleum alpiunum*, *Scabiosa columbaria*, *Leucanthemum vulgare*, *Succisa pratensis*, *Stachys germanica*, *Prunella lanciniata*, *Luzula campestris*, *Ranunculus bulbosus* and *Trifolium repens*), while some were typical for a certain phase, that is, an aspect of the plant community.

The first aspect of the flora is characterized by 53 species. *Festuca rupicola*, *Festuca bosniaca*, *Potentill*, *Succisa pratensis* and *Leuchanthenum vulgare* had the highest cover values.

In the second floristic aspect, 62 species were found. Some additional species appeared: *Lathyrus pratensis*, *Laucanthemum vulgare*, *Rhinanthus minor* and *Trifolium pratense*. *Agrostis capillaris*, *Bromus erectus*, *Festuca bosniaca*, *Festuca rupicola*, *Poa alpina*, *Trifolium repens*, *Verbascum nigrum* and *Lathyrus pratensis* had great cover value.

The third aspect includes 60 species. The third aspect of the flora is characterized by the appearance of the late summer xerophilous grass *Botriochloa ischaemum*. Some species did not appear in the third aspect such as *Luzula campestris*, *Taraxacum officinale* and *Bupleurum falcatum*.

The participation of groups of plants differed significantly in certain aspects (Figure 1). The share of plants of excellent, very good and good quality was highest in the second aspect, medium quality plants in the third aspect, poor quality plants in the first phase, while the worthless and toxic in the first aspect.

According to the quality index, the best quality of this community was in the third phase (3.375), then in the second (3.172) and the worst in the first phase (2.599). The total quality index of this community was 3.577, which represents moderate quality because the maximum value for this index is 5.

Table 1. Floristic composition and structure of plant community in different phases. I, II, III plots; Braun-Blanquet (1964) cover scale: r – 3-5 individuals; + - few individuals; 1 – less than 5%; 2 – 5%-25%; 3 – 25%-50%; 4 – 50%-75%). Only species with cover value equal or bigger than 1 are given in the table.

	Phase I			Phase II			Phase III		
Species	I	II	III	I	II	III	I	II	III
Achillea millefolium L.	1	1	1	+	+	+	1	+	+
Agrimonia eupatoria L.	-	1	1	-	1	1	-	1	1
Agrostis capillaris L.	-	-	-	1	2	2	2	2	2
Alchemilla xanthochlora Rothm.	1	+	+	1	1	+	2	+	+
Artemisia absinthium L.	-	-	+	-	1	+	-	1	+
Bromus erectus Huds.	1	1	1	2	2	2	1	2	2
Bupleurum falcatum L.	+	1	-	+	1	-	+	+	-
Carex caryophyllea Latourr.	1	1	1	1	1	1	+	+	+
Carlina acaulis L.	+	+	1	+	+	+	-	+	r
Carum carvi L.	-	1	+	-	+	+	-	-	-
Cirsium eriophorum (L.) Scop.	-	1	1	-	1	1	-	1	1
Colchicum autumnale L.	-	1	1	-	1	+	-	+	+
Convolvulus arvensis L.	-	+	1	-	+	+	-	+	+
Cynosurus cristatus L.	-	-	-	-	1	1	-	1	1
Dactylis glomerata L.	-	-	-	-	1	1	-	+	+
Dianthus deltoides L.	-	-	-	1	+	1	+	-	+
Euphorbia myrsinites L.	1	-	-	1	-	-	1	-	-
Festuca bosniaca Kumm. & Sendtn.	1	1	1	1	1	1	1	1	1
Festuca rupicola Heuff.	3	2	2	3	2	2	2	2	2
Galium anisophyllon Vill.	+	+	+	1	1	+	1	1	1
Koeleria splendens C. Presl.	-	-	-	2	2	1	1	1	1
Lathyrus pratensis L.	-	1	+	-	2	+	-	2	1
Linaria vulgaris Mill.	2	+	1	1	+	1	-	+	+
Luzula campestris (L.) DC.	1	1	-	1	1	-	+	+	
Medicago falcata L.	-	1	1	-	+	+	-	+	+
Phleum alpinum L.	1	1	1	+	+	+	+	1	+
Pilosella bauhini (Schult.) Arv.-Touv.	1	+	1	1	+	1	+	+	+
Plantago lanceolata L.	2	1	1	1	+	1	+	+	+
P. media L.	1	+		+	+	+	+	1	+
Poa alpina L.	-	2	2	-	1	1	1	1	1
Potentilla crantzii (Crantz) Beck ex Fritsch	2	1	1	1	+	+	-	-	-
Prunella laciniata (L.) L.	1	+	1	1	+	+	1	+	+
Rhinanthus minor L.	-	1	-	-	1	1	-	1	+
Rumex acetosella L.	-	-	1	-	-	1	-	-	+
Scabiosa columbaria L.	1	1	2	1	+	1	+	1	2
Schedonorus pratensis (Huds.) P. Beauv.	-	-	-	-	1	2	-	1	1
Stachys germanica L.	+	1	1	+	+	+	+	+	+
Succisa pratensis Moench	2	+	1	2	+	1	2	1	1
Thymus longicaulis C. Presl	2	1	+	2	1	+	1	+	+
Trifolium pratense L.	-	1	1	-	+	+	-	+	+
T. repens L.	2	2	1	1	2	2	+	1	1
Trisetum flavescens (L.) P. Beauv.	-	-	+	-	3	2	-	+	1
Verbascum nigrum L.	-	2	2	-	2	2	-	1	1
Veronica chamaedrys L.	-	2	2	-	2	2	-	2	1
Vicia cracca subsp. *incana* (Gouan) Rouy	-	+	-	-	1	-	-	+	-
Viola tricolor L.	-	-	2	-	-	1	-	-	+

Chemical composition of the pasture biomass

As expected, the chemical composition of the pasture biomass has changed throughout the grass phenological phases (Table 2). The content of DM increased during research period. Differences are significant among all phases ($p < 0.05$).

The content of the total DM, total crude fibers and fat content increase during the experimental period and differences were significant among tested phenological phases. The low content of DM that results in reduced energy intake is the main limiting factor for high milk production in pastures.

Significant differences were found in the protein content, but the protein content decreased during assesment. The difference in ash content was significant only in the first phase.

Table 2. Chemical composition of pasture biomass. Values followed by different letters have significant difference according to LSD test (p < 0.05). 1: Early vegetative phase; 2: late vegetative phase; 3: reproductive phase.

Phenological phase	DM	Crude protein	Total fat	Crude fibers	Ash
	%	g kg^{-1} DM	g kg^{-1} DM	g kg^{-1} DM	g kg^{-1} DM
1	20.81 ± 2.9^a	196.84 ± 2.2^a	12.33 ± 1.1^a	222.16 ± 3.7^a	72.82 ± 3.6^a
2	30.15 ± 3.3^b	100.97 ± 1.8^b	15.42 ± 2.4^b	286.11 ± 2.6^b	65.37 ± 1.7^b
3	46.82 ± 4.0^c	88.64 ± 2.3^c	15.99 ± 1.8^c	312. 31 ±3.5^c	68.20 ± 1.9^b

Chemical composition of milk

The content of milk fat, protein, lactose and solids-nonfat in milk was included in the analysis (Table 3). The fat content significantly increased during this period (3.50%-4.35%). Significant differences were found in the content of fat in the third phase. The protein content varied slightly (3.28%-3.39%). The phenological phase did not significantly affect content of protein. Content of lactose was very constant during this period (4.46%-4.25%) and the influence of the phenological phase was nonsignificant. Solids-nonfat consists of proteins, lactose and a small part of the minerals, so neither the phenological phase had any influence on its content (8.47%-8.42%).

The content of the observed parameters in milk was quite good in all three phases, especially in the third phase, when was the largest share of fat and protein in milk. As expected, the amount of milk on the control day decreased with the maturing of the pasture.

Table 3. Average milk chemical composition. Values followed by different letters have significant difference according to LSD test (p < 0.05). 1: Early vegetative phase; 2: late vegetative phase; 3: reproductive phase; SNF: solids-nonfat; DMY: DM yield.

Phenological phase	Fat	Protein	Lactose	SNF	DMY
	%	%	%	%	kg
1	3.50 ± 0.54^a	3.28 ± 0.34^a	4.46 ± 0.38^a	8.47 ± 0.62^a	14.90 ± 3.46^a
2	4.04 ± 0.42^b	3.28 ± 0.21^a	4.50 ± 0.24^b	8.42 ± 0.41^a	12.40 ± 2.50^a
3	4.35 ± 0.41^c	3.39 ± 0.38^a	4.25 ± 0.32^b	8.42 ± 0.44^a	10.00 ± 2.49^b

Fatty acid composition

The content of 35 FAs was determined, 17 saturated and 18 unsaturated (Table 4). In the first phase, the total saturated fatty acids (SFAs) content was 74.67%. In all phases C16:0 has the highest amount, then C18:0 and C14:0. More than 1% has C10:0, C12:0 and C15:0 while other SFAs account for less than 1% of the total FA content. The total content of SFAs increased in the second phase (85.16%). This increase is mostly reflected in the increase in the content of C16:0. The similar FA content was in the third phenological phase.

Table 4. Milk fatty acid (FA) content (% of total fatty acid methyl esters). *Significant difference between mean values $p < 0.05$. SFA: Saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; ^{ns}: nonsignificant differences. Phenological phases I: early vegetative phase; II: late vegetative phase; III: reproductive phase.

	Phenological phase		
	I	II	III
FA	Mean ± SD	Mean ± SD	Mean ± SD
	SFA		
C4:0	1.93 ± 0.12*	2.07 ± 0.11*	1.65 ± 0.05*
C6:0	2.20 ± 0.11*	2.14 ± 0.09*	1.84 ± 0.06*
C8:0	1.63 ± 0.08*	1.52 ± 0.07*	1.33 ± 0.05*
C10:0	3.69 ± 0.22*	3.52 ± 0.21*	3.00 ± 0.16*
C11:0	0.17 ± 0.01*	0.16 ± 0.01 ^{ns}	0.14 ± 0.01*
C12:0	3.35 ± 0.24*	4.08 ± 0.21*	3.56 ± 0.18*
C13:0	0.17 ± 0.01*	0.14 ± 0.00*	0.14 ± 0.00*
C14:0	13.39 ± 0.56*	14.45 ± 0.45*	14.15 ± 0.40*
C15:0	2.22 ± 0.10*	2.11 ± 0.08*	2.38 ± 0.11*
C16:0	28.96 ± 0.41*	36.16 ± 0.54*	34.45 ± 0.46*
C17:0	1.21 ± 0.03*	1.12 ± 0.04*	1.30 ± 0.04*
C18:0	15.9 ± 0.71*	17.09 ± 0.70*	19.02 ± 0.54*
C20:0	0.26 ± 0.01*	0.24 ± 0.01*	0.27 ± 0.01*
C21:0	0.05 ± 0.00*	0.06 ± 0.00 ^{ns}	0.05 ± 0.00*
C22:0	0.12 ± 0.00*	0.11 ± 0.00*	0.12 ± 0.00*
C23:0	0.06 ± 0.00*	0.07 ± 0.00*	0.06 ± 0.00*
C24:0	0.06 ± 0.00*	0.06 ± 0.00*	0.06 ± 0.00*
Σ	74.67	85.16	83.58
	MUFA		
C14:1	0.39 ± 0.03*	0.41 ± 0.03*	0.39 ± 0.03*
C16:1	0.60 ± 0.03*	0.54 ± 0.03^{ns}	0.57 ± 0.02*
C18:1n9c	19.16 ± 0.72*	13.34 ± 0.48*	12.99 ± 0.43*
Σ	21.49	14.61	14.26
	PUFA		
C18:3n3	1.58 ± 0.03*	0.86 ± 0.05*	0.85 ± 0.02*
C18:2n6c	1.08 ± 0.02*	0.89 ± 0.06*	0.78 ± 0.03*
C20:3n6	0.05 ± 0.00*	0.05 ± 0.00*	0.05 ± 0.00*
C20:5n3	1.58 ± 0.03*	0.86 ± 0.05*	0.85 ± 0.02*
Σ	3.02	2.11	1.99

Only C:18 increased its share through phases, while C:8, C:10 and C:11 decreased from first to third phase. Six FAs, C:4, C:12, C:14, C:16, C:21 and C:23 had the highest content in second phase, and then a slight decrease in the third phase. A significant influence of the phenological phase was found for the majority of saturated FAs in the third phase, while for the first two phases there was no significance.

The content of nine monounsaturated fatty acids (MUFAs) was determined. In the first phase, the total content of MUFAs was 21.49%. The largest share has C 18:1n9c, 19.16%. The total content of MUFAs decreased in the second phase (14.61%) with a similar share of individual FAs. Slightly lower content of MUFAs was recorded in the third phase, 14.26% and five MUFAs were present in traces (C15:1, C17:1, C20:1, C22:1n9, C18:1n9t, C24:1). The highest content in second phase had C14:1. The greatest decrease in second phase was in C18:1n9c. A significant influence of the phenological phase was found for all MUFAs in the third phase, while there was no significance for the first two phases. The content of nine polyunsaturated fatty acid (PUFAs) was determined. In the first phase, the total content of PUFAs was 3.02%. The largest amount has C18:2n6c and C18:3n3, while other PUFAs account for less than 1% of the total FA content. These two fatty acids are also the most important PUFAs. The total content of PUFA was lower in the second phase (2.11%) with a lower content of C18:3n3. The lowest PUFAs content was recorded in the third phase, 1.99%. Five polyunsaturated FAs were present in traces (C18:3n6, C18:2n6t, C20:2, C20:3n3, C22:2). Three FA, C18:3n3, C20:3n6 and C20:5n3, had the highest content in the first phase and slightly decrease in the other phases. A significant influence of the phenological phase was found for all PUFAs in the third phase, while there was no significance for the first two phases. The quality of the biomass (low content of cellulose and high protein content) in the first phase influenced favorable FAs composition of the milk. A significant number of preferred FAs had the highest participation in the first phase, which means that in this phase, milk has the best FAcomposition. During the second and third phases, there was a significant decline in the quality of biomass, the participation of functional plant species changes, as well as the participation of high-value species. The reason for such a large decline in the content of PUFAs in milk in the second phase was the high temperatures during July and the sudden decline in the quality of biomass. Also, there is a coarsening of the existing biomass and a reduction of the available biomass yield. Nevertheless, the third phase was very similar to the second, so it can be concluded that it is justified to keep the cows on pasture so late, although the amount of milk indicates the need of introduction additional nutrients into their nutrition.

DISCUSSION

The extensive livestock system with traditional farming based on grazing during summers is typical for this area (Radonjic et al., 2019; Dubljević et al., 2020). Grazing is the best way to utilize extensive grasslands and contribute to the preservation of the entire agro-ecosystem (Tonn et al., 2022). Grazing dairy cows produce milk with high levels of unsaturated fatty acids (UFAs) and conjugated linoleic acid, which are able to reduce cardiovascular diseases and have some anticancer properties (Corazzin et al., 2019). Several authors examined the chemical composition of biomass from mountain pastures and the results obtained are analogous to these (Coppa et al., 2015; Corazzin et al., 2019). The same authors also state the content of fatty acids (FAs), with the fact that the content of saturated FA is similar to that in this study, while the content of UFAs is slightly higher. Numerous studies show that an increase in the proportion of green mass has a positive effect on the content of polyunsaturated FAs, while on the other hand Marín et al. (2018) showed that by reducing concentrate supplementation to grazing cows the level of milk monounsaturated fatty acid (MUFA) decreased.

Recent botanical research of this type of pasture is poor in Montenegro. This study confirmed a high diversity of species in this area, which is indicated by some old data (Milojević, 1955). The previously cited authors also state that the reasons for the lower yield and poorer quality of pasture in this area are: Soil, rocky terrain, harsh climate, abandonment and underutilization, and lack of repair measures. Studies of lowland dry pasture performed by Radonjic et al. (2019), indicate similar chemical composition of pasture biomass and slightly higher polyunsaturated fatty acid (PUFA) content in cow’s milk, especially in the second phase. Dubljević et al. (2020) did similar research but on wet pasture and obtain higher PUFA content in the second phase, and lower in the third.

Grazing represents a good strategy for improving milk fat composition because it increases the proportion of desirable FAs, mainly C18:1 c9, rumenic acid and *cis*-MUFA, and decreases the proportion of saturated fatty acids (SFA) compared to silage-based feeding (Hanuš et al., 2018). Loza et al. (2023) determined the positive influence of pasture diversity on the FA composition of milk. Pasture botanical composition and the phenological phase influence the content of the main nutritional n-3 precursor, α-linolenic acid which makes about 50%-75% of the total FA content of fresh grass. Also, increase in the content of conjugated linoleic acid in milk may be the result of the consumption of grasses rich in linoleic (C18: 2) and α-linolenic acid (C18: 3), precursors of conjugated linoleic acid (Hanuš at al., 2018; Samková et al., 2018). The highest level is in young plants at the first cut, and then it decreases during summer, particularly around flowering (De La Torre-Santos et al., 2020).

The diversity of this pasture in terms of the number of species is better compared to most research (Coppa et al., 2015; Radonjic et al., 2019; Dubljević et al., 2020), but the participation of certain desirable groups of plants such as Poaceae and Fabaceae was significantly lower. The obtained results on the chemical composition of milk are similar to numerous results that have been carried out on different types of pastures, but there are no results from this type of *Bromus erectus* dry pasture.

The influence of the phenological phase on the chemical composition of biomass, and indirectly on the composition of milk, has also been confirmed by Radonjic et al. (2019) and Dubljević et al. (2020). The same authors reported higher participation of favorable plant groups of lowland pastures in Montenegro. The content of saturated FAs increases during grassland biomass maturation in different pastures, while in this research a decline in the third phase was obtained. The level of MUFAs in grasses change slightly with grass maturation. Rutkowska et al. (2012) found higher content of MUFAs in the summer period compared to the spring in the milk of cows grazing on mountain pastures.

The changes in the PUFA content are in accordance with Coppa et al. (2015), who indicate a significant influence of the phenological phase on the PUFA content in grazing cows, while Rutkowska et al. (2012) found a significant increase in PUFAs content in the summer compared to spring. Reduction of the C18: 3n3 in biomass with maturation of pasture is reported by Revello-Chion et al. (2011).

During the grazing season, dairy cows are usually moved through different pastures to take full advantage of the available territory following the vegetation gradient and the so-called 'vertical transhumance' animal management strategy. Moreover, the use of pasture is considered as a tool to increase the added value of summer dairy products obtained from mountain farms (Romanzin et al., 2015).

Based on results presented here, it is necessary to improve pastures with some agricultural practices, especially in summer period when the quality and quantity of grass is significantly decreasing.

CONCLUSIONS

Consumers consider that production systems based on pastures are more acceptable for the environment and animal welfare, and the products are extremely valuable and beneficial for their health. This value is reflected in the participation of some antioxidative substances such as polyunsaturated fatty acids. This pasture has a great diversity of species, but worse quality in terms of some plant group participation, as well as milk composition and milk yield. All this is influenced by the extensive way of use and the natural conditions that prevail in this area (soil, climate, temperature).

Mountain pastures face many challenges such climate changes. Farmers have to fight against these impacts in different ways. One of them is by adding concentrated feed in very dry periods, in order to prevent a drop in production and product quality (late vegetative phase).

Author Contribution

Conceptualization: D.R. Methodology: S.A., M.M. Software: M.Đ. Validation: O.J. Formal analysis: D.R., M.M. Investigation: D.R. Resources: D.R., O.J. Data curation: M.Đ. Writing-original draft: D.R. Writing-review & editing: D.R. Visualization: D.R. Supervision: D.R. Project administration: M.M. Funding acquisition: D.R. All co-authors reviewed the final version and approved the manuscript before submission.

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