Fatty acid composition of summer and winter cows' milk and butter

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Summary

Fatty acid (FA) composition of summer and winter cows' milk and butter as well as pasture forage samples were analysed by gas chromatography. The FA contents in butter fat were very similar to those in milk fat from which butters were produced. The higher average contents of FA in the summer milk compared with the winter milk ($P < 0.001$) were found for health-beneficial FA, e.g. *cis*-9, *trans*-11 conjugated linoleic acid 8.0 mg.g^{-1} vs 4.1 mg.g^{-1}, *trans* vaccenic acid 17.8 mg.g^{-1} vs 8.5 mg.g^{-1}, α-linolenic acid 6.4 mg.g^{-1} vs 4.4 mg.g^{-1}, linoleic acid 20.4 mg.g^{-1} vs 18.4 mg.g^{-1} ($P < 0.01$), oleic acid 218 mg.g^{-1} vs 194 mg.g^{-1}, and for non-atherogenic stearic acid 102 mg.g^{-1} vs 90 mg.g^{-1} of total FA. On the other hand, the contents of health-risky palmitic acid and myristic acid were lower in the summer milk: 293 mg.g^{-1} vs 331 mg.g^{-1} ($P < 0.001$) and 102 mg.g^{-1} vs 112 mg.g^{-1} FA ($P < 0.01$), respectively. In comparison to the winter diet, the summer diet improved the rheological and nutritional properties of butter. The summer butter showed lower palmitic/oleic acids content ratio 1.34 compared with 1.71 ($P < 0.01$) for the winter butter, and improved nutrition value due to quartile reduction of atherogenicity index from 2.72 to 2.19 ($P < 0.001$).

Keywords

gas chromatography; fatty acid composition; pasture plants; summer cows' milk; winter cows' milk; butter; conjugated linoleic acid

Functional foods contain dietary components that have beneficial properties beyond their traditional nutrient value. The concept of dairy products as functional foods has gained much attention largely due to health benefits associated with conjugated linoleic acid (CLA) isomers, particularly *cis*-9, *trans*-11 CLA isomer also named rumenic acid (RA). RA is the most abundant (accounting for 75–90% of the total CLA content in milk fat) and most biologically active natural isomer of CLA in dairy products. The studies conducted on cell cultures, animal models and some human studies found RA to be a potent anticarcinogen, as well as having antiatherogenic, immune-modulating, antidiabetic, cholesterol-lowering properties and other health benefits [1]. Milk fat is also one of the few food sources of butyric acid, a potent inhibitor of cancer cell proliferation as well as an inducer of differentiation and apoptosis in a number of cancer cell lines [2,3].

Milk and milk products are the richest source of CLA that are both accessible and acceptable to most consumers. The average CLA content of commercial milk in the USA varies between 3 mg·g^{-1} and 6 mg·g^{-1} of the total fatty acid (FA) content [4]. The daily intake of CLA that would provide cancer protection, 3.5 g CLA per day [5], is 75% higher than the amount that is currently being consumed by humans [6]. In order to increase the uptake of CLA, humans need to either consume more foods containing CLA, or to increase the content of CLA in the products being consumed. The latter approach is more practical since it can be manipulated in particular through the diet and individual cows' variation, whereas the stage of lactation, parity and breed had little effect on CLA milk fat content. The average contents of RA in milk of pasture feeding cows are 2–3 times higher than those during barn feeding. With different feeding and management practices, the CLA contents in milk fat from mountain pastures varied up to 21.8 mg·g^{-1} of total fat [7].

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The effect of diet on the FA profile of milk fat is substantial and, in a study by LYNCH et al. [8], diet was responsible for 95% of the variance in milk fat. In the ruminants, CLA is produced naturally from dietary linoleic (LA), α-linolenic (ALA) and *trans* vaccenic acid (*trans*-11 18:1, TVA) FA. CLA synthesis occurs either in the rumen during ruminal biohydrogenation of dietary FA or in tissues by Δ-9 desaturase enzyme activity. CLA is synthesized in the rumen during biohydrogenation of LA, while TVA is synthesized during biohydrogenation of LA, ALA and by oleic acid isomerization [9]. TVA provides a substrate for endogenous synthesis of CLA through the activity of Δ-9 desaturase enzyme especially in the mammary gland and in other body tissues [9]. By increasing the intake of these FA it is possible to increase the production of CLA in the ruminants, and the human CLA consumption may increase without the need to consume more or larger portions of milk products.

Grazing is likely the best natural approach for increasing CLA content in milk of ruminants consuming all-forage diets. ALA and LA are the predominant unsaturated FA in forages with ALA content accounting for 50–75% of the total lipid fraction, depending on the stage of pasture plants development. Variability in FA content with plant development can affect the utility of forages in grazing systems. Nevertheless, the variability in forage FA throughout pasture season could be minimized in some plant species by management that maintains the forage in a vegetative state [10]. LOCK and GARNSWORTHY [11] revealed the seasonal variation in the CLA content of milk fat with the highest content of 17 $mg \cdot g^{-1}$ FA in grazing cows and the lowest content of 6.0 $mg \cdot g^{-1}$ fat in winter. ELGERSMA et al. [12] noted seasonal changes in cows' milk CLA content between winter and summer period from 3.0 $mg \cdot g^{-1}$ to 7.0 $mg \cdot g^{-1}$ FA. The authors found 300% decrease in CLA content (from 1.5% to 0.5% of the total fat content) in the past four decades due to the changes in feeding and management of animals, especially higher proportion of concentrates and silage, and lower proportion of forage. The potential of pasture plant breeding and management to increase the ALA content of pasture grass and clover species was reviewed by DEWHURST et al. [13].

LEDOUX et al. [14] analysed 54 French butters from local producers in different seasons. The average CLA levels in butter were 4.5 $mg \cdot g^{-1}$ FA in winter and 8.0 $mg \cdot g^{-1}$ FA in summer. The authors noted regional variations of CLA levels in French butters. The butters from mountain regions showed the highest annual average CLA levels and the greatest variation between the winter and summer. Similar variations were found for TVA.

Our study was conducted to analyse the content of individual FA in pasture plants, in summer and winter cows' milk, as well as from these milks produced summer and winter butter in Orava region (Slovakia) under common practice conditions. The objective was to compare the summer and the winter butter based on the content of health-affecting FA and to discuss the options for producing butter with higher CLA content and other health-promoting FA as it is the most commonly consumed animal fat.

MATERIALS AND METHODS

Six cows' milk farms were located in Jasenová (No. 1), Žaškov (No. 2), Oravská Poruba (No. 3), Bziny (No. 4), Veličná (No. 5), and Ludrová (No. 6). Butter was made of bulk milk obtained at ORAVA – MILK Leštiny, Slovakia. Production farms Nos. 1–5 were located in Orava region and No. 6 in Liptov region (Slovakia).

Milk producers raised mostly Holstein cows' herds in average of 200 animals with different numbers at lactation and different milk productivity. Each winter and summer milks of 6 producers were sampled 3-times during 2008–2009. Winter butters were sampled 5-times and summer butters 7-times during 2008–2009. Pasture plants from 6 cows' milk farms were sampled once in July, 2009. In the winter season, the cows received total mixed rations (TMR) with a very different feeding dose 31–51 kg per day per cow in association with a very different milk yield 6–38 l per day per cow. In summer, the cows with very high yield potential cannot meet their energy requirements from grass alone partly due to insufficient intake [15], therefore grazing was combined with TMR diet at different proportion.

During the butter production the raw milk was pre-heated at 45 °C and then centrifuged. Cream containing 35% fat was pasteurized in a batch heated up to 95 °C and then the cream was cooled with water and stored at 6 °C for 4 h. The cream was churned at 10 °C, and butter kernelled after 40 min. Butter milk was separated and the granules of butter were pressed to remove excess butter milk to 16% water content. Butter was finally wrapped in 125 g and 500 g packages.

Previously published procedures were used for FA analyses of plant pastures, milk and butter samples [16]. The lipids of plant, milk and butter samples were extracted using chloroform-methanol mixture (2 : 1). For the preparation of FA methyl esters, the base-catalysed methylation procedure with sodium methoxide solution in methanol was used [17]. GC-MS analyses of methylesters from milk and plant lipids were performed on an Agilent Technologies 6890N gas chromatograph with a 5973 Network mass-selective detector and flame ionization detector (Agilent Technologies, Waldbronn, Germany). Derivatized fat extracts were separated by programmed temperature gas chromatography using a capillary column 60 m × 0.25 mm × 0.25 μm coated with DB-23 stationary phase (J&W Scientific, Agilent Technologies, Santa Clara, California, USA). GC conditions: injector temperature 300 °C, injection volume 2 μl, split/splitless mode (split ratio 1 : 50), the inlet column pressure was 210 kPa of He, column temperature programme 70 °C maintained for 2 min, slope 25 °C·min^{-1} until 150 °C, slope 5 °C·min^{-1} until 240 °C maintained for 7 min, time of separation 30 min. For separation of TVA from *trans*-10 18:1, as well as separation of 17:0 from phytanic acid, isothermic separation at 150 °C in column 100 m × 0.25 mm i.d. × 0.20 μm of CP-Sil 88 (Varian, Palo Alto, California, USA) was used. Separated compounds were identified using reference materials, published retention data and mass spectrometry measurements. The chromatograms were evaluated quantitatively using published response factors for flame ionization detector for FA methyl esters [18]. Gas chromatographically unseparated triplet *trans*-7, *cis*-9 + *cis*-9, *trans*-11 + *trans*-8, *cis*-10 CLA isomers was resoluted by the chemometric deconvolution using GC/MS-SIM detection [19, 20].

Statistical analysis of FA contents in pasture plants, milk and butter samples was carried out from three parallel measurements. The effect of individual farm on the FA contents in pasture plants and summer and winter milk was evaluated by one-way ANOVA. Significances of differences among averaged summer and averaged winter milk and corresponding summer and winter butter products were evaluated employing t-tests. Significant differences were considered at the level $P < 0.05$.

RESULTS AND DISCUSSION

Composition of fatty acids in pasture plants

Milk FA composition is mainly influenced by feed composition and feeding strategy. Previous studies showed that the content of unsaturated fatty acids (UFA), in particular CLA, in milk fat was dependent on the content of polyunsaturated fatty acids (PUFA), mainly ALA and LA, in pasture plants. However, diets rich in ALA or LA may increase CLA content of milk when dietary FA is accessible to the rumen microorganism for biohydrogenation [4]. FA composition of pasture plants from six milk farms is presented in Tab. 1; individual farms are presented in the order of increasing ALA content in pasture. All plant pastures included four major FA: ALA, LA, palmitic acid (PA) and oleic acid (OA) with their total pasture content on average 90% of the total FA content. Nevertheless, the proportions of these FA in pastures were different. ALA was the most abundant FA with the content ranging from 379 mg·g^{-1} FA (Farm No. 1) to 496 mg·g^{-1} FA (Farm No. 6) ($P < 0.001$). LA was the next most abundant FA with reversed content trend of farms order compared to ALA. LA content in pasture ranged from 259 mg·g^{-1} (Farm No. 1) to 196 g·mg^{-1} (Farm No. 6; $P < 0.01$). OA pasture content decreased in a similar order of farms as LA content from 65 mg·g^{-1} (farm No. 1) to 18 mg·g^{-1} (Farm No. 6; $P < 0.001$). PA content (mean value, 180 mg·g^{-1} FA) was relatively stable in all the pastures analysed. The LA/ALA content ratio in pasture decreased almost two-fold from 0.68 (Farm No. 1) to 0.39 (Farm No. 6; $P < 0.001$). A similar reverse trends in the contents of ALA and LA in the herbage observed by Elgersma et al. [21] were associated with a longer re-growth period of the sward.

The ALA contents in pasture plants given in Tab. 1 were mostly lower than those published as 50–75% of the total lipid fraction [22]. The lower ALA content in investigated pastures may be in part associated with the pasture plant sampling timing in the beginning of July when the ALA content was unfavourable affected by environmental factors, such as maturity stage of pasture plants. Different composition of plant pasture species may be another reason. The number and timing of cuts or grazing cycles also affects the FA composition of forages. The FA content in herbage tends to be highest in the spring and autumn, with lowest values during the summer particularly around flowering. The management that inhibits the initiation of flowering by cutting increases FA levels. There is a potential for manipulating pasture FA through genetic manipulation of flowering times for obtaining maximum benefits from genetic plant species differences [13]. Soluble fibre and fermentable saccharides present in fresh grass may create an environment in the rumen without lowering the ruminal pH that is favourable for the growth of the microbes responsible for the increase in CLA and TVA contents in milk because ruminal pH is higher in cows grazing pasture compared to those fed with TMR.

Tab. 1. Fatty acid composition of July pasture herbage (mg·g^{-1} total FA).

Fatty acid	Jasenová No. 1	Žaškov No. 2	Or. Poruba No. 3	Bziny No. 4	Veličná No. 5	Ludrová No. 6	SEM	S
10:0	0.8	0.6	0.7	0.9	0.8	0.7	0.02	NS
12:0	2.9	2.6	3.3	3.0	3.1	2.4	0.03	NS
14:0	7.9	6.1	6.7	7.1	7.2	7.0	0.11	NS
14:1	0.1	0.1	0.1	0.1	0.2	0.2	0.01	NS
15:0	3.5^{a}	4.8	3.4^{a}	3.7^{a}	3.2^{a}	2.7^{a}	0.14	*
16:0 *iso*	0.8^{a}	1.4	0.6^{a}	0.5^{a}	0.5^{a}	0.4^{a}	0.1	*
16:0 PA	183.1	192.9	181.9	170.1	178.2	178.6	1.26	NS
16:1	16.6	20.9	15.0	16.8	16.4	19.4	0.45	NS
17:0	5.0	6.9	6.1	4.8	5.8	4.2	0.19	NS
18:0	30.5	29.9	27.1	20.2	24.2	20.9	0.9	NS
18:1 *trans*	6.7^{ab}	1.5^{a}	1.9^{a}	18.8	4.1^{a}	13.8^{b}	1.61	***
9*c*-18:1	65.3	36.9^{a}	26.9^{a}	35.0^{a}	30.7^{a}	18.1^{a}	3.43	**
c-18:1	6.7^{ab}	7.7^{ab}	8.4^{b}	5.5^{ab}	4.7^{ab}	3.5^{a}	0.38	*
18:2 *n*-6 LA	259.1^{a}	240.6^{ab}	242.9^{ab}	234.0^{b}	221.1^{bc}	195.8^{c}	4.05	**
18:3	2.7	3.2	3.2	2.8	3.4	3.6	0.07	NS
18:3 *n*-3 ALA	378.8^{a}	406.7^{a}	427.8^{ab}	442.5^{b}	462.3^{bc}	495.7^{c}	0.71	***
20:0	8.4	10.4	13.6	9.4	9.0	9.0	0.36	NS
22:0	9.0	9.7	14.3	10.8	11.2	10.0	0.35	NS
23:0	3.0	4.6	3.8	3.8	2.5	3.2	0.14	NS
24:0	9.0	12.6	12.2	10.3	11.3	11.1	0.23	NS
LA/ALA	0.68	0.59^{a}	0.57^{a}	0.53^{ab}	0.48^{b}	0.39	0.017	***

SEM – standard error of the mean, S – significance.
PA – palmitic acid, LA – linoleic acid, ALA – α-linolenic acid.
* – $P < 0.05$, ** – $P < 0.01$, *** – $P < 0.001$. NS – none of compared means differ significantly at $P < 0.05$ level.
The means marked with the same letter do not differ significantly at $P < 0.05$ level.

Composition of fatty acids in summer and winter cows' milk

Significant differences in the FA milk composition between some individual farms were found. Highest differences in the content of UFA and saturated fatty acids (SFA) in the winter milk were found between the Farm No. 5 and the Farm No. 4. The contents of CLA, ALA, OA, LA and stearic acid (SA) were higher by 50%, 33%, 22%, 10%, and 19%, respectively, and those of PA and myristic acid (MA) lower by 16% and 7%, respectively. Highest contents of these UFA and SFA in the summer milk were found in the milk from Farm No. 2.

The average contents of individual FA in summer and winter cows' milk obtained by measurements of 3 summer and 3 winter milks from 6 farms during the period 2008–2009 are presented in Tab. 2. The higher average FA contents in the summer milk compared to the winter milk ($P < 0.001$) were found for CLA 8.0 mg·g^{-1} vs 4.1 mg·g^{-1}, TVA 17.8 mg·g^{-1} vs 8.5 mg·g^{-1}, ALA 6.4 mg·g^{-1} vs 4.4 mg·g^{-1}, LA 20.4 mg·g^{-1} vs 18.4 mg·g^{-1} ($P < 0.01$), OA 218 mg·g^{-1} vs 194 mg·g^{-1}, and SA 102 mg·g^{-1} vs 89.8 mg.g^{-1} of total FA. The lower average FA contents in the summer milk compared with the winter milk were found for PA 293 mg·g^{-1} vs 331 mg·g^{-1} FA ($P < 0.001$), and for MA 102 mg·g^{-1} vs 112 mg·g^{-1} FA ($P < 0.01$).

The average CLA content in the summer milk was two-fold higher than that in the winter milk. The CLA-enriching effect of pasture has been attributed to the effects on biohydrogenation and the provision of ALA as a lipid substrate for the formation of TVA in the rumen and its subsequent desaturation to RA in the mammary gland [23]. The problem of increasing delivery forage PUFA into milk is with a general increase in the extent of biohydrogenation with increasing proportion of forage in the diet because of the predominant role of the fibrolytic bacterium *Butyrivibrio fibrisolvens* in rumen biohydrogenation. Therefore, the CLA content in the summer milk from individual cows' farms was not directly proportional to the ALA pasture content in individual farms (Tab. 1 and Tab. 2). The difference was mainly related to a variable proportion of pasture in the summer cows' diet, as the increased proportion of grain or concentrate lowers the CLA content in milk. CLA production in the rumen may affect the type and

Tab. 2. Fatty acid composition of cows' summer and winter milk and butter ($mg \cdot g^{-1}$ total FA).

Fatty acid	Milk				Butter				S
	Summer		Winter		Summer		Winter		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
4:0	27.9	3.4	27.6	4.0	27.6	3.9	26.1	2.8	NS
6:0	16.4	3.3	17.6	2.2	17.4	2.0	17.3	1.6	NS
8:0	9.01	0.69	10.4[a]	1.1	10.1[a]	0.7	10.5[a]	0.8	
9:0	0.29	0.20	0.34	0.10	0.31	0.04	0.38	0.07	NS
10:0	23.0[a]	2.3	26.5[b]	3.0	25.6[ab]	1.2	27.2[b]	1.9	
10:1	2.23	0.34	2.76[a]	0.33	2.51[a]	0.11	2.71[a]	0.25	
11:0	0.48[a]	0.20	0.66[b]	0.20	0.60[a]	0.10	0.74[ab]	0.14	
12:0	29.3[a]	3.2	33.6[b]	3.9	31.4[ab]	1.0	34.6[b]	2.0	
12:1	0.65	0.12	0.86	0.14	0.75	0.03	0.86	0.10	NS
13:0 *iso*	1.01	0.13	1.09	0.15	1.07	0.10	1.10	0.07	NS
13:0 *anteiso*	0.12	0.05	0.12	0.04	0.08	0.01	0.10	0.01	NS
13:0	1.05	0.28	1.25	0.22	1.15	0.10	1.37	0.20	NS
14:0 *iso*	1.26	0.24	1.35	0.32	1.25	0.08	1.29	0.10	NS
14:0	102.3[a]	6.8	111.8[b]	5.7	104.7[a]	1.5	113.1[b]	2.9	
14:1	9.09	1.37	10.1	1.0	9.13	0.46	10.1	0.6	NS
15:0 *iso*	2.85	0.25	2.94	0.97	2.86	0.06	2.71	0.12	NS
15:0 *anteiso*	5.07	0.43	4.76	0.63	5.06	0.10	4.68	0.16	NS
15:0	11.7	1.7	12.5	1.4	12.00	0.9	13.0	1.1	NS
15:1	0.13	0.03	0.13	0.03	0.13	0.03	0.13	0.03	NS
16:0 *iso*	2.82	0.32	3.14	0.72	2.81	0.15	3.14	0.21	NS
16:0	292.7[a]	19.8	331.3[b]	21.2	293.8[a]	6.4	331.5[b]	9.0	
6-9*t*-16:1	1.32[a]	0.45	0.91[b]	0.15	1.37[a]	0.15	0.82[b]	0.15	
10-13*t*-16:1	2.36[a]	0.27	2.01[b]	0.16	2.48[a]	0.11	2.01[a]	0.14	
9*c*-16:1	14.9[a]	1.2	15.7[b]	1.2	15.0[a]	0.4	15.9[b]	0.6	
10-12*c*-16:1	0.38	0.28	0.46	0.24	0.21	0.13	0.29	0.08	NS
c-16:1	0.49	0.20	0.38	0.05	0.41	0.04	0.42	0.04	NS
17:0 *iso*	4.78[a]	0.53	3.99[b]	0.73	4.91[a]	0.44	3.88[b]	0.69	
17:0 *anteiso*	4.41	0.19	4.34	0.49	4.49	0.11	4.36	0.13	NS
phytanic acid	1.65	0.20	1.89	0.70	1.55	0.14	1.65	0.04	NS
17:0	5.97	0.35	5.69	2.12	5.60	0.23	4.94	0.05	NS
9*c*-17:1	2.71	0.29	2.45	0.28	2.77	0.18	2.63	0.17	NS
18:0 *iso*	0.69	0.11	0.64	0.12	0.68	0.04	0.64	0.07	NS
18:0	102.0[a]	10.2	90.3[b]	10.6	99.2[a]	2.8	89.8[b]	7.3	
5*t*-9*t*-18:1	5.71[a]	0.76	4.70[b]	0.97	4.74[b]	0.70	4.59[b]	0.45	
10,11,12*t*-18:1	22.0[a]	6.1	10.7[b]	2.5	16.7[a]	1.4	10.7[b]	1.5	
9*c*/10*c*-18:1	217.8[a]	15.9	194.4[b]	18.7	218.5[a]	6.4	194.6[b]	11.7	
11*c* + 15*t*-18:1	9.44	1.34	7.17[a]	0.90	7.17[a]	0.53	7.19[a]	0.22	
12*c*-18:1	2.95	0.47	3.14	0.73	2.96	0.13	3.07	0.38	NS
13*c*-18:1	1.41	0.63	0.96	0.17	1.26	0.12	1.11	0.08	NS
16*t*-18:1	4.11[a]	0.96	3.06[b]	0.54	4.04[a]	0.24	2.99[b]	0.19	
15*c*-18:1	2.90[a]	0.55	2.17[b]	0.48	2.72[a]	0.18	2.14[b]	0.19	
8*t*12*c*-18:2	0.86[a]	0.27	0.62[b]	0.16	0.79[a]	0.09	0.58[b]	0.14	
9*t*13*c*-18:2	1.06	0.30	0.99	0.31	1.02	0.09	0.87	0.13	NS
9*c*12*t*-18:2	0.76	0.29	0.76	0.12	0.64	0.15	0.72	0.17	NS
9*t*12*c*-18:2	0.42[a]	0.13	0.24[b]	0.08	0.48[a]	0.13	0.34[b]	0.12	
18:2 *n*-6 LA	20.4[a]	1.6	18.4[b]	2.1	20.5[a]	0.3	18.3[b]	0.5	
11*t*15*c*-18:2	1.73	0.21	1.77	0.27	1.66	0.07	1.89	0.24	NS
9*c*15*c*-18:2	0.65[a]	0.24	0.43[b]	0.10	0.56[a]	0.05	0.42[b]	0.11	
19:0	0.32	0.21	0.46	0.36	0.19	0.04	0.50	0.44	NS
18:2	0.33	0.24	0.13[a]	0.10	0.12[a]	0.10	0.08[a]	0.02	
18:3 *n*-6 GLA	0.34	0.12	0.26	0.11	0.26	0.05	0.29	0.11	NS
18:2 + 19:1	0.65	0.44	0.60	0.55	0.29	0.25	0.71	0.49	NS
cyclo 19:0	0.69	0.17	0.77	0.17	0.89	0.23	0.69	0.10	NS
18:3 *n*-3 ALA	6.43[a]	1.99	4.39[b]	0.97	6.12[a]	0.60	4.09[b]	0.64	

Tab. 2. continued

Fatty acid	Milk				Butter				S
	Summer		Winter		Summer		Winter		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
9*c*11*t*-18:2 CLA	8.03[a]	2.59	4.14[b]	0.85	8.35[a]	1.05	4.38[b]	0.35	
ct CLA	0.22	0.12	0.10	0.05	0.23	0.07	0.08	0.04	NS
20:0	1.61	0.26	1.62	0.31	1.57	0.16	1.51	0.22	NS
tt CLA	1.05	0.21	1.14	0.21	1.20	0.10	1.12	0.06	NS
9*c*-20:1	0.76	0.37	0.59	0.26	0.80	0.13	0.56	0.07	NS
20:2	0.19	0.06	0.19	0.03	0.24	0.07	0.31	0.19	NS
21:0	0.51	0.28	0.31	0.20	0.42	0.07	0.26	0.10	NS
20:3 *n*-6	0.68[a]	0.24	0.91[b]	0.13	0.78[a]	0.09	0.89[ab]	0.07	
20:4 *n*-6 AA	1.16[a]	0.24	1.48[b]	0.23	1.34[a]	0.08	1.43[ab]	0.13	
22:0	0.59	0.24	0.55	0.16	0.62	0.07	0.56	0.05	NS
20:5 *n*-3 EPA	0.52	0.17	0.38	0.09	0.48	0.07	0.39	0.05	NS
23:0	0.24	0.13	0.22	0.10	0.22	0.07	0.24	0.14	NS
24:0	0.30	0.19	0.33	0.11	0.35	0.12	0.35	0.08	NS
22:5 *n*-3 DPA	0.74	0.24	0.96[a]	0.13	0.89[a]	0.11	0.96[a]	0.11	
24:1	0.07	0.04	0.07	0.04	0.05	0.03	0.06	0.01	NS
22:6 *n*-3 DHA	0.18	0.13	0.12	0.09	0.32	0.18	0.44	0.14	NS
SSFA 4:0-24:0	627.3[a]	24.3	675.1[b]	24.3	634.4[a]	7.5	675.6[b]	12.8	
SSFA 4:0-10:0	76.6[a]	7.2	82.5[b]	9.6	81.0[ab]	7.6	81.5[b]	7.1	
SSFA 12:0-16:0	437.2[a]	30.1	490.5[b]	28.0	443.1[a]	8.7	493.5[b]	13.2	
BSFA	22.3	8.8	21.7	13.2	22.5	6.2	21.3	6.1	NS
MUFA	301.44[a]	19.6	262.7[b]	21.6	293.7[a]	6.8	262.9[b]	12.4	
PUFA	46.3[a]	5.9	38.0[b]	3.7	46.3[a]	1.7	38.3[b]	0.9	
LA/ALA	3.17[a]	1.01	4.19[b]	1.05	3.34[a]	0.32	4.48[b]	0.72	
Atherogenicity index	2.10[a]	0.16	2.70[b]	0.22	2.19[a]	0.08	2.72[b]	0.14	

SD – standard deviation, S – significance.

LA – linoleic acid, GLA – γ-linolenic acid, ALA – α-linolenic acid, cyclo 19:0 – 2-octyl-cyclopropaneoctanoic acid, 9*c*11*t*-18:2 CLA – the sum content of *cis*-9, *trans*-11 + *trans*-7, *cis*-9 + *trans*-8, *cis*-10 conjugated linoleic acid (CLA) isomers, AA – arachidonic acid, EPA – eicosapentaenoic acid, DPA – docosapentaenoic acid, DHA – docosahexaenoic acid, SSFA – straight-chain saturated fatty acids, BSFA – branched-chain saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids.

The means marked with the same letter do not differ significantly at $P < 0.05$ level. NS - none of compared means differ significantly at $P < 0.05$ level.

source of dietary saccharide that may influence the rates of microbial fermentation altering the rate of CLA production or utilization by rumen microbes. The latter may be associated with different cutting date and cutting interval, i.e. different stages of pasture plant development through pasture season [13]. The differences in the CLA content in the summer milk were more than three-fold (4.4 $mg \cdot g^{-1}$ to 15.2 $mg \cdot g^{-1}$ FA) whereas those in the winter milk were less than two-fold (2.8 $mg \cdot g^{-1}$ to 4.9 $mg \cdot g^{-1}$ FA) because of more reproducible winter TMR diet compared to the summer diet containing variable proportion of pasture and TMR, and different plants development. The overlaps in CLA content between the summer and the winter milk reflect a very low proportion of grazing in some cow farms.

A higher ALA content in the summer milk compared with the winter milk was associated with the higher ALA content in the summer diet because milk ALA comes exclusively from ALA non-hydrogenated in the rumen. The summer increase in ALA intake also induced an increase in the ruminal bypass of its biohydrogenation intermediates TVA and SA and their availability within the mammary epithelial cell [24]. Thus the increase in the ALA, TVA and SA availability within the mammary epithelial cell implied an increase in CLA, ALA, TVA, OA and SA contents in the summer milk.

The milk content of *trans* FA, especially TVA, increased with transition from winter to summer diet, i.e. in proportion with fresh grass. The average TVA content in summer milk was higher

(17.8 mg·g^{-1}) than that in winter milk (8.5 mg·g^{-1}; $P < 0.001$), and also the average content of *trans*-10 18:1 in summer milk was higher (5.6 mg·g^{-1}) than that in winter milk (3.1 mg·g^{-1}; $P < 0.001$). TVA being the most abundant *trans* FA in milk serves as the major precursor for RA synthesis in the mammary gland and other tissues. The higher TVA content in summer milk suggests inhibitory effects of PUFA on the reduction of *trans* 18:1 to 18:0 in the rumen as LA-rich lipids serve as substrates for ruminal *trans* 18:1 synthesis [9].

Composition of pasture No. 1 measured in July showed the highest OA content and pasture No. 6 the lowest OA content (Tab. 1). Correspondingly, July milk samples of cows grazing on pastures No. 1 and No. 6 were investigated on the content of CLA isomers by GC/MS-SIM with chemometric deconvolution of gas chromatographically unseparated CLA isomers [19]. The 3.6-fold higher OA content in pasture No. 1 compared with pasture No. 6 led to 1.9-fold higher (0.27 mg·g^{-1} vs 0.50 mg·g^{-1} FA; $P < 0.01$) content of *trans*-7, *cis*-9 CLA in milk fat of pasture No. 1, because OA is a precursor of this CLA isomer. In the rumen, OA from fodder is isomerized to *trans* 18:1 isomers, and *trans*-7 18:1 isomer is converted to *trans*-7, *cis*-9 CLA isomer via endogenous synthesis by Δ-9 desaturase [25].

Nutritional properties of summer and winter butter

The average content of FA in summer and winter butter based on the analysis of 7 summer and 5 winter butters during the period 2008–2009 are also presented in Tab. 2. Tab. 3 presents the average contents of selected health affecting FA in analysed summer and winter butter and milk samples. Comparison of average FA composition of summer butter and milk as well as winter butter and milk confirmed that FA contents in butter fat samples are very similar to those of the milk fats from which these butters were produced.

The content of SFA in summer butter was prevailingly lower. The content of 4:0 – 24:0 SFA was by 6.5% lower ($P < 0.001$) compared with winter butter. The content of 4:0 – 10:0 SFA with positive health effects was similar in both summer and winter butter. The content of 12:0 – 16:0 was in the summer butter by 11% lower compared to winter butter ($P < 0.001$). The sum content of PA and MA, which increase the risk of cardiovascular disease (CVD), was by 12% lower in the summer butter ($P < 0.001$). The sum content of positive health-affecting branched-chain saturated fatty acids (BSFA) was by 6% higher ($P < 0.05$) in the summer butter.

The sum content of monounsaturated fatty acids (MUFA) and PUFA was higher in the summer butter than that in winter butter. The MUFA content was higher by 12% ($P < 0.01$), most significantly for OA by 12%, in the summer butter. Oleic acid is considered to be favourable for health because it lowers both plasma cholesterol, LDL-cholesterol and triacylglycerol contents, reduces risk for coronary artery disease and shows cancer-protective effects. The PUFA content in the summer butter was higher by 21% ($P < 0.001$), mostly for RA by 91%, ALA 49% and LA by 12%. The LA/ALA content ratio was more favourable for the summer butter compared to the winter butter, i.e. 3.3 vs 4.5 [26].

The TVA content in the summer butter was 200% higher than that in winter butter, and also the average content of *trans*-10 18:1 in summer butter was on 80% higher than that in winter butter. In general, *trans* FA are associated with carcinogenic effects but TVA, being the most

Tab. 3. Average contents of selected health-affecting fatty acids in summer and winter milk and butter (mg·g^{-1} total FA).

Fatty acid	Summer milk	Winter milk	Δm	Summer butter	Winter butter	Δb
CLA	8.0	4.1	3.9	8.4	4.4	4.0
TVA	17.8	8.5	9.3	17.3	8.5	8.8
ALA	6.4	4.4	2.0	6.1	4.1	2.0
LA	20.4	18.4	1.9	20.5	18.3	2.2
OA	217.8	194.4	23.4	218.5	194.6	23.9
SA	292.8	331.3	–38.6	293.8	331.5	–37.6
MA	102.3	111.8	–9.5	104.7	113.1	–8.4
PA	102.0	90.3	11.7	99.2	89.8	9.4

Δm – summer minus winter milk, Δb – summer minus winter butter.
CLA – the sum content of *cis*-9, *trans*-11 + *trans*-7, *cis*-9 + *trans*-8, *cis*-10 CLA isomers, TVA – *trans* vaccenic acid, ALA – α-linolenic acid, LA – linoleic acid, OA – oleic acid, SA – stearic acid, MA – myristic acid, PA – palmitic acid.

abundant *trans* FA in milk fat, is not carcinogenic. It has been suggested that an increase in CLA content in summer butter compared to winter butter may partially offset the impact of the increase in *trans* FA content on the consumer [24].

The contents of health-promoting FA in summer butter compared with winter butter are higher and the contents of FA, which may have negative health effects, are lower. Thus, summer butter compared to winter butter gives improved nutritional composition of FA for human consumption.

In a consumers' view, the summer butter may be superior to the winter butter not only for a higher content of potentially health-promoting FA but also for more accessible sensoric properties (rancidity), rheological and thermal properties (firmness and melting in mouth). The summer butter shows a lower 16:0/18:1 content ratio than the winter butter (1.32 vs 1.74; $P < 0.01$) which is associated with reduced butter hardness and improved melting in mouth. In addition, the summer butter has a better nutrition value than the winter butter (2.19 vs 2.72; $P < 0.001$) owing to quartile reduced atherogenicity index (sum of 12:0 + 16:0 + 4× 14:0 contents divided by unsaturated FA content) [24]. Summer and winter butters showed similar contents of 4:0 and 6:0 FA, thus the flavour and odour (rancing character) of both butters were similar. This is in contrast to data published by Couvreur et al. [24] who suggested decreased rancidity with increasing proportion of fresh grass in the diet consumed by cows. According to these authors, the higher summer butter spreadibility was correlated with higher UFA content in butter fat and reduced milk fat globule size induced by grass diet.

CONCLUSIONS

Our study demonstrated that the summer butter compared with the winter butter had higher contents of potentially health-promoting unsaturated FA (CLA, OA, TVA, ALA and LA) and lower contents of saturated FA (PA and MA), which may increase the risk of cardiovascular disease. The studied summer and winter butter had CLA contents very similar to those found in French butter [14]. The results suggest that it is possible to increase CLA contents in butter without oil supplementation by improving consumed pasture through oversowing the pastures with plant species with higher and stable ALA contents throughout the pasture season using cutting optimization as well as by breeding the cows with higher CLA content in milk. Some economic analyses show benefits of grazing depending on the farm size and spatial situation, as conserved feed is more expensive than fresh herbage. Grassland has a potential for butter differentiation and production of CLA-enriched butter in increasingly competitive markets [12].

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