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Fatty acid profile of the milk of cows reared in the mountain region of Poland

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An appropriate composition of milk fatty acids (FA) improves the nutritional value of milk and milk products, and improves milk processing. Polish dairy farms in the mountainous region are rather small, animal nutrition there is based on locally produced forages and this, together with the transitional climate zone brings about seasonal changes in FA composition of milk. The aim of the study was to evaluate the composition of FA in bovine milk fat in relation to fat intake in forages and their FA profiles. The study involved 5 herds reared in low-input mountain farms located at an altitude of 670–780 m above sea level (Beskid Mountains). The cows were fed forages produced locally. FAs in forages and milk samples were subjected to gas chromatography. Highest fat intake observed in grazing season (4·2–4·7%) and high amounts of polyunsaturated FA in forages from that period (51·8–64·1 g/100 g FA) resulted in a markedly high content of valuable FAs: *t-11* C18:1 (3·22 g/100 g FA), *c-9, t-11* C18:2 (CLA; 1·20 g/100 g FA) in milk. Lower fat intake of forages containing high amount of SFA (32·42–38·83 g/100 g FA) in the indoor period resulted in changes in milk composition. The content of total short-chain saturated FA (SCFA) was highest in winter and early spring samples (14·10 and 13·44 g/100 g FA, respectively), like the amounts of myristic C14:0 and palmitic C16:0 acids (11·80 and 37·92 g/100 g FA). Total odd- and branched-chain fatty acids (OBCFA; 6·58 g/100 g FA) content was highest at the beginning of the grazing period. Fresh grass consumed by cows promoted the activity of Δ^9-desaturase in mammary gland as evidenced by higher C14:1 : C14:0 (0·054) and C16:1 : C16:0 (0·026) ratios in grazing than in the indoor periods.

Keywords: Dairy cow milk, fatty acid composition, CLA, Δ^9-desaturase, mountainous region.

The composition of bovine milk fat is essential for the milk value regarding its nutritive value and processability. Milk fat is one of the most complex natural sources of fatty acids (FA) from C2 to C28, including even- and odd-numbered, saturated, monounsaturated, polyunsaturated *cis* and *trans*, linear and branched and various keto- and hydroxy-FA (Sommerfeld, 1983; Ledoux et al. 2005). Some of these FA, like short-chain saturated FA (SCSFA) and positional and geometrical isomers of octadecadienoic acid with conjugated double bonds called CLA, have beneficial biological, physiological and nutritional properties (Watkins et al. 1999; Pariza et al. 2001).

Major CLA isomer, *c-9, t-11* C18:2, was recently shown as potentially beneficial for human health having, e.g., potent cancer-fighting properties (Pariza et al. 2001). The richest dietary sources of CLA are ruminant products such as bovine meat, milk and milk products. Although CLA is known to be generated in the rumen, there is good evidence that much of it present in bovine milk is actually synthesised within the mammary gland from *t-11* 18:1. This is mediated by the stearoyl-CoA desaturase (Δ^9-desaturase), an enzyme capable of adding a *cis*-9 double bond to *t-11* C18:1, to render *c-9, t-11* C18:2 (CLA). The results obtained by Corl et al. (2001) indicated that rumen is a source of CLA in milk fat, rumen production of *t-11* C18:1, a substrate for the endogenous synthesis of CLA, being predominant.

The composition of bovine milk fat is influenced by many factors both internal (cattle breed, age, stage of lactation, etc.) and external (feeding systems, seasonal changes, milking frequency and milking system; cf. Jensen, 2002; Kalač & Samková, 2010; Morales-Almaráz et al. 2010). Another important factor is geographical, which determines the plant variety underlying the feeding of ruminants. The effects of seasonal and geographical factors on FA composition of the bovine milk fat were reported by many authors (Faulkner et al. 1986; Lock & Garnsworthy, 2003;

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Alonso et al. 2004; Thorsdottir et al. 2004; Frelich et al. 2009; Rutkowska & Adamska, 2011).

A specific FA composition of milk from mountainous regions, e.g. in Switzerland, France, Italy and in Czech Republic was recently reported (Leiber et al. 2004; Collomb et al. 2008; Frelich et al. 2009). Mountainous regions in Poland also seem very promising for producing milk due to a high quality of forage devoid of concentrates and with low content of corn silage commonly used in lowland farms. Moreover, mountainous regions represent an important grassland zone and are, therefore, well suited for milk production. However, Polish mountainous regions substantially differ from the Alpine ones—over 80% of farms in the former are small-scale and the arable land is of low fertility. The economic status of those farms is low and, for that reason, the dairy cow husbandry is based on extensive breeds resistant to poor climatic conditions. Animal nutrition is based exclusively on locally produced feeds as the human relations in those areas are very strong. The aim of this study was thus to evaluate the fatty acid profile of milk fat in mountain farms in Poland in relation to lipid intake in feeds and their FA composition.

Materials and Methods

Sampling and sample treatment

The study was carried on 5 cow herds reared in low-input mountain farms located at altitudes of 670–780 m above sea level (Beskid Mountains, Poland), 9 cows per herd, on average. The cows were of Black-White breed, their average milk yield amounting to 4465 kg per standard lactation (305 d). The farms were typical of the Polish mountainous region in terms of area and in the number of reared animals. The nutrition of cows was typical of that region and depended on soil and climatic conditions and on the economical status of farms. From 1st May to 15th October the cows were pastured with supplementation of fodder maize in colder periods. In the evenings, when back to the stalls, the cows were administered coarse grains (triticale and oat). During the indoor period (from mid-October to the end of April), the cows were fed corn silage and hay prepared from grass cut in June and July. The details on cow feeding are presented in Table 1. Samples of every forage were pooled across the 5 studied farms.

Cows were milked twice daily at 06:00 and 18:00 using bucket machine. The pooled evening and morning milk were stored at 4 °C in temperature-controlled bulk tanks and on the following morning milk samples were taken and transported in cooled box to the laboratory. Milk fat was immediately extracted. A total of 83 milk samples were collected from November 2009 to November 2010 and analysed by the season. One milk sample was taken from each herd every month in the indoor feeding period, i.e. 17 samples in the winter (November–February) and 10 samples in early spring (March, April). In the grazing period, two samples were taken from each herd every month, i.e. 10 samples in late spring (May), 30 samples in the summer (June–August), and 16 samples in the autumn (September, October). Less samples were taken in the autumn due to approaching calving of some cows and in the winter—due to suspected cold-related disorders.

Extraction of lipids from milk and from forage samples

Milk lipids were extracted and determined using the Röse–Gottlieb method (AOAC No 905.02). Well ground samples of forage (3 g) were homogenised in chloroform-methanol 2:1 v/v and filtered (Folch et al. 1957). The filter was washed several times with small amounts of that mixture, the total filtrate was supplemented with 20% water and shaken for 5 min in a separating funnel with deaeration, then covered with aluminium foil and left for 2 h to separate layers. The chloroform layer was dried with sodium sulphate, filtered into a 150-ml round-bottomed flask, chloroform was evaporated on a pressure evaporator and the residue was dried till constant weight up to 3 decimal places. Dry mass of samples (5 g) was determined in duplicates in a preheated and cooled weighing bottle, dried for 2 h at 105 °C, then cooled for 30 min and weighed. Drying and cooling was repeated until constant weight (within 1 mg).

Fatty acid analysis of lipids in milk and in forage samples

Methyl esters of FA (FAME) were prepared by transmethylation of fat samples using a mixture of concentrated H_2SO_4 (95%) and methanol (AOCS Official Method Ce 2-66); FAMEs were analysed by gas chromatography (GC) using an Agilent 6890N (USA) chromatograph equipped with flame ionisation detector (FID), a split/splitless injector, operated with a split ratio of 1:50, and capillary column with stationary phase of high polarity (100 m × 0·25 mm I.D., film thickness 0·1 µm; Rtx 2330 Restek). Oven temperature was initially 120 °C for 40 min then ramped to 155 °C at 1·5°/min and held for 50 min, then ramped again at 2°/min to 210 °C and held for 35 min. Injector and detector temperatures were maintained at 250 °C, the carrier gas (helium) flow rate being 0·9 ml/min.

Peak areas were corrected by the response factors for FAME responses of FID, and area% of FAME was appropriately converted to weight% of FA. A butter reference standard (CRM 164; Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium) was used to determine the recovery rates and correction factors for individual FAs in milk samples. In addition, the Supelco 37 No. 47885-U standard (Sigma Aldrich) was applied for fatty acid identification and recoveries of FAME. The retention times of unsaturated FAME: linoleic—*c-9 c-12* C18:2, α-linolenic—*c-9 c-12 c-15* C18:3, γ-linolenic C18:3 n-6) were confirmed by those recorded for natural plant oils (rapeseed, sunflower, evening primrose), for which those acids are characteristic. The contents of individual FAs were expressed as g/100g FA.

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Table 1. Composition of the feed ration (kg fresh weight per cow and day) administered to cows (n = 45)

	Period				
Feed	Winter	Early Spring (March, April)	Late Spring (May)	Summer	Autumn
Grazed pasture sward	–	–	35	50	30*
Hay	10	8	4	–	12
Corn silage	22	22	10	–	–
Fresh-cut corn	–	–	–	–	10
Cereal grains (triticale 70% and oat 30%)	4	3	3	3	3

*From 1st September to 15th October

Because of the analytical techniques used, the term 'fatty acid' or its acronym 'FA' means actually methyl ester of the respective acid (FAME). Thus, the absolute or relative contents of FAs are those of FAME throughout the text. The activity of Δ^9-desaturase was assessed from the product: substrate ratio according to Lock & Garnsworthy (2003).

Data analysis

The between-season differences in individual FA in milk samples were assessed using one-way ANOVA and Tukey's HSD *post-hoc* test for uneven sample sizes. Statistica 9PL software (StatSoft Inc., 2010) was used, the level of $P \leqslant 0{\cdot}05$ being considered significant.

Results

Fatty acid composition of forages and fat intake by cows

Highest lipid content was found in fresh grass (4·2–4·7%; Table 2). Other forages contained much less fat (1·5–2·4%) and for that reason highest fat intake by cows was noted in the grazing season (Fig. 1), mainly during summer, when the animals were grazing 10–12 h daily and the grass intake amounted to about 50 kg (Table 1). Grass from the grazed pasture was rich in polyunsaturated FA (PUFA)—64·1 and 51·8 g/100 g FA in the summer and autumn seasons, respectively; PUFA content in other forages was lower and ranged from 22·57 (hay) to 32·74 g/100 g FA (grains; Table 2). Grass from various seasons differed with respect to individual PUFA: May grass (late spring) contained more α-linolenic acid than the summer one – 50·54 and 40·54 g/100 g FA, respectively. Regarding linoleic acid, its content in autumn grass was drastically lower compared with that from summer or late spring (1·36 vs. 12·95 g/100 g FA, respectively).

Seasonal differences were also found in saturated FA (SFA; mainly C16:0 and C18:0): green forages (grass and corn) contained less SFA (16·11–22·27 g/100 g FA) than grains, corn silage or hay (24·52–38·83 g/100 g FA). Moreover, cereal grains and fresh corn were rich in oleic acid (*c-9* C18:1; Table 2).

Fatty acid composition of milk

Application of the high-resolution GC enabled measurement of the levels of 47 FA in milk fat. The composition of milk fat in relation to season is shown in Tables 3 and 4.

Short-chain saturated FA (SCSFA) were represented by C4:0 – C12:0 compounds; in general, their content was highest in winter (14·10 g/100 g FA) and lowest in summer (11·32 g/100 g FA). Samples from the winter period were significantly ($P \leqslant 0{\cdot}05$) higher in the contents of butyric (C4:0), caproic (C6:0) caprylic (C8:0) and capric (C10:0) acids compared with the summer ones.

The levels of major long-chain saturated FA (LCSFA) in milk fat (C14:0, C16:0 and C18:0) varied significantly throughout the year. The contents of palmitic (C16:0) and myristic (C14:0) acids were higher in winter: (37·92 and 11·80 g/100 g FA) than in summer (25·00 and 9·80 g/100 g FA, respectively). An opposite trend was observed in case of stearic acid (C18:0) whose highest content was found in summer (11·33 g/100 g FA), and lowest in winter and early spring (7·64 g/100 g FA).

Oleic acid (*c-9* C18:1) levels were higher in the grazing season (20·50 and 19·64 g/100 g FA in the summer and spring, respectively) than in the indoor one (14·52 g/100 g FA). Both total *trans* C18:1 acids and vaccenic acid (*t-11* C18:1) were subject to high seasonal variations, highest concentrations being noted in summer and lowest in the winter and early spring months. The content of α-linolenic acid was twice as high in summer (0·77 g/100 g FA) than in the winter period (0·38 g/100 g FA). Greatest variability throughout the year was noted in the CLA content which was highest in summer and lowest in winter and early spring (1·20 and 0·33 g/100 g FA on average).

The contents of the main odd- and branched-chain FAs (OBCFA) isomers (tetradecanoic—*iso* C14:0, pentadecanoic—C15:0, *iso* C15:0 and *anteiso* C15:0, hexadecanoic—*iso* C16:0 and heptadecanoic—C17:0, *iso* C17:0 and *anteiso* C17:0) are presented in Table 4. Total OBCFA content differed significantly ($P \leqslant 0{\cdot}05$) between the grazing and indoor seasons: 6·97 and 5·92 g/100 g FA in late spring and in winter, respectively.

Assessment of Δ^9-desaturase activity

The activity of Δ^9-desaturase was assessed by comparing the product:substrate ratios of certain FA shown in Table 5. The ratios were season-dependent.

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Table 2. Mean content (g·100 g^{-1}FA) of major fatty acids in forages

Values are means ± SD

	Forage						
Fatty acid	Spring pasture	Summer pasture	Autumn pasture	Hay*	Grains	Corn Silage	Fresh Corn
C12:0	1·13 ± 0·03	1·53 ± 0·03	0·25 ± 0·01	1·10 ± 0·04	ND	0·79 ± 0·02	0·15 ± 0·01
C14:0	0·88 ± 0·03	0·98 ± 0·03	1·20 ± 0·02	1·38 ± 0·06	0·30 ± 0·01	1·30 ± 0·03	0·48 ± 0·01
C16:0	15·28 ± 0·41	17·75 ± 0·48	7·21 ± 0·19	32·21 ± 2·24	22·66 ± 0·61	26·18 ± 0·72	17·04 ± 0·48
C18:0	1·76 ± 0·04	2·01 ± 0·05	7·45 ± 0·22	4·14 ± 0·26	1·56 ± 0·04	4·15 ± 0·10	2·74 ± 0·07
∑ **SFA**	19·05 ± 0·42	22·27 ± 0·64	16·11 ± 0·45	38·83 ± 2·68	24·52 ± 0·71	32·42 ± 0·99	20·41 ± 0·60
C16:1 n-7	1·98 ± 0·03	2·04 ± 0·06	ND	1·14 ± 0·06	0·32 ± 0·01	0·62 ± 0·01	2·11 ± 0·05
C16:1 n-10	0·35 ± 0·01	0·37 ± 0·01	ND	0·62 ± 0·03	0·55 ± 0·01	0·70 ± 0·02	0·52 ± 0·01
c-9 C18:1	2·21 ± 0·06	2·46 ± 0·07	2·01 ± 0·03	9·92 ± 0·67	33·90 ± 1·01	5·43 ± 0·34	27·62 ± 0·81
∑ **MUFA**	4·54 ± 0·11	4·87 ± 0·12	2·01 ± 0·05	11·68 ± 0·79	34·77 ± 0·99	6·75 ± 0·46	30·25 ± 0·90
c-9 c-12 C18:2	13·23 ± 0·35	12·70 ± 0·32	1·36 ± 0·04	11·26 ± 0·76	31·09 ± 0·91	10·50 ± 0·34	24·76 ± 0·72
c-9 c-12 c-15 C18:3	50·54 ± 1·47	40·54 ± 1·19	48·26 ± 1·36	10·07 ± 0·68	1·47 ± 0·04	17·63 ± 0·93	5·10 ± 0·12
C18:3 n-6	0·33 ± 0·01	0·49 ± 0·01	2·18 ± 0·05	1·24 ± 0·06	0·18 ± 0·01	1·76 ± 0·08	0·62 ± 0·01
∑ **PUFA**	64·10 ± 1·92	53·73 ± 1·58	51·80 ± 1·54	22·57 ± 1·55	32·74 ± 0·96	29·89 ± 1·27	30·48 ± 0·91
Fat (%)	4·20 ± 0·10	4·70 ± 0·12	4·20 ± 0·10	2·00 ± 0·05	2·30 ± 0·06	1·50 ± 0·03	2·40 ± 0·06

*Collected from 31st May to 3rd June
ND – not detected

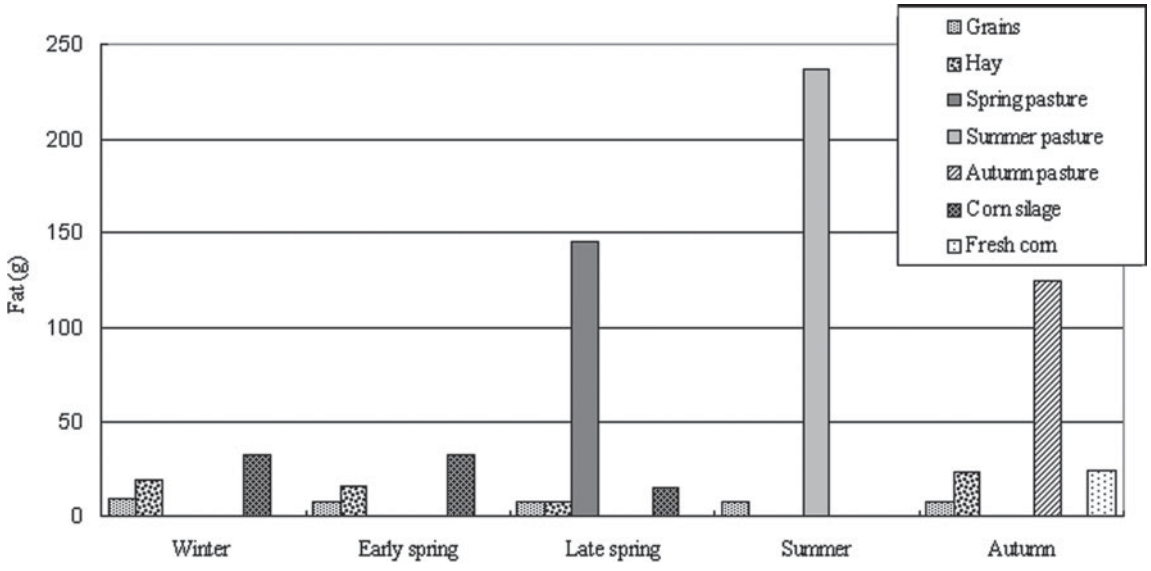

Fig. 1. Daily intakes (g/d) of fat from various forage sources

Discussion

The study was conducted under natural circumstances of farming and herd management in a Polish mountainous region which remains natural with respect to environmental conditions like climate, soil, vegetation period, etc. The nutrition of studied herds was based solely on forages planted locally, i.e. pasture supplemented with small amounts of grains in the grazing season, and hay and corn silage in the winter season. Those season-related feeding regimes were, obviously, reflected in the FA profiles of milk and forages.

Fat intake was highest in summer (249 g/d) due to a high content of fat in grass and lowest in early spring (55·7 g/d). It should be emphasised that grazed grass was in late spring markedly rich in PUFA, α-linolenic acid being its principal component. However, hay produced from grass in that period contained only one quarter of the α-linolenic acid, the losses being due to wilting and drying. Namely, a mechanical damage to plants combined with air access bring about extensive oxidation of PUFA, especially α-linolenic acid, as reported by Kalač & Samková (2010). In the winter season, cereal grains, hay and corn silage were the sources of PUFA but the content of α-linolenic acid was low in them (17·63 g/100 g FA in corn silage).

The effect of PUFA on FA profile of bovine milk is of utmost importance due to the fact that PUFA are substrates

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Table 3. Fatty acid composition of bovine milk fat (g·100 g^{-1}FA) from a mountainous region in Poland

Values are means ± SD

Fatty acid	Season				
	Winter	Early Spring	Late Spring	Summer	Autumn
Saturated short- and medium-chain, even-numbered					
C4:0	4·68 ± 0·20^{a}	4·30 ± 0·19^{b}	3·91 ± 0·08^{bc}	3·70 ± 0·13^{c}	4·06 ± 0·16^{b}
C6:0	2·45 ± 0·10^{a}	2·29 ± 0·14^{ab}	1·92 ± 0·24^{b}	2·00 ± 0·18^{b}	2·14 ± 0·13^{b}
C8:0	1·27 ± 0·07^{a}	1·22 ± 0·08^{ab}	1·04 ± 0·14^{bc}	1·05 ± 0·15^{bc}	1·16 ± 0·11^{ac}
C10:0	2·56 ± 0·06^{a}	2·58 ± 0·13^{ab}	2·28 ± 0·24^{ab}	2·11 ± 0·26^{b}	2·23 ± 0·11^{b}
C12:0	3·14 ± 0·27	3·06 ± 0·14	2·74 ± 0·36	2·56 ± 0·25	2·20 ± 0·20
Total	**14·10** ± 0·48^{a}	**13·44** ± 0·55^{b}	**11·89** ± 0·96^{bc}	**11·32** ± 0·97^{c}	**12·10** ± 0·35^{d}
Saturated, even-numbered					
C14:0	11·80 ± 0·29^{a}	11·80 ± 0·14^{a}	10·50 ± 0·98^{b}	9·80 ± 0·99^{b}	9·98 ± 0·59^{b}
C16:0	37·92 ± 1·46^{a}	37·20 ± 1·13^{a}	29·15 ± 1·49^{c}	25·00 ± 0·80^{b}	28·02 ± 1·02^{c}
C18:0	7·34 ± 0·20^{a}	7·94 ± 0·34^{a}	10·26 ± 0·38^{b}	11·33 ± 1·27^{c}	10·60 ± 0·62^{bc}
C20:0	0·11 ± 0·01^{a}	0·12 ± 0·01^{ab}	0·13 ± 0·02^{b}	0·12 ± 0·01^{ab}	0·11 ± 0·01^{ab}
C24:0	0·05 ± 0·01^{a}	0·06 ± 0·02^{a}	0·09 ± 0·01^{c}	0·08 ± 0·01^{b}	0·06 ± 0·01^{a}
Total	**57·20** ± 3·40^{a}	**57·12** ± 2·36^{a}	**50·13** ± 2·56^{b}	**46·32** ± 1·80^{c}	**48·85** ± 2·20^{b}
Monounsaturated, even-numbered					
C10:1	0·31 ± 0·04^{a}	0·30 ± 0·07^{a}	0·27 ± 0·05^{ab}	0·24 ± 0·05^{b}	0·23 ± 0·01^{b}
C14:1	0·39 ± 0·03^{a}	0·45 ± 0·05^{b}	0·57 ± 0·05^{c}	0·49 ± 0·02^{b}	0·40 ± 0·05^{a}
C16:1 *n-10*	0·40 ± 0·08^{a}	0·51 ± 0·11^{ab}	0·61 ± 0·09^{b}	0·64 ± 0·11^{b}	0·51 ± 0·06^{ab}
C16:1 *n-7*	1·42 ± 0·16	1·36 ± 0·13	1·45 ± 0·10	1·26 ± 0·08	1·30 ± 0·10
trans isomers of C18:1	1·09 ± 0·18^{a}	0·96 ± 0·24^{a}	2·23 ± 0·36^{b}	3·70 ± 0·37^{c}	3·40 ± 0·30^{c}
***t-11* C18:1**	0·85 ± 0·14^{a}	0·74 ± 0·19^{a}	1·98 ± 0·31^{b}	3·22 ± 0·30^{c}	2·90 ± 0·28^{c}
c-9 C18:1	14·52 ± 0·91^{a}	14·91 ± 2·54^{a}	19·64 ± 1·83^{b}	20·50 ± 1·89^{b}	19·89 ± 1·75^{b}
c-11 C18:1	0·40 ± 0·08^{a}	0·40 ± 0·11^{a}	0·64 ± 0·13^{b}	0·74 ± 0·12^{b}	0·65 ± 0·16^{b}
C20:1	0·16 ± 0·01	0·18 ± 0·02	0·17 ± 0·01	0·17 ± 0·02	0·16 ± 0·03
Total	**19·55** ± 0·56^{a}	**19·81** ± 2·99^{a}	**27·57** ± 3·54^{b}	**30·97** ± 2·81^{c}	**29·42** ± 2·21^{bc}
Polyunsaturated					
C18:2 *n-6*	0·10 ± 0·01^{a}	0·10 ± 0·00^{a}	0·26 ± 0·09^{b}	0·39 ± 0·08^{c}	0·30 ± 0·01^{b}
c-9 c-12 C18:2	0·83 ± 0·06^{a}	0·80 ± 0·08^{ad}	0·95 ± 0·12^{ab}	1·12 ± 0·10^{c}	1·02 ± 0·12^{d}
C18:3 *n-6*	0·19 ± 0·03	0·23 ± 0·02	0·22 ± 0·04	0·23 ± 0·02	0·21 ± 0·02
c-9 *c*-12 *c*-15 C18:3	0·38 ± 0·09^{a}	0·31 ± 0·01^{a}	0·60 ± 0·06^{b}	0·77 ± 0·01^{c}	0·62 ± 0·05^{b}
9*c*11*t* C18:2 (CLA)	0·38 ± 0·10^{a}	0·28 ± 0·05^{a}	0·80 ± 0·07^{b}	1·20 ± 0·11^{c}	0·98 ± 0·05^{bc}
C20:2	0·06 ± 0·02^{a}	0·07 ± 0·01^{a}	0·09 ± 0·03^{ab}	0·16 ± 0·02^{c}	0·12 ± 0·03^{b}
C20:3 *n-6*	0·09 ± 0·01^{a}	0·12 ± 0·02^{ab}	0·13 ± 0·01^{b}	0·14 ± 0·02^{b}	0·11 ± 0·02^{ab}
C20:3 *n-3*	0·10 ± 0·03	0·09 ± 0·00	0·11 ± 0·01	0·11 ± 0·02	0·11 ± 0·01
C20:5 *n-3*	0·07 ± 0·01^{a}	0·08 ± 0·01^{ab}	0·09 ± 0·01^{ab}	0·11 ± 0·01^{b}	0·08 ± 0·02^{ab}
Total	**2·2** ± 0·34^{a}	**2·07** ± 0·08^{a}	**3·29** ± 0·09^{b}	**4·18** ± 0·16^{c}	**3·55** ± 0·09

Values with different superscripts significantly ($P \leqslant 0·05$) differ from each

of biohydrogenation of the essential fatty acids—vaccenic and a range of conjugated linoleic acids in which the *c-9 t-11* C18:2 constitutes 75–90% of total CLA (Bauman et al. 2003). Vaccenic acid is the predominant *trans*-FA in milk fat (Griinari et al. 1998). Vaccenic acid can be converted to *c-9 t-11* C18:2 – CLA *via* the Δ^9-desaturase. Several authors demonstrated that human beings can convert about 20% of vaccenic acid into CLA, thereby doubling the CLA supply (Palmquist et al. 2005). In this study, the concentration of vaccenic acid in the summer/autumn milk increased over fourfold compared with the winter/early spring season and the same was true for total *trans* C18:1 isomers, the increase being nearly fourfold. Those differences could be explained by a higher supply of PUFA during summer (Kepler & Tove, 1967; Couvreur et al. 2006). Our study confirmed earlier observations (Bargo et al. 2006; Couvreur et al. 2006; Ferlay et al. 2008) that high content of total *trans* C18:1 in milk fat and of vaccenic acid were due to grazing. Similar variations in total *trans* C18:1 isomers were found in other studies (Lock & Garnsworthy, 2003; Collomb et al. 2008). Similar changes were also shown by us for CLA as confirmed by others: Collomb et al. (2002, 2008), Bargo et al. (2006), Couvreur et al. (2006), Frelich et al. (2009).

Due to the differences in PUFA intake, the total PUFA content in milk tended to fluctuate throughout the year; summer and winter milk samples contained 4·23 and 2·20 g/100 g FA, respectively. Also, α-linolenic acid showed seasonal variation; its significantly higher content in

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Table 4. Fatty acid composition of odd- and branched-chain (OBCFA) of bovine milk fat (g/100 g FA) from a mountainous region in Poland means ± SD

	Season				
Fatty acid	Winter	Early Spring	Late Spring	Summer	Autumn
C11:0	0·04 ± 0·00	0·04 ± 0·00	0·03 ± 0·01	0·03 ± 0·01	0·04 ± 0·01
anteiso C12:0	0·04 ± 0·00 ^{a}	0·05 ± 0·00^{ab}	0·05 ± 0·00^{ab}	0·06 ± 0·01^{b}	0·05 ± 0·01^{ab}
iso C12:0	0·08 ± 0·01^{a}	0·08 ± 0·01^{a}	0·07 ± 0·03^{a}	0·06 ± 0·01^{b}	0·05 ± 0·02^{b}
anteiso C13:0	nd	nd	0·01 ± 0·00	0·02 ± 0·00	0·01 ± 0·00
iso C13:0	0·05 ± 0·01^{a}	0·05 ± 0·01^{a}	0·06 ± 0·01^{a}	0·09 ± 0·01^{b}	0·09 ± 0·02^{b}
C13:0	0·11 ± 0·00	0·10 ± 0·01	0·10 ± 0·01	0·09 ± 0·02	0·08 ± 0·02
iso C14:0	0·20 ± 0·04^{a}	0·28 ± 0·03^{b}	0·23 ± 0·05^{a}	0·22 ± 0·02^{a}	0·15 ± 0·04^{c}
anteiso C15:0	0·63 ± 0·03^{ac}	0·78 ± 0·06^{b}	0·85 ± 0·07^{b}	0·81 ± 0·07^{b}	0·63 ± 0·06^{a}
iso C15:0	1·05 ± 0·06^{a}	1·11 ± 0·02^{b}	1·03 ± 0·05^{ab}	0·82 ± 0·00^{c}	0·84 ± 0·12^{c}
C15:0	1·44 ± 0·05^{c}	1·50 ± 0·07^{ab}	1·60 ± 0·15^{b}	1·50 ± 0·10^{ab}	1·33 ± 0·10^{c}
iso C15:1	0·03 ± 0·00	0·03 ± 0·00	0·04 ± 0·00	0·05 ± 0·01	0·03 ± 0·00
C15:1	0·36 ± 0·03^{a}	0·40 ± 0·02^{ab}	0·49 ± 0·06^{b}	0·39 ± 0·03^{a}	0·36 ± 0·04^{a}
iso C16:0	0·16 ± 0·03^{ab}	0·21 ± 0·02^{bc}	0·19 ± 0·03^{bc}	0·20 ± 0·01^{bc}	0·14 ± 0·03^{a}
anteiso C17:0	0·49 ± 0·04^{a}	0·54 ± 0·04^{ab}	0·65 ± 0·05^{c}	0·58 ± 0·04^{b}	0·47 ± 0·07^{a}
iso C17:0	0·17 ± 0·02	0·05 ± 0·00	0·07 ± 0·03	0·12 ± 0·02	0·10 ± 0·02
C17:0	0·72 ± 0·03^{a}	0·80 ± 0·01 ^{ab}	0·87 ± 0·05^{b}	0·90 ± 0·06^{b}	0·74 ± 0·04^{a}
C17:1	0·34 ± 0·04^{ac}	0·40 ± 0·02^{ab}	0·46 ± 0·01^{b}	0·43 ± 0·05^{b}	0·37 ± 0·03^{ac}
C21:0	0·08 ± 0·01	0·08 ± 0·01	0·12 ± 0·02	0·14 ± 0·04	0·13 ± 0·02
C23:0	0·04 ± 0·01^{a}	0·07 ± 0·01^{b}	0·07 ± 0·01^{b}	0·07 ± 0·01^{b}	0·04 ± 0·01 ^{a}
Total	**5·92 ±** 0·17^{a}	**6·62 ±** 0·18^{b}	**6·97 ±** 0·42^{c}	**6·55** ± 0·25^{b}	**5·48 ±** 0·51^{a}

nd = not detected
Values with different superscripts significantly ($P \leqslant 0·05$) differ from each other

Table 5. Product:substrate ratios reflecting Δ^{9}-desaturase activity in the mammary gland

Values are means±SD

Ratios product:substrate	Winter	Early Spring	Late Spring	Summer	Autumn
C14:1 9*c*/C14:0	0·033 ± 0·003^{a}	0·038 ± 0·001^{a}	0·054 ± 0·005^{b}	0·050 ± 0·005^{b}	0·038 ± 0·006^{a}
C16:1 9*c*/C16:0	0·011 ± 0·004^{a}	0·014 ± 0·003^{ab}	0·021 ± 0·004^{cd}	0·026 ± 0·005^{c}	0·018 ± 0·002^{bd}
C18:1 9*c*/C18:0	1·978 ± 0·226	1·878 ± 0·131	1·915 ± 0·223	1·820 ± 0·153	1·870 ± 0·152
C18:2 9*c*11*t*/C18:1 11*t*	0·438 ± 0·118	0·385 ± 0·043	0·401 ± 0·065	0·374 ± 0·043	0·352 ± 0·119

Values with different superscripts significantly ($P \leqslant 0·05$) differ from each other

summer milk was reported for Switzerland (Collomb et al. 2008) it was attributed to a high content of PUFA in grass and legume silages in contrast to a lower one in concentrates which, in turn, led to development of specific rumen bacteria of intense activity. The feeding regimen based only on fodder prepared from own crops and on grazing (May till mid-October) led to increasing the content of α-linolenic acid in milk fat, and the resulting biohydrogenated FA, CLA and vaccenic acid (Dewhurst et al. 2006; Collomb et al. 2008). These effects are amplified by a specific mobilisation of body fat in cows with alpine hypoxia, as well as by a reduced ruminal biohydrogenation due to energy shortage or to secondary plant ingredients like polyphenols and terpenoids that inhibit hydrogenating microorganisms in the rumen (Leiber et al. 2004, 2005, Collomb et al. 2008).

During the indoor period, the nutrition of cows was based mainly on corn silage and hay supplemented with grains. Those fodders contained much more SFA compared with green products – 38·83 and 32·42 g/100 g FA in hay and corn silage, respectively. In that period, the intake of long-chain SFA was higher than in the summer season which resulted in correspondingly higher content of those SFA in milk – 57·21 and 46·32 g/100 g FA, respectively. Similar results were reported for mountain farms by Ferlay et al. (2008) in France and Frelich et al. (2009) in Czech Republic.

As compared with other reports (Lock & Garnsworthy, 2003; Collomb et al. 2008; Frelich et al. 2009; Nałęcz-Tarwacka et al. 2009) on various regions and feeding regimens, the content of C4:0 in milk of cows studied by us was much higher but season-related (3·70 and 4·68 g/100 g FA in summer and winter, respectively). Similar season-

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dependence of the C4:0 content in milk was reported for Spain (Alonso et al. 2004), UK (Lock & Garnsworthy, 2003), France (Ledoux et al. 2005) and Poland (Rutkowska & Adamska, 2011) but a reverse trend was observed in a mountainous area in the Czech Republic (Frelich et al. 2009). Seasonal changes in the content of other SCFAs (C6:0–C10:0) in milk are worth interest since they are synthesised in part by non-malonyl CoA mechanisms, i.e. not involving acetyl-CoA carboxylase, thus being less likely to be affected by differences in the intakes of PUFA between grazing vs. winter feeding periods (Ferlay et al. 2008).

As far as the long-chain SFA are concerned, lower levels of the rather controversial FA (C14:0 and C16:0) in milk were noted by us in the summer than in winter samples. This was particularly evident in case of palmitic acid whose content decreased dramatically from winter to summer (37·92 vs. 25·0 g/100 g FA, respectively). Similar seasonal trends for C16:0 were reported from a mountainous region in France (Ferlay et al. 2008), Czech Republic (Frelich et al. 2009) and Switzerland (Collomb et al. 2008) but the differences were smaller. Those changes might be beneficial for human health since only the C12:0, C14:0 and C16:0 acids adversely affect plasma cholesterol levels.

The content of stearic acid (C18:0) in ruminant feed is known to be low amounting to 3·50 g/100 g FA on average (Doreau & Chilliard, 1997); thus, an increased concentration of C18:0 acid in milk compared with that in the animal diet is due to extensive biohydrogenation of PUFAs in the rumen (Polan et al. 1964; Lock & Garnsworthy, 2003). In this study, the concentration of C18:0 in milk fat was significantly increased in summer when the cows stayed all day on pasture. We thus confirmed that fresh grass contained high concentrations of α-linolenic acid convertible in the rumen to vaccenic and stearic acids. The majority of C18:0 is a product of PUFA hydrogenation in rumen (Chilliard et al. 2001; Jenkins et al. 2008). Similar seasonal effects of mountainous pastures on C18:0 content in milk was also reported by other authors (Ferlay et al. 2008; Frelich et al. 2009), as well as for other areas (Asturia, Spain; Alonso et al. 2004). Higher content of C18:0 in butter was also noted in the summer than in winter by Polish and French authors (Ledoux et al. 2005; Rutkowska & Adamska, 2011).

The interest in OBCFAs is steadily increasing due to their anticarcinogenic effects and for being potential indicators of dairy product intake by human beings (Vlaeminck et al. 2006). Although OBCFAs in milk fat are largely derived from rumen bacteria, we attributed the variability in milk OBCFA levels to the composition of fodders. Fresh grass feeding resulted in a higher total content of OBCFA in milk compared with indoor feeding. Similar effect was also reported by Collomb et al. (2008), Ferlay et al. (2008) and Frelich et al. (2009) in milk samples originated from mountains in the Switzerland, France and Czech Republic, respectively. Our results confirmed earlier observations of Ferlay et al. (2008) that microbial synthesis of branched-chain FA (BCFA) seems to be enhanced by fibre-rich diets since the relative content of fibre in the forage was closely related to the relative content of *anteiso* C15:0 in rumen bacteria. The content of *anteiso* C15:0 varied from 0·63 to 0·85 g/100 g FA and was generally higher compared with other reports (Collomb et al. 2008; Ferlay et al. 2008; Frelich et al. 2009) due probably to a high fibre content in the forage and low quantities of concentrates in the diet. We also noted higher contents of C17:0 and *anteiso* C17:0 in the period of intensive biohydrogenation of PUFA than reported by others (Vlaeminck et al. 2006).

In addition, direct and indirect effects of diet on the FA composition of milk fat and the role of Δ^9-desaturase ought to be considered. Mammary gland cells contain the Δ^9-desaturase complex (often referred to as stearoyl-CoA desaturase), responsible for catalysing the oxidation of acyl-CoA esters, that results in the introduction of a *cis* double bond between carbon atoms 9 and 10 (Shingfield et al. 2008). The 4 main products of Δ^9-desaturase activity in the mammary gland of ruminants (C14:1, C16:1, *c-9* C18:1 and CLA) are produced from C14:0, C16:0, C18:0, and *t-11* C18:1 acids, respectively.

The product:precursor ratio C14:1 : C14:0 was used as a proxy for the assessment of Δ^9-desaturase activity in the mammary gland regarding possible effects of season and dietary changes. That ratio is the most appropriate since the whole amount of the C14:0 in milk fat is produced *via de novo* synthesis in the mammary gland, hence all of the C14:1 in milk is produced *via* desaturation of C14:0 in the mammary gland (Corl et al. 2000). We noted significantly higher activities of Δ^9-desaturase during the late spring (May) and summer confirmed by higher C14:1 : C14:0 ratios (0·054) than in winter (0·033) and the same was true for the C16:1 : C16:0 ratio. Our observations were supported by the results of Lock & Garnsworthy (2003).

A marked seasonal change in the *c-9* C18:1 content in milk fat was noted. Highest supply of the *c-9* C18:1 acid took place in autumn because of two sources: fresh corn and grains (27·62 and 33·90 g/100 g FA, respectively). The *c-9* C18:1 : C18:0 ratio indicated no between-season differences in the Δ^9-desaturase activity due to the fact that a steady concentration of *c-9* C18:1 acid in milk fat throughout the year would arise only if maximal activity of Δ^9-desaturase had been achieved, since 40–50% of *c-9* C18:1 acid in milk fat is produced from C18:0 in the mammary gland *via* Δ^9-desaturase (Chilliard et al. 2000; Lock & Garnsworthy, 2003).

Summing up, milk fat was nutritionally more valuable in the grazing period, when fat intake was highest, and the FA profile of dietary fat was favourable, than in the winter season. Since the content of fatty acids beneficial for human health in milk was higher in the spring and summer, mainly because of the high quality of locally produced forages, increasing the population of dairy cattle in the mountainous regions, reared under natural pasture conditions, would increase the output of dairy products with health promoting properties and improve the economic status of households in such regions.

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