Fatty acid composition of cow milk fat produced on low-input mountain farms

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ABSTRACT: The fatty acid (FA) composition of bulk milk fat was examined on three mountain dairy farms in the Czech Republic. Milk samples were collected in the period of indoor grass silage feeding (November–April) and in the grazing period (May–October). In total fifty FAs were identified in the milk fat. The two-way ANOVA with factors of the farm and of the period of milk sample collection was used for the evaluation of variation in FA concentrations. Significant differences between the farms ($P < 0.01$) were found in the concentration of five FAs, which accounted for 30.40 g/100 g total FAs. Significant differences between the indoor and the grazing period ($P < 0.01$) were found in the concentration of sixteen FAs, which accounted for 63.86 g/100 g total FAs. The content of long-chain (> C16), mono- and polyunsaturated FAs in the milk fat was higher in the grazing period (49.22, 31.69 and 4.69 g/100 g total FAs) than in the indoor period (42.25, 27.55 and 4.15 g/100 g total FAs, respectively; $P < 0.01$). The proportion of conjugated linoleic acid (CLA) was also higher in the grazing period (1.09 g/100 g total FAs) than in the indoor period (0.74 g/100 g total FAs; $P < 0.01$). The medium-chain (C12–C16) and the saturated FAs were more abundant in the milk fat in the indoor period (48.91 and 67.16 g/100 g total FAs) than in the grazing period (41.31 and 62.16 g/100 g total FAs; $P < 0.001$ and $P < 0.01$; respectively). These results indicated a positive influence of seasonal grazing on the FA profile of cow milk fat as regards its potential health effects in consumers.

Keywords: milk; fatty acids; CLA; dairy cows; low-input farms; pasture

The modification of the milk fatty acid (FA) composition by altering the feed of ruminants attracts an attention of researchers regarding the possibility of improving a human diet (McGuire and McGuire, 2000; Lock and Bauman, 2004). While the consumption of saturated fat, mainly of C12:0, C14:0 and C16:0 FAs, is associated with cardiovascular diseases, the unsaturated FAs are regarded as beneficial for human health (Parodi, 2004).

Aside from the animal factors, like the breed, parity or stage of lactation (Jensen, 2002; Kelsey et al., 2003; Pietrzak-Fiećko et al., 2009), the nutrition substantially determines the FA composition in the milk fat (Jenkins and McGuire, 2006; Strusińska et al., 2006; Hanuš et al., 2008; Liu et al., 2008; Jalč et al., 2009; Kudrna et al., 2009; Veselý et al., 2009). The addition of forage, especially of fresh grass, into feeding rations was found to enhance the proportion of unsaturated FAs in cow milk fat compared to saturated FAs (Dewhurst et al., 2006; Elgersma et al., 2006). The intake of fresh grass elevated also the concentration of conjugated linoleic acid (CLA) (Chilliard et al., 2001; Bargo et al., 2006), the major 9-*cis*, 11-*trans* isomer of which was proved to be a biologically active compound with anti-carcinogenic and other health beneficial effects in animal models

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(Ip et al., 2003). The modification of the milk fat composition by grazing is thus appreciated as regards both the low feed expenses and the potential positive impact on consumers' health.

In the Czech Republic, seasonal grazing and the fresh-cut herbage supplementation of cows are frequently applied on low-input mountain farms. Milk yields achieved on these farms during the grazing period were higher than those obtained in the rest of the year (Frelich et al., 2006, 2009). The FA profile of the milk fat has recently been reported from milk of Holstein and Czech Fleckvieh cows, generally housed in a byre and reared in the lowlands (Pešek et al., 2005, 2006; Janů et al., 2007; Samková et al., 2009; Veselý et al., 2009). The aim of this study was to analyse the FA composition in the bulk milk fat produced on mountain farms and to evaluate the effect of seasonal grazing on the FA profile.

MATERIAL AND METHODS

Herd management and diet

Three dairy farms located in the Czech Republic at an altitude of 575, 793, and 730 meters above sea level (farm 1, 2, 3, respectively) were selected for the examination. The herd on farm 1 consisted of 51 Czech Fleckvieh and 55 Holstein cows with an average milk yield of 5.529 and 6.673 kg per standard (305-day) lactation (Czech Fleckvieh and Holstein, respectively). On farm 2 and 3, the Czech Fleckvieh cows were reared in the number of 89 and 161 cows with average milk production of 6.601 and 5.319 kg per standard lactation (farm 2 and 3, respectively). Calving was applied continuously throughout the year.

The composition of the feed ration is shown in Tables 1 and 2. The herbage (grazed and cut) formed a majority of the feed ration in the grazing period (May–October). The vegetation of pastures appertained to the *Lolio-Cynosuretum* association (Frelich et al., 2006). The fresh-cut herbage was offered to cows in the stalls during milking twice a day. At night, the cows returned to the pasture (farm 1 and 2) or they stayed in the stalls (farm 3). In the indoor period (November–April), cows were fed grass silage prepared from the pasture vegetation cut in late May and June.

Sampling and analysis

The chemical composition of forage and grain concentrates was analysed on the farms. Grass si-

Table 1. Composition of the feed ration on three farms in the indoor period (November–April) and in the grazing period (May–October)

Ingredients (kg fresh weight per cow and day)	Indoor period			Grazing period		
	farm 1	farm 2	farm 3	farm 1	farm 2	farm 3
Grass silage	20–25	25–30	30	–	–	–
Grazed pasture sward	–	–	–	*ad libitum*	*ad libitum*	*ad libitum*
Fresh-cut herbage[1]	–	–	–	20	–	15
Hay	1	3	1	–	2–3	–
Straw	–	–	2	–	–	–
Rapeseed	–	3	1	–	2	–
Wheat pollard	–	2–3	–	–	1–2	–
Brewery draff	–	–	10	–	–	10
Grain concentrates [2]	1–10	4–8	4–8	1–10	4–8	4–8
Mineral and vitamin supplements	0.5	0.5	0.5	0.5	0.5	0.5

[1]farm 1: May–July: grass/legume/red clover mixture (46/46/8%), August–September: red clover; farm 3: July–September: pasture sward

[2]offered to cows with daily milk production higher than 12 kg (farm 1) or 20 kg (farm 2)

Table 2. Chemical composition of feeds – mean concentrations are given for grass silage (n = 8), pasture sward (n = 12) and grain concentrates (n = 3)

Diet component		Farm 1	Farm 2	Farm 3	P
		(g/100 g dry matter)			
Grass silage	CP	13.99[a]	12.14[ab]	11.64[b]	< 0.05
	fat	2.15	2.67	2.60	< 0.05
	ADF	35.20[a]	34.44[a]	39.40[b]	< 0.01
Pasture sward	CP	11.41[a]	15.04[b]	11.23[a]	< 0.01
	fat	2.64[a]	2.14[b]	2.24[ab]	< 0.05
	ADF	31.02[ab]	29.83[a]	33.33[b]	< 0.05
Grain concentrates	CP	13.59	16.56	20.34	NS
	fat	2.92	2.63	3.36	NS
	ADF	10.63[ab]	8.16[a]	12.67[b]	< 0.05

CP = crude protein (N × 6.25); ADF = acid detergent fibre

[a,b]different superscripts indicate differences between farms (differences within rows); NS = not significant

lage samples were collected in March and November in 2007 and in January and February in 2008. The samples were taken both from a manger and from a silage pit (two samples on each date; n = 8). Pasture herbage was collected inside of one-hectare representative area of the pasture four times during the grazing season 2007. The herbage was cut 3 cm above the ground using a Husqvarna 323 R cutting machine from three 10 m^2 plots (three samples on each date; n = 12). The grain concentrates were collected in a manger in March, October and December 2007 (n = 3). The samples were dried for 6 hours at 105°C and analysed for nitrogen content by Kjeldahl method, fat content was determined by Soxhlet method and acid detergent fibre (ADF) was determined by an *in vitro* method according to Van Soest and Wine (1967).

Bulk milk was collected once a month, four samples in the indoor period (March, April and November 2007, February 2008) and five samples in the grazing period (May, June, July, August and September 2007). The samples were taken in the morning from bulk cooling tanks which contained milk from the morning milking and from the evening milking of a preceding day. Milk samples were transported in a cooled box to the laboratory, frozen and analysed later. Fat, protein, casein and lactose contents were determined using the spectrophotometric apparatus Milcoscan 4000 (Foss, Hillerød, Denmark). The mean concentrations of these milk components in the samples from the indoor and grazing period are given in Table 3.

Fatty acids were determined by a gas-chromatographic method (GLC) using a Varian 3800 appa-

Table 3. Composition of bulk milk samples (the arithmetic means) on three farms in the indoor period (November–April) and in the grazing period (May–October)

	Indoor period (n = 4, for each farm)			Grazing period (n = 5, for each farm)		
	farm 1	farm 2	farm 3	farm 1	farm 2	farm 3
Fat (%)	3.99	4.33	4.04	3.84	4.12	4.12
Protein (%)	3.18	3.28	3.25	3.21	3.25	3.30
Casein (%)	2.50	2.56	2.54	2.58	2.61	2.63
Lactose (%)	4.78	4.75	4.71	4.70	4.75	4.73

Table 4. Parameters of the chromatographic analysis of fatty acids

Parameter	Value
Column	omegawax 250; 30 m
Detector	FID
Temperature: – column	70°C for 3 min; 30°C/min up to 150°C; 3.0°C/min up to 240°C
– injection	250°C
– detector	250°C
Helium flow	1.5 ml/min
Injection	1 µl; split 10

ratus (Varian Techtron, USA) under the conditions given in Table 4. Milk fat was extracted with petroleum ether from freeze-dried milk samples. Fatty acids in isolated fat were re-esterified to their methyl esters by a methanolic solution of potassium hydroxide. The identification of fatty acid methyl esters was carried out using the analytical standards (Supelco, USA). In total, sixty-four FAs were observed and fifty of them were identified. The proportions of individual FAs were calculated from the ratio of their peak area to the total area of all the observed FAs. The conjugated linoleic acid (CLA) refers to 9-*cis*, 11-*trans* and 9-*trans*, 11-*cis* isomers of C18:2 (the GLC method does not allow to distinguish between the two isomers).

Differences in the chemical composition of feeds (Table 2) were evaluated by the one-way ANOVA ($P < 0.05$). The two-way ANOVA and Tukey's HSD post-hoc test ($P < 0.05$) were used for the evaluation of differences in concentrations of FAs between the farms and between the feeding periods (Statistika, StatSoft, Inc., 2005).

RESULTS

The FA composition of examined milk fat is given in Table 5. Palmitic (C16:0), oleic (9-*cis* C18:1), stearic (C18:0) and myristic (C14:0) acid contributed most to total FAs. Their concentration was 27.79, 22.07, 12.07 and 9.86 g/100 g total FAs, respectively ($n = 27$). The saturated FAs contributed by 64.38 g, monounsaturated FAs by 29.85 g, polyunsaturated FAs by 4.45 g and branched-chain FAs by 2.12 g/100 g total FAs. The content of CLA was 0.93 g/100 g total FAs.

Significant differences between the farms in concentrations of individual FAs ($P < 0.01$) were found in five FAs, which accounted for 30.40 g/100 g total FAs. Significant differences between the indoor and the grazing period ($P < 0.01$) were found in sixteen FAs, which accounted for 63.86 g/100 g total FAs. The milk fat produced in the grazing period contained more of long-chain FAs (49.22 vs. 42.25 g/100 g total FAs; $P < 0.01$) and less of medium-chain FAs (41.31 vs. 48.91 g/100 g total FAs; $P < 0.001$) than in the indoor period. In the grazing period, the milk fat contained less of saturated FA (62.16 vs. 67.16 g/100 g total FAs; $P < 0.01$) and more of monounsaturated FA (31.69 vs. 27.55 g/100 g total FAs; $P < 0.01$) and polyunsaturated FAs (4.69 vs. 4.15 g/100 g total FAs; $P < 0.01$) than in the indoor period. Myristic and palmitic acids were more abundant in the milk fat from the indoor period (10.73 and 30.74 g/100 g total FAs) than in the grazing period (9.17 and 25.43 g/100 g total FAs; $P < 0.01$ and $P < 0.001$; respectively). Stearic and oleic acids were more abundant in the grazing period (12.89 and 23.32 g/100 total FAs) than in the indoor period (11.05 and 20.51 g/100 g total FAs; $P < 0.001$ and $P < 0.05$; respectively).

The concentration of CLA was higher in the grazing period (1.09 g/100 g total FAs) than in the indoor period (0.74 g/100 g total FAs; $P < 0.01$). A higher concentration of this FA (1.08 g/100 g total FAs) was found in milk fat produced on farm 3 than on the other farms (0.78 and 0.89 g/100 g total FAs, on farm 1 and 2 respectively; $P < 0.05$).

DISCUSSION

The contribution of individual FAs to total FAs in milk fat found in this study was in accordance with the previously obtained results on the cow milk fat composition reported in the Czech Republic

Table 5. Milk fatty acid composition (g/100 g total fatty acids) in bulk milk samples[1] on three farms and in two feeding periods

	Farm			Period		Total	SEM	*P*		
	1	2	3	indoor	grazing			F	S	F × S
C4:0	2.65	2.54	2.62	2.34	2.87	2.63	0.06	0.418	< 0.001	0.811
C5:0	0.03	0.03	0.02	0.02	0.03	0.03	0.00	0.400	0.039	0.315
C14:0	10.53	9.80	9.52	10.73	9.17	9.86	0.29	0.275	0.006	0.671
Iso C14:0	0.13	0.12	0.13	0.13	0.12	0.12	0.00	0.666	0.007	0.273
C14:1	0.73	0.69	0.65	0.77	0.61	0.68	0.03	0.337	0.002	0.564
C15:0	1.17[a]	0.97[b]	1.13[a]	1.17	1.02	1.09	0.03	0.002	0.001	0.133
Anteiso C15:0	0.49	0.44	0.47	0.44	0.49	0.47	0.01	0.088	0.006	0.044
C16:0	30.51[a]	26.86[b]	26.89[b]	30.74	25.43	27.79	0.79	0.007	< 0.001	0.025
C16:1	1.39	1.30	1.27	1.35	1.30	1.32	0.02	0.033	0.170	0.038
C17:0	0.71[a]	0.60[b]	0.65[b]	0.65	0.66	0.65	0.01	0.001	0.884	0.527
Iso C17:0	0.48	0.47	0.44	0.40	0.53	0.47	0.02	0.356	< 0.001	0.613
Anteiso C17:0	0.45[a]	0.42[b]	0.44[a]	0.42	0.46	0.44	0.01	0.022	< 0.001	0.111
C17:1 n7	0.32	0.28	0.28	0.28	0.30	0.29	0.01	0.040	0.276	0.138
C18:0	10.93[a]	12.51[b]	12.47[b]	11.05	12.89	12.07	0.32	0.012	< 0.001	0.028
Iso C18:0	0.06	0.05	0.05	0.06	0.05	0.05	0.00	0.493	0.003	0.853
9-*cis* C18:1	19.73	23.00	23.00	20.51	23.32	22.07	0.63	0.022	0.011	0.191
C18:1[2]	4.12[a]	4.86[ab]	5.23[b]	3.96	5.51	4.82	0.23	0.032	< 0.001	0.380
C18:2 n6	2.04	2.39	2.35	2.11	2.41	2.28	0.06	0.012	0.004	0.918
C18:2 /9,11/ (CLA)	0.78	0.89	1.08	0.74	1.09	0.93	0.06	0.029	0.001	0.452
C18:3 n3	0.96	0.78	0.70	0.83	0.79	0.81	0.04	0.005	0.469	0.037
C19:0	0.07	0.07	0.07	0.04	0.09	0.07	0.01	0.881	0.002	0.881
C20:5 n3	0.07[a]	0.06[ab]	0.05[b]	0.06	0.05	0.06	0.00	0.001	0.136	0.687
C22:5 n3	0.10[a]	0.09[ab]	0.08[b]	0.10	0.07	0.09	0.00	0.055	0.001	0.761
C23:1	0.03	0.04	0.03	0.02	0.04	0.03	0.00	0.963	0.036	0.772
Unidentified	1.30	1.32	1.27	1.14	1.45	1.32	0.04	0.891	< 0.001	0.452
SCFA	8.21	7.73	7.63	7.69	8.02	7.88	0.16	0.260	0.898	0.968
MCFA	48.66[a]	43.45[ab]	43.22[b]	48.91	41.31	44.69	1.17	0.030	< 0.001	0.195
LCFA	41.84[a]	47.50[ab]	47.88[b]	42.25	49.22	46.12	1.21	0.022	0.001	0.234
SAFA	67.47[a]	63.38[ab]	63.13[b]	67.16	62.16	64.38	0.88	0.029	0.001	0.453
MUFA	26.99[a]	30.79[ab]	31.08[b]	27.55	31.69	29.85	0.79	0.023	0.002	0.363
PUFA	4.24	4.50	4.51	4.15	4.69	4.45	0.09	0.288	0.004	0.425
BCFA	2.18	2.05	2.09	2.02	2.19	2.12	0.03	0.078	0.008	0.124

P = level of statistical significance; F = effect of farm; S = effect of season; F × S interaction between farm and season effect

[1]fatty acids without significant effect ($P < 0.05$) of farm or season are not given in this table (i.e. C6:0, C8:0, C9:0, C10:0, C10:1, C11:0, C12:0, C12:1, C13:0, iso C15:0, C15:1, iso C16:0, C18:3 n6, C19:1, C20:0, C20:1 n9, C20:2 n9, C20:3 n3, C20:3 n6, C20:4 n3, C20:4 n6, C21:0, C22:0, C22:1 n9, C23:0, C24:0)

[2]unidentified isomers of C18:1; CLA = conjugated linoleic acid (9-*cis*, 11-*trans* and 11-*cis*, 9-*trans* C18:2); SCFA = short-chain fatty acids (C4–C11); MCFA = medium-chain fatty acids (C12–C16); LCFA = long-chain fatty acids (C17–C24) ; SAFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; BCFA = branched-chain fatty acids (iso and anteiso)

[a,b]different superscripts indicate differences between farms (differences within rows)

(Komprda et al., 2005; Pešek et al., 2005, 2006; Janů et al., 2007; Samková et al., 2009). The majority of FAs in milk fat was represented by saturated FAs (64 g/100 g total FAs) while unsaturated FAs were present by 34 g/100 g total FAs. Although the breed composition of herds and the feed ration differed between the farms, the farm-specific factors affected the milk fat composition less than the seasonal effects. Significant seasonal differences were found in 64 g/100 g total FAs, whereas the between-farm differences related to 30 g/100 g total FAs. The seasonal alternation of the feed ration between the grass-silage and the fresh-herbage one thus influenced the FA composition significantly. The proportion of long-chain FAs (C17–24) increased from 42 g/100 g total FA in the indoor period to 49 g/100 g total FAs in the grazing period. This was accompanied by a decline in the proportion of medium-chain FAs (C12–16) from 49 g/100 g total FAs in the indoor period to 41 g/100 g total FAs in the grazing period. The ratio between the concentration of unsaturated and saturated FAs increased from 0.47 in the indoor period to 0.59 in the grazing period. These results are in accordance with other studies (Jahreis et al., 1996; Ferlay et al. 2008), where the intake of fresh grass was found to elevate the concentration of long-chain and unsaturated FAs in cow milk fat when compared to the feeding of preserved forage.

The proportion of CLA in milk fat was higher in the grazing period (1.09 g/100 g total FAs) than in the indoor period (0.74 g/100 g total FAs). The elevation of CLA concentration as a result of the addition of fresh grass to ruminant diet was documented by many authors (e.g. Collomb et al., 2002; Elgersma et al., 2004; Bargo et al., 2006; Couvreur et. al., 2006; Floris et al., 2006). Because the long-chain FAs (>C16) are synthesised very little in ruminants, they must be ingested if they are to be secreted in milk. The fresh grass fat contains a high concentration (55–65%) of α-linolenic acid, 9-*cis*, 12-*cis*, 15-*cis* C18:3 (Chilliard et al., 2001), which is biohydrogenated in the rumen to vaccenic (11-*trans* C18:1) and stearic acid. These are further desaturated in the mammary gland to CLA and to oleic acid, and released in milk (Griinari et al., 2000; Bauman et al., 2006; Elgersma et al., 2006). Grazing thus modifies the cow milk fat towards its more desirable composition as regards the health beneficial effects in humans. This effect was observed also in this study on the Czech mountain farms. The specific composition of milk fat produced by grazing cows is worth the attention of milk processors and distributors and it may be positively evaluated by consumers.

CONCLUSIONS

The seasonal alternation between the grass-silage based and the fresh-herbage based diet was identified as an important factor affecting the cow milk fat composition on Czech low-input mountain farms. A higher ratio of unsaturated vs. saturated fatty acids and a higher concentration of CLA was found in milk fat in the grazing period (May–October) compared to the indoor period (November–April). A more valuable milk fat composition as regards its potential impact on consumers' health was thus produced in the grazing period than in the period of indoor silage feeding.

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