Animal Science Papers and Reports vol. 30 (2012) no. 3, 219-229
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Seasonal variation in fatty acid composition of cow milk in relation to the feeding system*

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(Received March 2, 2012; accepted June 2, 2012)

The fatty acid (FA) composition of bulk milk fat was examined on three dairy farms applying the seasonal pasture and on two farms applying the permanently indoor silage feeding. The seasonal variation in the content of major FAs was investigated in relation to the effect of farm in each feeding system separately. Six samples in winter period and four samples in summer period were taken on each farm. In the grazing herds, the seasonal changes were found in FAs forming 90 g/100 g total FAs on average ($P<0.05$), generally without interactions with farm effect ($P>0.05$). Only several FAs, forming 14 g/100 g total FAs on average, were influenced by the season effect in the indoor herds ($P<0.05$). The seasonal increase in unsaturated FAs against the saturated FAs and omega-3 against omega-6 polyunsaturated FA indicated that the milk yielded in summer was more beneficial to consumers' health than that yielded in winter. No significant differences in these indicators of healthy milk were found compared to the milk yielded by herds kept indoors ($P>0.05$). The milk produced by grazing cows may be positively evaluated by consumers.

*Supported by the research project of Ministry of Education, Youth and Sports of the Czech Republic, No. MSM 6007665806, and by the of Ministry of Agriculture of the Czech Republic, No. NAZV QH 81210. We would like to thank the farmers for their help with realization of this study.
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KEY WORDS: CLA / dairy cows / fatty acids / feeding system / milk / pasture

The fatty acid (FA) composition of cow milk fat has been widely presented in relation to its potential health impact on the consumers [Mensink *et al.* 2003, Lock and Bauman 2004, German *et al*. 2009, Parodi 2009]. The saturated FAs (SAFA), mainly the C12:0, C14:0 and C16:0, are assessed negatively due to their relation with an elevated serum cholesterol level being a risk factor for a coronary heart disease. The Unsaturated FAs (USFA) and especially polyunsaturated FAs (PUFA) are considered favourable in a human diet implying their positive impact on health. Special attention as regards milk fat is paid to the conjugated linoleic acid (CLA) due to its anticancer and other health affecting properties [McGuire and McGuire 2000, Collomb *et al*. 2006] and linolenic acid (C18:3 n-3) as regards its beneficial effect in coronary heart disease [De Caterina and Zampolli 2001, Kristensen *et al*. 2001]. The n-6 to n-3 FA ratio is also an important parameter from nutritive point of view [Haug *et al*. 2007, Simopoulos 2008].

Strategies for improving the dietary lipid quality by elevating the percentage intake of PUFA n-3 and lowering the overall n-6/n-3 FA ratio have ben suggested by Rego *et al.* [2004], Chen *et al*. [2007], Pajor *et al* [2009], Strzałkowska *et al*. [2009] and Jóźwik *et al*. [2010]. The positive effect of forage, and especially of a fresh herbage in ruminant diet on the increase of proportion of UFAs of milk fat has been well documented [reviews: Dewhurst *et al.* 2006, Elgersma *et al.* 2006, Kalač and Samková 2010]. In the Czech Republic, the seasonal pasture of cows from May to October is applied in the mountain areas as an alternative to the all-year indoor feeding with silage. Generally, the milk production of grazing cows exceeds that obtained in winter in low-input farming systems [Frelich *et al.* 2006, 2009b]. Although the milk fat composition has already been examined both in the grazing and the indoor fed herds [Janů *et al*. 2007, Frelich *et al.* 2009a, Samková *et al.* 2009, Węglarz *et al*. 2007], the comparative study on its simultaneous seasonal changes in the two feeding systems has not been carried out so far.

The aim of this study was to examine the effect of season on proportional changes in milk FAs content in relation to the farm-specific environment, mainly the feeding system and the diet composition. The seasonal intake of a fresh herbage by cows on farm with the pasture was expected to result in more pronounced seasonal changes in the FAs content than on farms with all-year-through indoor silage feeding. The study focused on the major FAs and FA classes which may be relevant in evaluation of milk fat.

Material and methods

Herds management and diets

Used were Czech Fleckvieh cows and /or Holstein cows on three farms kept on seasonal pasture (farms 1, 2 , 3) or all-year-round kept on silage-based diet (farm 4,

Table 1. General characteristics and milk composition on farms with seasonal grazing (farm 1, 2, 3) and on farms with all-year-round indoor feeding (farm 4, 5)

Item		1		3	4	5
General characteristics						
Sea level (m)		575	793	730	622	826
Breed [1]		C, H	C	C	C	C
Herd size (number of cows)		98	78	122	148	229
305-day milk production (kg)		5 511	6 035	5 738	7 308	7 128
Milk composition (g/100 g)						
Fat	summer	3.63±0.20	4.03±0.87	3.71±0.23	3.80±0.91	4.50±1.55
	winter	4.02±0.20	3.78±0.30	3.87±0.21	4.21±0.50	3.87±1.16
Protein	winter	3.32±0.02	3.39±0.13	3.31±0.02	3.44±0.07	3.42±0.15
	summer	3.23±0.07	3.30±0.16	3.30±0.08	3.41±0.23	3.53±0.17
Lactose	winter	4.76±0.08	4.75±0.16	5.15±1.01	4.90±0.10	4.86±0.12
	summer	4.84±0.06	4.84±0.04	4.76±0.04	4.73±0.27	4.87±0.08

[1]C – Czech Fleckvieh; H – Holstein.

Table 2. Components of the feed ration used on the farms with seasonal grazing (farm 1 , 2, 3) and on the farms with all-year-round indoor feeding (farm 4, 5)

Components[1] (kg fresh weight per cow daily)	1	2	3	4	5
Grass silage	20-25 (W)	25-30 (W)	30 (W)	20	25
Maize silage	-	-	-	20	10
Grazed pasture sward	ad lib. (S)	ad lib. (S)	ad lib. (S)	-	-
Fresh-cut herbage [2]	20 (S)	-	15 (S)	-	-
Hay	1 (W)	3	1 (W)	0.5	-
Straw	-	-	2 (W)	-	-
Rapeseed	-	3 (W), 2 (S)	1 (W)	-	-
Wheat pollard	-	3 (W), 2 (S)	-	-	-
Brewery draff	-	-	10	-	1
Molasses	-	-	-	-	1
Potatoes [3]	-	-	-	7 (II–VI)	-
Grain concentrates	1-10	48	4-8	39	4-8
Mineral and vitamin supplements	0.5	0.5	0.5	0.5	0.5

[1]W – offered to cows in the winter feeding period (November-April); S – offered to cows in the summer feeding period (May-October).
[2]Farm 1 – May-July: grass/legume/red clover mixture; August-September: red clover; Farm 3 – July-September: pasture sward
[3]Offered to cows from February to June.

5) – Table 1. On farm 1, cows of both breeds were in similar numbers, and bulk milk was composed of milk of cows of both breeds. The composition of the feed ration is given in Table 2. In herds kept on pasture (farm 1, 2, 3), the feed ration was based on a grass silage in the winter and on a fresh grass grazed *ad libitum* in the summer, which was supplemented by a fresh-cut herbage offered to cows during milking on farm 1 and 3. In indoor-fed herds (farm 4 and 5), the diet consisted mainly of grass and maize silage during all the year. The grain concentrates in different amounts were offered to cows on all five farms.

Sampling and analyses

Bulk milk samples were collected on five farms on the same date. On each farm, six samples were taken in the winter feeding period (in January, March – two samples, April, November in 2009 and in November in 2010) and four samples in the summer feeding period (in June, July, September in 2009 and in June in 2010). Milk samples were transported in a cooled box to the laboratory, freezed and analysed later. Fat, protein, casein and lactose contents were determined using the spectrophotometric apparatus Milcoscan 4000 (FOSS, Hillerřd, Denmark). The mean concentration of the mentioned milk components in the samples from winter and from summer period are given in Table 1. Fatty acids were determined by a gas-chromatgraphy (GLC) using an apparatus Varian 3800 (VARIAN TECHTRON, USA) according to conditions quoted in Table 3. Milk fat was extracted with petroleum ether from freeze-dried milk samples. Fatty acids of isolated fat were re-esterified to their methyl esters by methanolic solution of potassium hydroxide. The identification of fatty acid methyl esters was carried out using the analytical standards (SUPELCO, USA) and acetonitrile chemical ionization mass spectrometry (VARIAN MS 4000 detector). 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.

Table 3. Parameters of chromatographic analysis of fatty acids

Parameter	Value
Column	CP-Select CB for FAME, 50m x 0.25mm, 0.25μm tickness
Detector	FID
Temperature:	
column	55°C for 5 min; 40°C /min up to 170°C; 2.0°C /min up to 196°C; 10.0°C /min up to 210°C
injection	250°C
detector	250°C
Helium flow	1.8 ml/min
Injection	1ml, split 10

The variation in concentration of selected FAs was statistically evaluated. These FAs formed 88.48 -91.75 g/100 g total FAs in the samples. The lauric acid (C12:0), myristic acid (C14:0), C15:0, palmitic acid (C16:0), stearic acid (C18:0), vaccenic acid (11-*trans* C18:1), oleic acid (9-*cis* C18:1), linoleic (C18:2 n-6), linolenic acid (C18:3 n-3) and the conjugated linoleic acid, CLA (mixture of 9-*cis*, 11-*trans* and 9-*trans*, 11-*cis* isomers of C18:2) were evaluated individually. The following health parameters were calculated from these selected FAs and used in the analysis: the rate of monounsaturated to saturated FAs (MUFA/SAFA), polyunsaturated to saturated FAs (PUFA/SAFA), polyunsaturated to monounsaturated FAs (PUFA/MUFA) and n-6 to n-3 PUFA (n-6/n-3). The two-way ANOVA and Tukey´s HSD post-hoc test ($P < 0.05$) were used for the evaluation of differences in concentration of FAs between the farms in each feeding system separately and between the summer (May – October) and the winter (November – April) period (StatSoft CR s r.o., 2008):

$$yij = \mu + Fi + Sj + eij$$

where:

yij – value of measured trait*, i.e.* concentration of FAs;

Fi – fixed effects of the i-ith farm;

Sj – fixed effect of the j-th season;

eij – the residuum.

Results and discussion

In herds kept on pastures (Tab. 4), significant inter-farm differences were found in 82 g/100 g total FAs (P<0.05). This concerned all the evaluated FAs except the short chain fatty acids (SCFA) group, which did not differ between the farms (P>0.05). The seasonal changes were found in FAs forming 90 g/100 g total FAs on average, *i.e.* in all the evaluated FAs. Short- and medium-chain FAs (C4 – 16) showed higher concentrations in winter than in summer, whereas the long-chain (C18) FAs had higher proportions in the summer period (P<0.05). The interaction between farm and season effects appeared significant only for myristic acid C14:0 (P=0.009).

The CLA concentration differed significantly between the seasons and between the farms (P<0.001). In the summer its mean concentration was 2.2 times higher than in winter (1.36 and 0.62, respectively). The concentration of both linoleic (C18:2 n-6) and linolenic (C18:3 n-3) acids increased in the summer (P<0.001), but their ratio (n-6/n-3) decreased from 3.28 in the winter to 2.83 in the summer period (P<0.05). The concentration of both the FAs as well as n-6/n-3 differed also between farms (P<0.01), the mean n-6/n-3 being 2.2 times higher on farm 3 than on farm 1 (4.43 *vs*. 1.98, respectively) and 1.6 times higher on farm 3 than on farm 2 (4.43 and. 2.76, respectively). The interaction between the season and the farm effects was insignificant (P>0.05).

Table 4. The effect of farm and season on milk fatty acid composition (g /100 g total fatty acids) in bulk milk samples at three farms with grazing herds

Fatty acid and groups of fatty acids	Farm						Season		Total	SEM	P		
	1		2		3								
	summer	winter	summer	winter	summer	winter	summer	winter			F	S	F × S
SCFA[1]	7.89	10.02	7.28	9.27	6.15	10.42	7.11	9.91	8.79	0.42	0.677	0.001	0.364
C12:0	3.45	4.10	3.09	3.91	2.43	3.91	2.99	3.97	3.58	0.13	0.026	<0.001	0.124
C14:0	10.68	12.48	10.37	12.61	8.61	12.24	9.89	12.44	11.42	0.28	0.001	<0.001	0.009
C15:0	1.17	1.26	1.07	1.12	1.09	1.25	1.11	1.21	1.17	0.02	0.037	0.013	0.514
C16:0	25.79	32.30	24.30	28.67	24.16	27.66	24.75	29.54	27.63	0.66	0.019	<0.001	0.382
C18:0	11.94	8.60	12.28	10.59	12.99	10.49	12.40	9.89	10.90	0.32	0.011	<0.001	0.232
11-trans C18:1	3.28	1.50	4.03	1.92	4.45	2.44	3.92	1.95	2.74	0.21	0.001	<0.001	0.778
9-cis C18:1	20.81	17.48	22.08	20.01	24.43	19.61	22.44	19.04	20.40	0.45	<0.001	<0.001	0.107
C18:2 n-6	2.10	1.58	2.23	1.71	2.81	1.92	2.38	1.74	1.99	0.09	0.008	<0.001	0.406
C18:3 n-3	1.04	0.83	0.97	0.54	0.70	0.40	0.90	0.59	0.71	0.05	<0.001	<0.001	0.097
CLA	1.10	0.53	1.39	0.54	1.59	0.79	1.36	0.62	0.91	0.08	<0.001	<0.001	0.147
MUFA/SAFA	0.40	0.28	0.45	0.33	0.52	0.33	0.46	0.32	0.37	0.02	<0.001	<0.001	0.032
PUFA/SAFA	0.07	0.04	0.08	0.04	0.09	0.05	0.08	0.04	0.06	0.00	0.004	<0.001	0.064
PUFA/MUFA	0.18	0.16	0.18	0.13	0.18	0.14	0.18	0.14	0.16	0.01	0.507	0.001	0.491
n-6/n-3	2.02	1.93	2.31	3.21	4.15	4.71	2.83	3.28	3.10	0.22	<0.001	0.028	0.136

P – level of statistical significance; F – effect of farm; S – effect of season; F x S interaction between farm and season effect;
[1]SCFA – short-chain fatty acids (C4:0, C6:0, C8:0 and C10:0); MUFA – monounsaturated fatty acids (trans C18:1 and 9-cis C18:1); SAFA – saturated fatty acids (C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C18:0); PUFA – polyunsaturated fatty acids (CLA, C18:2 n-6, C18:3 n-3); n-6 = C18:2 n-6; n-3 = C18:3 n-3.

Table 5. The effect of farm and season on milk fatty acid composition (g /100 g total fatty acids) in bulk milk samples on two farms with indoor kept herds

Fatty acid and groups of fatty acids	Farm				Season		Total	SEM	P		
	1		2								
	summer	winter	summer	winter	summer	winter			F	S	F × S
SCFA[1]	8.11	10.52	8.42	10.69	8.26	10.61	9.67	0.50	0.802	0.025	0.945
C12:0	4.21	5.18	4.05	4.39	4.13	4.79	4.52	0.17	0.124	0.039	0.301
C14:0	12.88	13.67	12.66	12.63	12.77	13.15	13.00	0.20	0.116	0.328	0.297
C15:0	1.34	1.44	1.17	1.12	1.26	1.28	1.27	0.04	0.003	0.746	0.284
C16:0	33.06	32.37	32.31	30.13	32.68	31.25	31.82	0.48	0.118	0.133	0.423
C18:0	8.26	7.26	9.68	8.93	8.97	8.10	8.45	0.31	0.009	0.111	0.822
11-trans C18:1	1.36	1.19	1.60	1.83	1.48	1.51	1.50	0.07	<0.001	0.728	0.017
9-cis C18:1	17.57	16.28	17.71	18.43	17.64	17.36	17.47	0.47	0.243	0.764	0.304
C18:2 n-6	2.35	2.21	2.08	1.94	2.21	2.07	2.13	0.06	0.021	0.195	0.998
C18:3 n-3	0.44	0.42	0.49	0.44	0.46	0.43	0.44	0.02	0.253	0.380	0.644
CLA	0.39	0.30	0.56	0.59	0.48	0.44	0.46	0.04	<0.001	0.547	0.238
MUFA/SAFA	0.28	0.25	0.28	0.30	0.28	0.27	0.28	0.01	0.149	0.690	0.231
PUFA/SAFA	0.05	0.04	0.05	0.04	0.05	0.04	0.04	0.00	0.763	0.161	0.574
PUFA/MUFA	0.17	0.17	0.16	0.15	0.17	0.16	0.16	0.01	0.191	0.679	0.403
n-6/n-3	5.46	5.35	4.35	4.41	4.90	4.88	4.89	0.20	0.014	0.957	0.820

P – level of statistical significance; F – effect of farm; S – effect of season; F × S interaction between farm and season effect; [1]SCFA – short-chain fatty acids (C4:0, C6:0, C8:0 and C10:0); MUFA – monounsaturated fatty acids (trans C18:1 and 9-cis C18:1); SAFA – saturated fatty acids (C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C18:0); PUFA – polyunsaturated fatty acids (CLA, C18:2 n-6, C18:3 n-3); n-6 – C18:2 n-6; n-3 = C18:3 n-3.

The MUFA/SAFA, PUFA/SAFA and PUFA/MUFA ratios were higher in the summer than in the winter ($P<0.001$). The former two indicators differed also between the farms ($P<0.01$), contrary to PUFA/MUFA which was not significantly different on particular farms ($P>0.05$). There was no significant interaction between the farm and the season effects in any of these health parameters ($P>0.05$).

In the milk of the indoor kept herds (Tab. 5), both the inter-farm and the inter-season differences in individual FAs concentration were found in FAs forming 14 g/100 g total FAs on average ($P<0.05$). The seasonal changes were found in SCFA and in the lauric acid, C12:0 ($P<0.05$), the contents being always higher in the winter than in the summer period. The effect of farm was significant in C15:0, stearic (C18:0), vaccenic (11-*trans* C18:1), linoleic (C18:2 n-6) and CLA ($P<0.05$). The interaction farm effect x season effect was significant only for vaccenic acid ($P<0.05$). MUFA/SAFA, PUFA/SAFA, PUFA/MUFA and n-6/n-3 changed significantly neither between the farms nor between the seasonal periods ($P>0.05$).

The seasonal change between the pasture-based and silage-based diets was found to be a principal cause of the changes in the milk fat profile shown in this study. Despite a high variation in the FAs concentration between the individual pastured herds, the effect of season did not interact with the farm effect (except for myristic acid). The fresh herbage intake resulted in the increase in long-chain FAs (stearic and unsaturated FAs) and the decrease in the short- and medium-chain FAs including the hypercholesterolemic lauric, myristic and palmitic acids. This is in accordance with the earlier results reported on executed farms 1-3 by Frelich *et al*. [2009a], and other studies dealing with the impact of the herbage on milk fat profile [Couvreur *et al*. 2006, Dewhurst *et al*. 2006, Elgersma *et al*. 2006, Floris *et al*. 2006, Kalač and Samková 2010].

The concentration of oleic acid – the major MUFA of a cow milk – was higher in the pasture period than in the winter period. The substitution of saturated fatty acids with oleic acid is desirable because it reduces the risk of a coronary heart disease [Mensink 2003]. The mean CLA concentration in milk was more than twice as high in the pasture period than during the winter period. There was a notable variation in CLA content also between the farms. Significant differences between the grazed farms were found also in n-6/n-3 ratio. Diverse farm specificity, genetics (two breeds on farm 1), feed, management, or botanical (sward composition) factors may be responsible for this inter-farm variation [Kelsey *et al*. 2003]. However, the effect of farm did not interact with the effect of season, in none of the individual PUFAs or the health parameters.

An increase in CLA and n-3 PUFA concentration of milk when fresh forage was fed to cows has been been well documented [Gardzina *et al.* 2005, Rego *et al*. 2004, Dhiman *et al*. 2005, Bargo *et al*. 2006, Collomb *et al.* 2006, Dewhurst *et al.* 2006, Floris *et al.* 2006]. A high concentration of linolenic acid in plants, the precursor of other C18 acids produced by biohydrogenation in the rumen and by mammary gland (CLA, oleic acid), may be responsible for these changes in milk fat composition [Chilliard *et al.* 2007, Bauman *et al.* 2006]. The linolenic is biohydrogenated in the rumen to the vaccenic and stearic acids, which are further desaturated in the mammary gland to CLA and to the oleic acid and released in milk. This probably caused also a higher concentration of vaccenic acid in milk of grazing herds in the summer compared to the winter period in this study. As a result, the milk beneficial to health, as indicated by MUFA/SAFA, PUFA/SAFA, PUFA/MUFA and n-6/n-3, is produced by cows in the pasture period compared to the winter feeding. No such seasonal trend in these health indicators was identified in the indoors herds.

A higher mono- or polyunsaturated to saturated FAs and polyunsaturated to monounsaturated FAs ratios and a lower n-6 to n-3 ratio in milk in the summer period than in the winter period in pastured herds indicate a milk potentially more beneficial to consumers' health. The seasonal grazing was hereby confirmed as the main factor affecting the seasonal variation of milk FAs composition, which may be positively evaluated by the consumers.

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