The effect of feeding diets markedly differing in the proportion of grass and maize silages on bovine milk fat composition

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ABSTRACT: Ten Czech Pied cows in the mid-lactation stage were fed diets based on grass silage and maize silage. The composition of milk fats differed. The proportions of even-chain saturated fatty acids (SFAs) up to $C_{14:0}$ were insignificant and the content of $C_{16:0}$ was significantly higher ($P < 0.05$) when feeding a diet based on maize silage, while the proportions of the individual polyunsaturated fatty acids (PUFAs) were significantly (except for $C_{18:2}$) higher when feeding a diet based on grass silage. The total SFA proportions were 67.60 and 62.93% ($P < 0.05$) of maize and grass silages, respectively, while an opposite relation was observed for the sum of PUFAs (3.56 and 4.74%; $P < 0.001$). Feeding of grass silage resulted in a significantly lower proportion of hypercholesterolaemic fatty acids $C_{12:0}$, $C_{14:0}$ and $C_{16:0}$ (49.38 and 44.98%, respectively; $P < 0.05$) and in lower values of the atherogenic index (3.03 and 2.44; $P < 0.05$). Thus, the results could be used for the improvement of milk fat composition.

Keywords: Czech Pied cattle; feeding diet; milk fat; fatty acids

The established negative role of saturated fatty acids (SFAs) for the development of cardiovascular diseases (Lock and Bauman, 2004; Parodi, 2004) has initiated the continuing effort in the research on animal fat composition. Milk fat has been a representative of that group as it contains all so-called hypercholesterolaemic fatty acids at a considerable level – lauric ($C_{12:0}$), myristic ($C_{14:0}$) and palmitic ($C_{16:0}$) acid – Collomb et al. (2004), Pešek et al. (2006, 2008). Moreover, it is distinguished by a relatively high level of the atherogenic index (AI), calculated as the ratio of the sum of lauric acid + 4 × myristic acid + palmitic acid contents to the sum of unsaturated fatty acids (UFAs) content. Myristic acid content is included four times due to its most detrimental health effects (Ulbricht and Southgate, 1991; Zock et al., 1994; Bobe et al., 2003).

The milk fat composition is not constant. It can fluctuate relatively widely depending on several factors. Diet composition and nutrition of dairy cows have been regarded as the most important factors that have often been utilised. The aim of the optimisation of milk fat composition has been the production of fat with more appropriate technological and/or health properties (Perdrix et al., 1996; Ashes et al., 1997).

The effects of different cow nutrition were reported. As regards forages, the feeding of different silages, mainly those produced from grasses and maize, affected considerably the proportion of preferred fatty acids with shorter chain

Supported by the Ministry of Education, Youth and Sports of the Czech Republic (Project No. MSM 6007665806) and by the Ministry of Agriculture of the Czech Republic (Project No. MZE QH81210).

Table 1. Characteristics of 10 Czech Pied cows – milk yield and composition

	Diet with predominant								P
	grass silage				maize silage				
	$\bar{x}$	s_x	x_{min}	x_{max}	$\bar{x}$	s_x	x_{min}	x_{max}	
Parity	2.3	0.7	2	4	3.3	0.7	3	5	–
Days in milk	182	38	121	243	158	42	91	243	0.0528
Milk yield (kg/day)	17.76	6.47	11.80	34.00	23.17	7.35	12.30	38.60	0.1507
Fat (%)	4.49	0.87	2.94	5.93	4.66	0.68	3.73	5.98	0.6646
Protein (%)	3.57	0.27	2.96	3.97	3.26	0.46	2.79	4.06	0.1300
Lactose (%)	4.79	0.17	4.50	5.00	5.00	0.14	4.80	5.20	0.0004

length (Martin et al., 2002; Floris et al., 2006). Feeding grass, red clover and clover-grass silages, Dewhurst et al. (2003) reported a significant increase in linolenic acid ($C_{18:3}$) and a decrease in palmitic acid proportion in milk fat of cows receiving clover silage.

In concentrates, their different treatments (e.g. thermal, grinding, extrusion, seed extraction) should be taken into consideration as they can increase the proportion of UFAs and concurrently decrease the level of SFAs (Sarrazin et al., 2004; Liu et al., 2008). Significant changes in the milk fat composition followed feeding of additives based on fodder fats and oils (e.g. Loor et al., 2005; Strusińska et al., 2006).

The aim of this study was to find out the extent to what fatty acid composition and atherogenic index of cow milk fat can be affected by diets widely used

Table 2. Chemical composition of used feeds

	Maize silage	Grass silage		Hay[1]	Mashed oats	Production feed mixture[2]
		1	2			
Dry matter (DM; g/kg)	286	324	385	897	870	883
Composition (DM; g/kg)						
Crude protein[3]	75.5	117.6	142.9	71.4	132.2	252.6
Crude fat	31.0	32.5	21.4	18.9	42.5	70.4
Crude fibre	250.7	280.6	259.3	309.2	141.4	67.5
Crude ash	44.8	75.3	91.0	63.2	33.6	72.3
NE_L (MJ/kg)[4]	6.09	5.46	5.87	4.18	6.83	7.41
Ca	2.13	5.12	13.53	3.69	1.49	13.21
P	1.96	3.02	2.83	2.38	4.37	8.00
Na	0.07	0.25	0.10	0.21	0.46	6.18
Mg	1.33	1.98	2.99	1.20	1.38	6.74

[1]permanent grassland hay from late cut with prevailing *Deschampsia cespitosa, Agrostis tenuis, Agrostis stolonifera, Alopecurus pratensis*;

[2]mixture consisted of 20, 20, 12, 25, 20 and 3% (w/w) of barley, wheat, oats, extracted rapeseed meal, extracted soybean meal and a mixture of minerals and vitamins;

[3]N × 6.25; [4]net energy for lactation (Sommer et al., 1994)

in farming, based on different proportions of grass and maize silages.

MATERIAL AND METHODS

Sampling and feeding

Ten lactating Czech Pied cows together stabled in a byre with stanchions for 200 animals on the Čejkovice farm were used (Table 1). Raw milk for the determination of milk and fatty acid composition was sampled within the afternoon regular testing of milk efficiency during the winter period in January and November 2006.

Diets were formulated according to the DLG-Futterwerttabellen, Wiederkäuer (1997) and calculated for the mean live weight of 600 kg, milk fat content of 4.2% and milk protein content of 3.5%. Diets consisted of silages widely used currently in Czech farming practice (maize and grass silages) – Table 2. The cows received primarily a diet based on grass silage (27 kg/day; 56.4% of dry matter) and then a diet based on maize silage (21 kg/day; 35.6% of dry matter) – Table 3. Both the diets were fed at least for six weeks before sampling.

Table 3. Intake of matter and diet composition

	Diet with predominant	
	grass silage	maize silage
Intake (kg/day)		
Fresh matter	37.6	38.9
Dry matter (DM)	15.5	16.6
Diet composition (% of DM)		
Maize silage	8.6	35.6
Grass silage 1	56.4	–
Grass silage 2	–	25.1
Hay[1]	4.4	4.1
Mashed oats	5.6	5.3
Production feed mixture:	23.3	28.0
–barley	4.8	5.8
–wheat	4.8	5.8
–oat	2.9	3.4
–extracted soybean meal	6.0	7.2
–extracted rapeseed meal	4.8	5.8
Mineral and vitamin mixture[2]	1.7	1.9
Chemical composition		
Dry matter (g)	411	427
Crude protein (g/kg of DM)[3]	144	146
NE_L (MJ/kg)[4]	6.0	6.3

[1]permanent grassland hay from late cut with prevailing *Deschampsia cespitosa, Agrostis tenuis, Agrostis stolonifera, Alopecurus pratensis*;

[2]mixture contained per kilogram: 210, 30, 100, 70 g of calcium, phosphorus, sodium, magnesium, resp.; 750, 30, 80, 2 730 mg of copper, selenium, iodine, vitamin E, resp.; 500 000 and 75 000 IU of vitamin A and D_3, resp.

[3]N × 6.25; [4]net energy for lactation (Sommer et al., 1994)

Chemical and statistical analyses

The chemical composition of feeds used in the diets was analyzed using valid methods according to Decree No. 124/2001 of the Ministry of Agriculture of the Czech Republic (2001). The content of crude protein was determined according to the Kjeldahl method (N × 6.25). The value of NE_L was estimated according to Sommer et al. (1994).

Fat, protein and lactose contents in milk samples (n = 20) were determined spectrophotometrically using a Milcoscan 4000 device (Foss Electric). Milk fat was extracted with petroleum ether. Fatty acids in isolated fat were reesterified to their methyl esters by a methanolic solution of potassium hydroxide. Methyl esters of fatty acids were determined by a gas-chromatographic method (GLC)

Table 4. Parameters of chromatographic analysis of fatty acids

Parameter	Value
Column	omegawax 530; 30 m
Detector	FID
Temperature:	
– column	40°C for 3 min; 20°C/min up to 150°C; 2.5°C/min up to 240°C
– injection	250°C
– detector	250°C
Nitrogen flow	6 ml/min
Injection	1 µl

using a Varian 3300 apparatus under conditions shown in Table 4. The identification of fatty acids was carried out using the analytical standards (Supelco). In total, 45 fatty acids were observed, out of which 33 were identified. The proportions of individual fatty acids were calculated from the ratio of their peak area to the total area of all the observed acids.

The analytical data were assessed statistically using Statistica CZ 6.1 program (Statsoft CR). For significance testing, *t*-test for dependent samples was used.

RESULTS AND DISCUSSION

Statistically insignificant differences ($P = 0.1507$) in daily milk yield (Table 1) were observed between the testing periods in January and November 2006 in ten cows of Czech Pied cattle. The differences could be caused by different parity (2.3 and 3.3 in January and November, respectively). As reported by Townsend et al. (1997) and Secchiari et al. (2003), parity had no effect on the fatty acid composition contrary to the stage of lactation. In our opinion, the changes in the spectrum of fatty acids of these ten lactating cows described below were caused primarily by different proportions of silages in diets.

Both the tested diets have been widely used in the Czech farming practice. The replacement of the diet based on grass silage (27 kg/day) by the diet with prevailing maize silage (21 kg/day) caused expected changes within all groups of milk fatty acids (Figures 1–3 and Table 5).

Feeding the diet based on grass silage resulted in a statistically significant (except for linoleic acid – $C_{18:2n6}$) increase in contents of 12 identified polyunsaturated fatty acids (PUFAs). Important changes were observed in the proportion of $C_{18:2n6}$ (1.81 versus 1.65%) after feeding the diets based on grass silage and maize silage, respectively, and those of individual n-3 fatty acids (Table 5).

The ratio of n-6 to n-3 fatty acids in human nutrition should be maximally 5:1 (WHO/FAO, 2003). While the intake of n-6 fatty acids from plant oils was sufficient and even exceeded, dietary sources of n-3 acids were usually limited. In the tested milk fat the ratio values were 3.18 and 3.53 ($P < 0.05$) after feeding the diets based on grass silage and maize silage, respectively (Figure 1). Literature data of the ratio in milk fat vary more widely, e.g. between 1.16 and 4.37 (Komprda et al., 2000; Collomb et al., 2004). However, it should be taken into consideration that the number of identified n-3 and n-6 fatty acids was different in different papers. For instance, Leiber et al. (2005) used the contents of only three n-6 and four n-3 acids for the ratio calculation, while Collomb et al. (2004) used nine and seven acids, respectively.

From the nutritional aspect, great attention has been focused on conjugated linoleic acids (CLA) with their preventive health roles (Parodi, 2004; Bauman et al., 2006). The determined proportions in milk fats were 0.92 and 0.48% ($P < 0.001$) after feeding the diets based on grass silage and maize silage, respectively (Table 5). Considerable differences ($P < 0.001$) were also observed in total proportions of PUFAs, 4.74 and 3.56%, respectively (Figure 2).

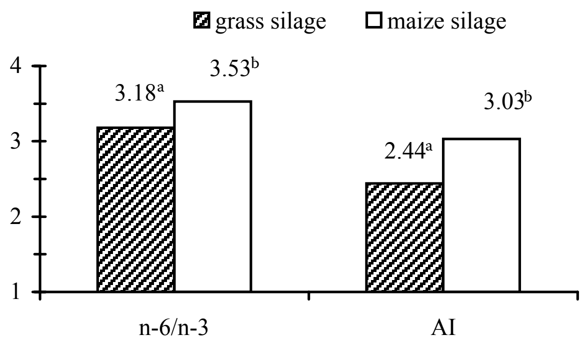

Figure 1. The ratio of polyunsaturated fatty acids *n-6* to polyunsaturated fatty acids n-3(n-6/n-3) and the atherogenic index ($AI = (C_{12:0} + (C_{14:0} \times 4) + C_{16:0})/UFA$) in milk fat of Czech Pied cows fed diets based on grass and maize silage; [a,b]means with different superscripts differ significantly at 0.05

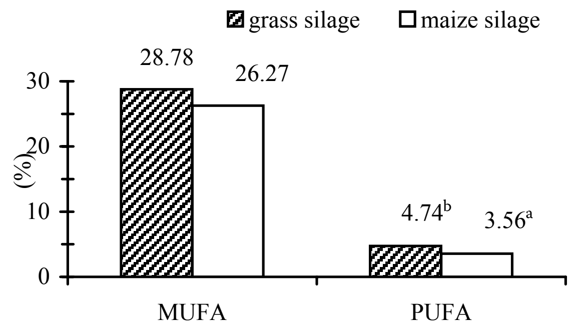

Figure 2. Proportions of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in milk fat of Czech Pied cows fed diets based on grass and maize silage; % of total fatty acids (w/w); [a,b]means with different superscripts differ significantly at 0.001

Table 5. Effects of feeding different diets on the fatty acid composition (% of the total 45 fatty acids, w/w) in milk fat of Czech Pied cows

Fatty acid	Diet with predominant								*P*
	grass silage				maize silage				
	$\bar{x}$	s_x	x_{min}	x_{max}	$\bar{x}$	s_x	x_{min}	x_{max}	
Saturated									
$C_{4:0}$	2.19	0.48	1.41	2.83	2.43	0.40	1.92	3.09	0.2305
$C_{6:0}$	1.87	0.38	1.24	2.27	2.04	0.22	1.73	2.34	0.2286
$C_{8:0}$	1.29	0.23	0.80	1.54	1.48	0.15	1.26	1.70	0.0621
$C_{10:0}$	3.24	0.51	1.98	3.81	3.73	0.51	3.13	4.58	0.0804
$C_{11:0}$	0.06	0.02	0.04	0.09	0.08	0.02	0.05	0.11	0.0716
$C_{12:0}$	3.75	0.55	2.37	4.22	4.25	0.77	3.43	5.67	0.1826
$C_{13:0}$	0.13	0.02	0.10	0.16	0.14	0.05	0.10	0.25	0.3438
$C_{14:0}$	11.99	1.45	8.39	13.68	13.15	1.20	10.88	14.45	0.0931
$C_{15:0}$	1.31	0.09	1.20	1.50	1.12	0.23	0.83	1.57	0.0696
$C_{16:0}$	29.24	1.46	26.70	31.92	31.99	3.58	26.33	39.37	0.0423
$C_{17:0}$	0.76	0.13	0.65	1.10	0.57	0.04	0.52	0.65	0.0012
$C_{18:0}$	6.72	1.82	4.02	10.38	6.38	0.84	5.44	7.73	0.6386
$C_{20:0}$	0.23	0.03	0.18	0.27	0.15	0.02	0.12	0.18	0.0000
$C_{22:0}$	0.08	0.01	0.06	0.09	0.05	0.01	0.02	0.07	0.0000
$C_{24:0}$	0.07	0.01	0.04	0.08	0.04	0.01	0.01	0.06	0.0000
Monounsaturated									
$C_{10:1}$	0.34	0.08	0.13	0.41	0.33	0.06	0.25	0.46	0.9832
$C_{12:1}$	0.20	0.10	0.06	0.40	0.09	0.03	0.04	0.15	0.0181
$C_{14:1}$	1.14	0.30	0.45	1.59	1.01	0.26	0.77	1.57	0.2762
$C_{16:1}$	1.81	0.28	1.55	2.27	1.92	0.52	1.28	2.67	0.4938
$C_{18:1}$	24.94	2.28	22.65	30.86	22.72	3.16	20.04	29.82	0.1399
$C_{20:1}$	0.35	0.02	0.31	0.38	0.20	0.02	0.15	0.23	0.0000
Polyunsaturated									
$C_{16:2n4}$	0.21	0.12	0.08	0.52	0.11	0.03	0.08	0.15	0.0323
$C_{16:3n4}$	0.40	0.08	0.35	0.61	0.32	0.08	0.20	0.46	0.0390
$C_{18:2n6}$	1.81	0.34	1.35	2.46	1.65	0.22	1.30	2.04	0.2585
CLA[1]	0.92	0.14	0.63	1.13	0.48	0.07	0.40	0.61	0.0000
$C_{18:3n6}$	0.09	0.02	0.06	0.13	0.07	0.01	0.05	0.09	0.0233
$C_{18:3n3}$	0.52	0.10	0.43	0.73	0.42	0.07	0.31	0.51	0.0120
$C_{20:3n6}$	0.16	0.04	0.12	0.22	0.10	0.03	0.07	0.17	0.0055
$C_{20:4n6}$	0.19	0.04	0.15	0.26	0.14	0.04	0.11	0.24	0.0005
$C_{20:4n3}$	0.05	0.02	0.02	0.09	0.02	0.01	0.01	0.04	0.0066
$C_{20:5n3}$	0.05	0.01	0.04	0.07	0.04	0.01	0.03	0.05	0.0241
$C_{22:4n6}$	0.07	0.01	0.05	0.10	0.03	0.01	0.02	0.06	0.0000
$C_{22:5n3}$	0.11	0.02	0.09	0.13	0.09	0.01	0.08	0.11	0.0027

[1]mixture of isomeric conjugated linoleic acids ($\Delta^{9,11}$*cis*-, *trans*; $\Delta^{9,11}$*trans*-, *cis*-octadecadienoic)

The diets affected also the contents of individual monounsaturated fatty acids (MUFAs). Feeding a diet based on grass silage caused the increased contents of all MUFAs except for palmitoleic acid ($C_{16:1}$), the proportion of which decreased insignificantly. Proportions of $C_{12:1}$ (0.20 and 0.09%, P < 0.05) and $C_{20:1}$ (0.35 and 0.20%, P < 0.001) after feeding the diets based on grass silage and maize silage, respectively, were determined. Statistically insignificant differences were observed both in oleic acid ($C_{18:1}$) proportions (24.94 and 22.72%) and in total MUFA proportions (28.78 and 26.27%, respectively) (Table 5 and Figure 2).

An increase in MUFA and PUFA proportions in milk fat is certainly compensated by a decrease in the SFA proportion (Figure 3). In this study, feeding of the diet based on grass silage resulted in an insignificant decrease in all even-chain acids between $C_{4:0}$ and $C_{14:0}$, and in a significant decrease in $C_{16:0}$ (29.24 and 31.99; P < 0.05). The proportions of acids with longer chains increased significantly (P < 0.01 and P < 0.001). As mentioned above, changes in the proportion of hypercholesterolaemic acids $C_{12:0}$, $C_{14:0}$ and $C_{16:0}$ can have a very detrimental health impact. As may be seen from Figure 3, the diet based on grass silage decreased significantly their sum value (44.98 and 49.38%; P < 0.05).

The determined decrease in hypercholesterolaemic acids along with the increase in MUFA and PUFA proportions resulted in a significant decrease in the values of atherogenic index (2.44 and 3.03; P < 0.05) – Figure 1.

The comparison of our results with literature data was complicated due to different experimental conditions and participating factors. However, the papers of Chilliard et al. (2001, 2007) reported the following results and knowledge of the effects of maize silage feeding on the milk fat composition. Maize silage as the main forage of a diet leads to the proportions 12–14%, 30–34% and 18–23% of $C_{14:0}$, $C_{16:0}$ and $C_{18:1}$, respectively, in milk fat. Our results (Table 5) showed to be well comparable with those levels. Moreover, according to Chilliard et al. (2001), feeding of maize silage as compared with grass silage causes an increase in all SFAs between C_6 and C_{12}. This was also observed in our study (Table 5). And finally, the proportions of CLA determined in our study are consistent with results of Chouinard et al. (1998) and Bayourthe et al. (2000), who reported the CLA proportion of 0.4–0.6% when feeding maize silage, while the levels for grass silage feeding were twice higher.

According to Chilliard et al. (2001), the differences in PUFA proportions between milk fats produced by cows fed maize or grass silage are not very large. The linoleic acid proportion varies between 1.8 and 2.8% in maize silage feeding, while it is lower in grass silage feeding. Data in Table 5 differ from these generalised conclusions. However, some other factors should be reasoned. The milk fat composition can be affected by different vegetation stages of ensiled forage (Vanhatalo et al., 2007), different botanical composition and origin of ensiled grasses (Collomb et al., 1999; Leiber et al., 2005) or by different hybrids of ensiled maize (LaCount et al., 1995). For instance, Michalet-Doreau and Doreau (1999) reported the effect of maize silages containing up to 30–40% of grains with a high content of linoleic acid. Such sources then increased the linoleic acid proportion in milk fat.

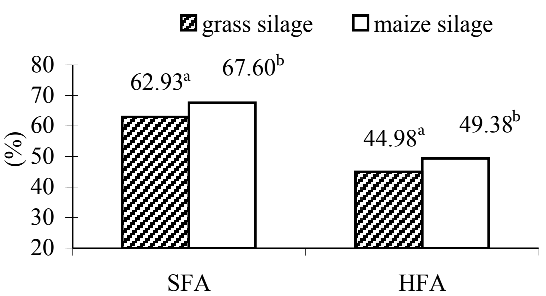

Figure 3. Proportions of saturated fatty acids (SFA) and hypercholesterolaemic fatty acids (HFA = $C_{12:0}$ + $C_{14:0}$ + $C_{16:0}$) in milk fat of Czech Pied cows fed diets based on grass and maize silage; % of total fatty acids (w/w); [a,b]means with different superscripts differ significantly at 0.05

CONCLUSIONS

The content and composition of all identified fatty acids in milk fat of Czech Pied cows differed with feeding diets based on grass or maize silage. Significant differences were found mainly in PUFA proportions. The proportion of salubrious CLA was significantly higher when feeding the diet based on grass silage as compared with the diet based on maize silage (0.92 and 0.48, respectively, P < 0.001). Contents of even-chain SFAs up to $C_{14:0}$ were insignificantly higher with maize silage feeding and the content of $C_{16:0}$ was significantly higher (29.24 and 31.99; P < 0.05). Significantly higher values as compared with fat of cows fed grass silage were observed both in the total proportion of hypercholesterolaemic fatty acids $C_{12:0}$, $C_{14:0}$ and $C_{16:0}$ (44.98 and 49.38%, $P < 0.05$) and in the atherogenic index of milk fat (2.44 and 3.03; $P < 0.05$).

The results confirm the presumption that the feeding of appropriate forage can considerably reduce the proportion of hypercholesterolaemic fatty acids in milk fat and the value of the atherogenic index. Thus, the information could be used for the improvement of milk fat composition for healthier nutrition.

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Received: 2008–05–06
Accepted after corrections: 2008–11–04

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