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Eighteen-carbon fatty acids in milk fat of Czech Fleckvieh and Holstein cows following feeding with fresh lucerne (*Medicago sativa* L.)*

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Changes in selected FAs level as influenced by inclusion of fresh lucerne (*Medicago sativa* L.) into a feeding ration were assesed in Czech Fleckvieh (dual purpose) and Holstein cows. Each of the balanced groups of 12 cows in mid-lactation was fed with a ration containing 12.7 kg (11.7% of dry matter) of fresh lucerne and then with a ration with preserved forage (maize and grass silages) only. Total content of nutritionally desirable C18 acids was 34.1 and 30.5% of total fatty acids in Czech Fleckvieh and Holstein cows, respectively (P=0.0693). The response of the breeds on the change in feeding differed. The changes in fatty acid composition in the Czech Fleckvieh cows were less extensive in stearic and oleic acids, while more extensive in essential linoleic and linolenic acids than those in the Holstein cows. Addition of lucerne into ration in both breeds has caused greatest changes in polyunsaturated fatty acids (P<0.01), particularly alpha-linolenic acid (P<0.001).

KEY WORDS: breed / cows / fatty acids / feeding / lucerne / milk fat

Milk is a valuable source of energy, protein, minerals and vitamins. It supplies also an important proportion of fat intake in human nutrition in many countries. Milk

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products provide consumers with about 15-25% of total fat intake and about 25-35% of total saturated fatty acids (FAs) [O'Donnel 1993]. Nevertheless, milk and milk products are an important source of essential FAs [Haug 2007].

Dewhurst *et al.* [2006] recommended for humans the level 15-30% of total fat and the proportions <10, 5-8, 1-2 and <1% within total energy intake of saturated FAs, n-6 polyunsaturated FAs (PUFA), n-3 PUFA and *trans*-isomers of unsaturated FAs, respectively. The recommendation was corroborated by FAO [2010] with similar respective values of 20-35, 10, 2.5-9, 0.5-2 and <1%.

In bovine milk fat, usual levels are 70-75, 20-25 and some 5% of saturated FAs, monounsaturated FAs and PUFA, respectively, from total FAs. FAs composition is affected by numerous factors, which are usually classified in two categories: animal factors and nutritional factors [Palmquist *et al.* 1993; Jensen 2002]. Breed and cow´s individuality are the important factors of the former category [Samková *et al.* 2012], while level of nutrition and ration composition of the latter one [Kalač and Samková 2010; Sterk *et al.* 2011]. The breed effects were recently proved by numerous authors, e.g. Poulsen *et al.* [2012], Larsen *et al.* [2012], Communod *et al.* [2012].

Rations for milking cows consist of forages (fresh or preserved) and concentrates. As reviewed by Kalač and Samková [2010], forages have often been the major and also the cheapest source of FAs in ruminant diets, however, the FAs content in different forages is relatively low. Moreover, losses of FAs occur during forage ensiling or drying.

On the other hand, feeding of fresh forages or pasture can widely change milk fat composition [Frelich *et al.* 2012]. Homolka and Kudrna [2007] reported that feeding of fresh forages changed composition of FAs produced *de novo* in mammary gland. The proportion of short-chain FAs increased to the exclusion of nutritionally undesirable lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids. In a study of Whiting *et al.* [2004], total proportion of saturated FAs decreased, whereas the proportion of desirable FAs (mainly n-3 PUFA) increased following feeding with fresh lucerne as compared to feeding with lucerne silage.

Comparison of FAs composition of milk fat from cows fed with either maize and grass silages or grazing lucerne and white and red clovers revealed higher proportion of vaccenic acid, conjugated linoleic acids (CLA) and alpha-linolenic acid (C18:3n-3) in milk fat of cows grazing legumes than from those fed with silages [Wiking *et al.* 2010]. Also Morel *et al.* [2006] prefer feeding a mixture of fresh lucerne and grasses to other fresh forages.

Thus, legumes (fresh and even ensiled or dried) in feeding rations showed positive effects on the proportion of desirable FAs in bovine milk fat [e.g. Van Dorland *et al.* 2008; Steinshamn 2010]. FA composition of milk fat from cows fed with legume silages was nutritionally comparable to that of milk fat from cows either grazed or fed with fresh forage [Samková 2011].

The aim of this study was to evaluate the eighteen-carbon (C18) FAs content and selected FAs groups in bovine milk fat of Czech Fleckvieh and Holstein breeds depending on inclusion of fresh lucerne into daily ration.

Material and methods

Feeding and sampling

Twenty four lactating cows (4 heifers and 20 multiparous – parity 2.17±0.72) of Czech Fleckvieh (n = 12) and Holstein (n = 12) breeds stabled together at Čejkovice farm, South Bohemia. Individual raw milk samples for determination of milk yield and composition (Tab. 1) and FAs composition was obtained within the afternoon regular testing of milk efficiency during the summer period in June and July 2006. In total, 48 of milk samples were taken.

Diets were formulated according to DLG-Futterwerttabellen [1997] and calculated for the mean live weight of 650 kg, milk fat content of 4.2% and milk protein content of 3.5%. Diets predominantly consisted of maize and grass silages widely used in the Czech farming practice (Tab. 2).

Table 1. Milk yield and composition in cows of two breeds fed two diets

Item	Czech Fleckvieh		Holstein	
	Fresh[1]	Silage[2]	Fresh[1]	Silage[2]
Days in milk	157±60	180±60	154±102	177±102
Milk yield (kg/d)	19.2±3.7	16.3±4.2	25.3±6.5	24.3±6.2
Fat (%)	4.61±0.88	4.36±0.70	4.19±0.81	4.29±1.05
Protein (%)	3.54±0.32	3.57±0.34	3.51±0.28	3.48±0.52
Lactose (%)	4.84±0.33	4.73±0.25	4.79±0.25	4.84±0.28

[1]Fresh – diet with maize and grass silages (47.0% of dry matter) + fresh lucerne (11.7% of dry matter).
[2]Silage – diet with maize and grass silages (58.8% of dry matter).

Table 2. Chemical composition of diet components

Item	Maize silage	Grass silage	Fresh lucerne	Hay[1]	Mashed oats	PM[2]
Dry matter (g/kg)	356	327	170	897	870	884
Concentration (g/kg DM)						
crude protein[3]	79.0	133.8	219.0	71.4	132.2	242.0
crude fat	42.4	19.8	31.0	18.9	42.5	19.3
crude fibre	179.5	262.1	238.0	309.2	141.4	39.1
crude ash	41.5	98.5	106.0	63.2	33.6	75.2
NEL (MJ/kg)[4]	6.62	4.93	5.82	4.68	6.83	8.02
Ca	1.88	10.01	10.00	3.69	1.49	8.60
P	1.97	3.65	2.82	2.38	4.37	6.12
Na	0.06	0.45	0.18	0.21	0.46	4.93
Mg	1.36	2.42	2.88	1.20	1.38	5.29

[1]Permanent grassland hay from late cut with prevailing *Deschampsia cespitosa, Agrostis tenuis, Agrostis stolonifera, Alopecurus pratensis*.
[2]PM – production mixture consisted of 37, 31, 28 and 4 % (w/w) of wheat, barley, extracted soybean meal and a mixture of minerals and vitamins.
[3]Crude protein – N x 6.25.
[4]NEL – net energy for lactation [Sommer *et al.* 1994].

The cows received primarily a diet (FRESH) based on silages (25.3 kg/d; 47.0% of dry matter) with 12.7 kg/d (11.7% of dry matter) fresh lucerne (alfalfa; *Medicago sativa* L.) and then a diet (SILAGE) based only on silages (31.5 kg/d; 58.8% of dry matter) – see Table 3. Both diets were fed at least for three weeks before sampling.

Table 3. Composition and intake of diets

Item	FRESH	SILAGE
Intake (kg/d)		
fresh matter	46.6	40.1
dry matter (DM)	18.4	18.4
Composition of diets (% of DM)		
maize silage	24.5	36.9
grass silage	22.5	21.9
fresh lucerne	11.7	-
hay	2.6	2.5
mashed oats	4.7	4.7
production mixture	33.4	33.2
mineral and vitamins in mixture[1]	0.8	0.8
Chemical composition (g/kg DM)		
dry matter (g)	395	460
crude protein[2]	164	147
nXP[3]	153	152
crude fat	27.3	28.9
crude fibre	158	151
NEL (MJ/kg DM)[4]	6.52	6.62

[1]Mixture consisted per kg: 210, 30, 100, 70 g of calcium, phosphorus, sodium, magnesium; 750, 30, 80, 2,730 mg of copper, selenium, iodine, vitamin E; 500,000 and 75,000 IU of vitamin A and D_3, respectively.
[2]Crude protein – N x 6.25.
[3] nXP – protein utilized in the intestine [DLG-Futterwerttabellen 1997].
[4]NEL – net energy for lactation [Sommer *et al.* 1994].

Chemical and statistical analyses

The chemical composition of feeds used in the diets was determined 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 Kjeldahl (N×6.25). The value of NEL was estimated according to Sommer *et al.* [1994]. Fat, protein and lactose contents in milk samples (n=48) were determined spectrophotometrically using a MILCOSCAN 4000 device (FOSS ELECTRIC).

Milk fat was extracted with petroleum ether. FAs in isolated fat were reesterified to their methyl esters by a methanolic solution of potassium hydroxide. Methyl esters of FAs were determined by a gas-chromatographic method (GLC) using a Varian 3300 apparatus under conditions given by Samková *et al.* [2009]. The identification of FAs was carried out using the analytical standards (SUPELCO). In total, 45 FAs were observed, out of which 33 were identified. The proportions of individual FAs 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 testing of significant differences between the breeds (Czech Fleckvieh; Holstein), *t*-test for independent samples, and within the breed (effect of feeding ration), *t*-test for dependent samples (within-group variation) were used.

Results and discussion

The effect of breed

Overall, FAs with 18 carbons (C18) have been assessed nutritionally as very desirable [German *et al.* 2009]. These acids are preformed as they originate from dietary lipids and adipose tissues reserves [Harvatine *et al.* 2009]. It is therefore supposed that composition of a ration and following biohydrogenation in the rumen both affect milk fat composition in a greater extent than does breed [Ferlay *et al.* 2011]. However, within the breed factors, cow´s individuality can have a role [Samková *et al.* 2012], particularly on monounsaturated FAs [Communod *et al.* 2012] and CLA levels. Such animal factors are ascribed to the activity of stearoyl-CoA desaturase (SCD) [Milanesi *et al.* 2008; Marchitelli *et al.* 2013]. For instance, major source of CLA in milk fat is endogenous synthesis by SCD in the mammary gland and other tissues from vaccenic acid [Mosley *et al.* 2006]. Differences in CLA contents observed Berry *et al.* [2012] with values of 0.92, 1.15 and 1.12% of total FAs in Danish Jersey, Swedish Red and Danish Holstein, respectively.

Table 4. Level of selected C18 fatty acids (FA) and groups of FAs (g/100 g of total FA) in milk fat of Czech Fleckvieh and Holstein cows as related to addition of fresh lucerne into feeding ration

FA[1]	Czech Fleckvieh					Holstein					
	Fresh[2]		Silage[3]		P (F)	Fresh[2]		Silage[3]		P (F)	P (B)
	mean	SD	mean	SD		mean	SD	mean	SD		
C18:0	8.8	1.2	9.2	1.9	0.3063	8.7	2.1	7.2	2.3	0.0012	0.0614
C18:1	22.1	5.4	22.6	6.6	0.3891	20.6	4.4	19.5	3.4	0.0923	0.1209
C18:2n-6	1.87	0.47	1.57	0.28	0.0057	1.78	0.21	1.54	0.24	0.0020	0.5319
C18:3n-3	0.54	0.13	0.34	0.09	0.0001	0.48	0.09	0.34	0.09	0.0001	0.5056
CLA	0.45	0.18	0.45	0.14	0.9729	0.35	0.11	0.31	0.11	0.1414	0.0046
SFA	67.8	6.5	68	7.2	0.7526	69.8	4.4	70.9	3.1	0.0968	0.1276
MUFA	25.4	5.7	25.9	6.7	0.3798	24.1	4.3	23.6	3	0.3876	0.2161
PUFA	3.9	1	3.2	0.6	0.0047	3.4	0.5	2.8	0.4	0.0027	0.0563

SD – standard deviation; P (F) – P value for effect of feeding ration; P (B) – P value for effect of breed.
[1]CLA – isomer of conjugated linoleic acid (9,11 *cis, trans* C18:2); SFA – saturated FA; MUFA – monounsaturated FA; PUFA – polyunsaturated FA.
[2]Fresh – diet with maize and grass silages (47.0% of dry matter) + fresh lucerne (11.7% of dry matter).
[3]Silage – diet with maize and grass silages (58.8% of dry matter).

Different SCD activity between and within breeds occurred probably also in this work. The effect of breed was proved only for CLA ($P = 0.0046$), while it was insignificant in all other C18 acids including the groups of saturated FAs, monounsaturated FAs and PUFAs (Tab. 4). Nevertheless, the observed proportions of individual C18 acids, monounsaturated FAs and PUFA were higher in milk of Czech Fleckvieh cows. This could be caused also by different milk yield and fat content between the breeds, as Stoop *et al.* [2008] reported a negative genetic correlation (-0.35) between fat yield and the proportion of FAs > C18.

In this report, the total C18 level of 30.5 and 34.1% at fat yield of 1042 and 803 g/d was found for Holstein and Czech Fleckvieh, respectively (Fig. 1). Correlation coefficients (not tabulated) between fat yield and selected C18 FAs varied between -0.10 ($P>0.05$) and -0.46 ($P< 0.01$).

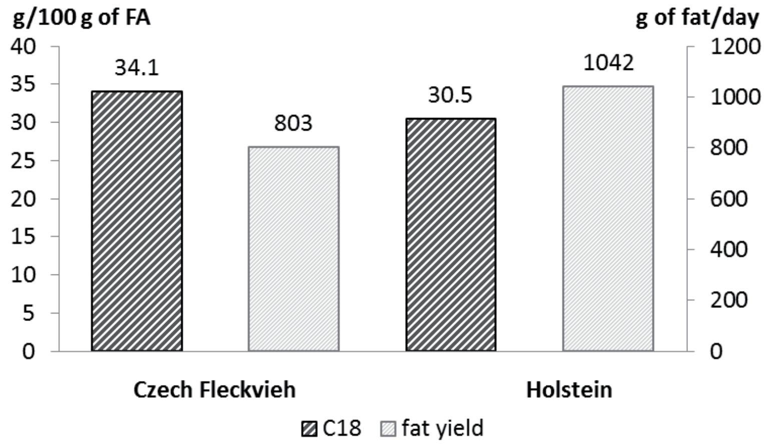

Fig. 1. Comparison of total content of C18[1] fatty acids (FAs, g/100 g of total FA) and fat yield[2] in milk samples of Czech Fleckvieh and Holstein cows.
[1]C18 – sum of C18:0, C18:1, C18:2n-6, C18:3n-6, C18:3n-3, and 9,11 *cis, trans* C18:2
[2]Fat yield = fat content ($g.kg^{-1}$) x milk yield ($kg.d^{-1}$).

The effect of ration

Changes in the amount of stearic acid (C18:0) following the change of the rations varied between the breeds. The mean levels were 8.8 and 9.2% from total FAs during feeding with the portion of fresh lucerne (FRESH) and with a mixture of maize and grass silages (SILAGE), respectively, in Czech Fleckvieh, while respective values were 8.7 and 7.2% in Holstein cows (Tab. 4). Similar trend was found in oleic acid (C18:1). Nevertheless, the differences between the breeds were statistically not significant, and the effect of ration was confirmed only in stearic acid for Holstein cows ($P<0.01$).

Feeding with fresh forages affected also the proportion of PUFA. An increase, especially of n-3 PUFA and CLA, was reported from many laboratories. Particularly fresh legumes had a positive effect [Van Dorland *et al.* 2008, Wiking *et al.* 2010], and even ensiled ones [Morel *et al.* 2006]. Similarly positive effect on elevated n-3 PUFA proportion had also grazing [Leiber *et al.* 2005; Frelich *et al.* 2012], forage botanical diversity [Collomb *et al.* 2002] or organic farming [O´Donell *et al.* 2010]. On the contrary, the mentioned feeding systems decreased the proportion of linoleic acid (C18:2n-6) and consequently of n-6 PUFA group [Samková *et al.* 2011].

Significantly higher levels (P<0.01 and P<0.001) of essential linoleic and alpha-linolenic acids were found in milk fats of both breeds fed with the portion of fresh lucerne as compared to feeding with the silages. This can be explained by an elevated content of linoleic acid in fresh lucerne in comparison with other forages [Morel *et al.* 2006]. The increase following feeding with fresh lucerne was apparent particularly in alpha-linolenic acid. Its proportion was 0.54 and 0.48% in Czech Fleckvieh and Holstein cows, respectively. The respective relative increase was 59 and 41% as compared to feeding the silages. Such changes are in accordance with results of Leiber *et al.* [2005] and Palladino *et al.* [2010].

Feeding with fresh lucerne naturally affected also the total of n-3 PUFA and n-6 PUFA. The response was again more extensive in Czech Fleckvieh than in Holstein cows (Fig. 2). The relative changes were 22 and 17% (P<0.01) in n-6 PUFA, 59 and 47% (P<0.001) in n-3 PUFA for Czech Fleckvieh and Holstein cows, respectively.

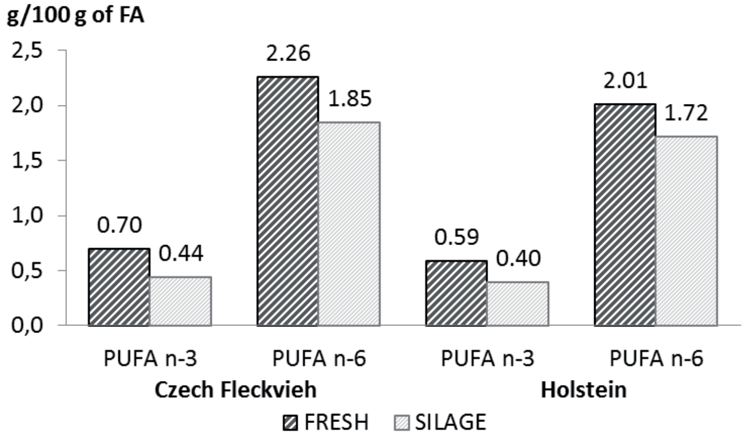

Figure 2. Proportion of polyunsaturated fatty acids n-3 (PUFA n-3)[1] and n-6 (PUFA n-6)[2] as related to addition of fresh lucerne into feeding ration (FRESH[3] *vs.* SILAGE[4]) in milk fat of Czech Fleckvieh and Holstein cows.
[1]PUFA n-3 – sum of C18:3n-3, C20:4n-3, C20:5n-3, and C22:5n-3
[2]PUFA n-6 – sum of C18:2n-6, C18:3n-6, C20:3n-6, and C20:4n-6
[3]FRESH – diet with maize and grass silages (47.0% of dry matter) + fresh lucerne (11.7% of dry matter);
[4]SILAGE – diet with maize and grass silages (58.8% of dry matter).

As mentioned above, mean proportions of CLA were 0.45 and 0.35% in fat of Czech Fleckvieh and Holstein cows, respectively. The proportion was the same at both the rations in Czech Fleckvieh cows, while slightly decreased in Holstein cows during feeding with the silages (Tab. 4). An increase of CLA reported by various authors after feeding with fresh forages was not thus proved. This can be explained by different type of fresh forage and by different level of fresh forage expressed as percentage in dry matter intake. Double and even higher increase in CLA proportion was found particularly in milk fat of grazed cows or after supplementation with plant oils [Flowers *et al.* 2008; Sterk *et al.* 2011].

The group of C18 FAs amounted about one third of total FAs in bovine milk fat. Stearic, oleic and linoleic acids prevail among saturated FAs, monounsaturated FAs and PUFA, respectively. All these FAs have been assessed as nutritionally desirable or neutral. If included also linolenic acid and CLA, the C18 group is indispensable and its proportion should be increased. As results from our experiment, total level of C18 FAs was higher in milk fat of Czech Fleckvieh cows as compared to Holstein cows´ milk fat. It was observed both after feeding the cows with silages and after the supplementation of the silages with fresh lucerne. Fresh lucerne increased proportion of total C18 FAs in both the breeds, however, with only mild differences between the breeds. The changes in stearic acid and oleic acid were somewhat more extensive in Holstein cows, while those of essential linoleic and linolenic acids in Czech Fleckvieh cows. The different breed response on fresh lucerne was probably affected by diverse fat yield of the breeds.

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