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NUTRITIONALLY DESIRABLE FATTY ACIDS INCLUDING CLA OF COW'S MILK FAT EXPLAINED BY ANIMAL AND FEED FACTORS

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Abstract

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This study evaluated the effects of four factors (breed, parity, month of lactation and feeding ration) on the total explained variation (R^2) determining composition of nutritionally important fatty acids. Individual milk samples were collected four times over an entire year (March, June, September, and December, respectively) on a conventional farm from two breeds: Czech Fleckvieh (dual-purpose, local) and Holstein (dairy, worldwide). In total, 145 samples (36; 38; 35 and 36, respectively) were analysed. Within the R^2, feeding ration and month of lactation were the main factors affecting milk fatty acids composition, whereas breed and parity showed a low effect. A high percentage of the R^2 was observed in rumenic acid (52 %), for example, whereas a low percentage was observed in both palmitic acid (30 %) and oleic acid (30 %). This may be due to intra-breed variability affected by different genetic predisposition of each cows, their performance or individual response to changes in feeding ration.

Keywords: cows, milk fatty acid, rumenic acid, breed, lactation, feeding ration

INTRODUCTION

Most of unsaturated fatty acids (FA) are more desirable in human nutrition than their saturated counterparts. The former group is subdivided into mono- and polyunsaturated FA. Moreover, polyunsaturated FA are nutritionally classified as n-6 and n-3 groups, according to the position of first double bond, numbered from the methyl end of carbon chain. The major unsaturated FA are non-essential oleic acid (18:1 *cis*-9) and essential linoleic (18:2 n-6) and alpha-linolenic acids (18:3 n-3), which have all-*cis* double bonds (Jensen, 2002).

Conjugated linoleic acid (CLA) is a group term for a series of conjugated dienoic positional and geometrical isomers of linoleic acid, which are relatively more abundant in the milk and fat tissue of ruminants (Dhiman *et al.*, 2005). In CLA, the most important isomer is *cis*-9, *trans*-11-linoleic acid (RA, rumenic acid). It forms about 75–90 % of all CLA isomers (Kramer *et al.*, 1998) and has putative health benefit (Palmquist *et al.*, 2005). RA in milk fat is primarily biosynthesized from monounsaturated vaccenic acid (18:1 *trans*-11) by the activity of the enzyme Δ9-desaturase in mammary gland. According to Mosley *et al.* (2006), this pathway produces about 80% of RA. The health effects of individual CLA isomers have been studied since the 1980s. RA has been associated with the prevention of a number of diseases, including cardiovascular diseases, obesity and diabetes (e.g. Riediger *et al.*, 2009; Sailas and Spener, 2009). However, most of the positive effects of CLAs have been observed in animals, and the evidence from clinical studies in humans is less convincing (Gebauer *et al.*, 2007; Yang *et al.*, 2015).

The fatty acid composition of bovine milk fat is affected by animal factors (Samkova *et al.*, 2012; Schwendel *et al.*, 2015) and feed factors (Chilliard *et al.*, 2001; Elgersma, 2015). For FA with up to 16 carbons, animal factors are expected to have a greater effect due to moderate coefficients of

I: *Distribution of milk samples according to breed, parity and month of lactation.*

	Breed		Parity			Month of lactation			Days in milk
	CF	**H**	**1**	**2**	**≥ 3**	**1-4**	**5-7**	**≥ 8**	**Mean ± SD**
March	18	18	12	15	9	19	9	8	128 ± 73
June	19	19	12	16	10	11	16	11	171 ± 81
September	18	17	9	16	10	10	11	14	177 ± 86
December	18	18	11	10	15	13	11	12	162 ± 102
Total	73	72	44	57	44	53	47	45	
P	0.9992			0.6521			0.2780		0.0684

P = probability, χ^2 for breed, parity and month of lactation, SD = standard deviation,
CF = Czech Fleckvieh, H = Holstein, Month of lactation: 1-4 (10-120 days in milk), 5-7 (121-210), 8-10 (211-345).

II: *Intake of matter, ingredient and chemical composition of feeding rations.*

Item	March	June	September	December
Intake (kg/day)				
Fresh matter	34.4	35.3	34.1	36.5
Dry matter (DM)	17.5	16.5	15.8	17.9
Ingredient composition (% of DM)				
Maize silage	39.0	24.3	19.5	28.1
Grass silage	24.5	22.2	21.6	26.8
CCM (corn cob meal silage)	-	-	-	12.4
Fresh lucerne (*Medicago sativa*)	-	11.4	-	-
Fresh maize	-	-	18.4	-
Hay	2.6	3.2	2.8	7.0
Mashed oats	4.9	5.3	5.4	4.9
Production feed mixture (PM)	27.9	32.5	31.1	19.8
Minerals and vitamins mixture (MM)	1.1	1.1	1.2	1.0
Chemical composition				
DM (g)	443	399	400	441
CP (g/kg of DM)	146	160	153	146
nXP	147	151	150	146
Crude fat	30.3	27.4	26.3	27.9
Crude fibre	167	159	176	166
NE_L (MJ/kg)	6.36	6.48	6.47	6.36

PM = mixture consisted of 20 (32; 9), 20 (37; 30), 12 (0; 0), 0 (0; 14), 20 (28; 7), 25 (0; 37) and 3 (3; 3) % (w/w) of barley, wheat, oats, lupin (*Lupinus albus*), extracted soybean meal, extracted rapeseed meal, and a mixture of minerals and vitamins in March (June – September; December), MM = mixture consisted per kg: 210, 30, 100, 70 g of calcium, phosphorus, sodium, magnesium, respectively; 750, 30, 80, 2,730 mg of copper, selenium, iodine, vitamin E, respectively; 500,000 and 75,000 IU of vitamin A and D_3, respectively, CP = N × 6.25, nXP = protein utilised in the intestine (DLG-Futterwerttabellen, 1997), NE_L = net energy for lactation (Sommer *et al.*, 1994).

heritability (Schennink *et al.*, 2008; Stoop *et al.*, 2008). Conversely, feed factors have the greatest effect when the carbon chain length is greater than 18 (Samkova *et al.*, 2014). For instance, CLA content can vary between 0.34 and 3.08 % of total FA depending especially on feeding (Dhiman *et al.*, 2005; Elgersma, 2015). The highest levels are observed under feeding with fresh forage (Collomb *et al.*, 2002), whereas breed and parity show lower effects (Stanton *et al.*, 1997).

Our work aimed at evaluating the extent of variability of four factors (breed, parity, month of lactation and feeding ration) affecting proportions of nutritionally desirable FA including RA in milk fat of dairy cows reared under farm management practices that are typical in the Czech Republic.

MATERIALS AND METHODS

Ethical committee hereby declares that experiments performed in the present study are according Act No 246/1992 Coll., on the protection of animals against cruelty of the Czech Republic. With regard to the type of study, no special permission is needed.

The study was carried out on a farm (420 meters above sea level), in the region of South Bohemia, Czech Republic. On the farm, cows of two breeds, Czech Fleckvieh (dual-purpose, local) and Holstein (dairy, worldwide) were housed together and milked twice a day. Individual milk samples were collected within the afternoon regular testing of milk efficiency in each of four months: March, June, September and December. Milk samples were taken with regard to breed, parity and month of lactation to obtain a balanced set of samples (Tab. I).

All cows were fed under the same conditions. Total mixed rations were formulated according to the German recommendations for ruminant feeding (DLG-Futterwerttabellen, 1997) and calculated for a mean live weight of 650 kg, milk fat content of 4.2 % and milk protein content of 3.5 %. Total mixed rations consisted of components widely used in the recent Czech farming practice (Tab. II). All feeding rations were fed at least for three weeks before sampling.

Milk samples were cooled (6 °C) immediately after collection and transported to the laboratory in a cool box. Fat, protein and lactose contents were determined spectrophotometrically using a MilcoScan 4000 apparatus (Foss Electric, Hillerød, Denmark). This instrument was regularly calibrated according to results of relevant reference methods (for fat content according to Roese-Gottlieb method, for crude protein content according to Kjeldahl method a for lactose monohydrate content according to polarimetric method) and took part in proficiency testing with good results. This was carried out in accordance with instrument operation manual. Milk fat was extracted with petroleum ether from freeze-dried milk samples. FA in isolated fat were reesterified to their methyl esters with a methanolic solution of potassium hydroxide. Methyl esters of FA were determined by a gas chromatographic method (GLC) using a Varian 3300 apparatus (Varian Techtron, USA) under conditions described in Tab. III.

The identification of FA was carried out using analytical standards (Supelco, USA). In total, 64 FA were observed, 50 of which were identified. The proportions of individual FA were calculated from the ratio of their peak area to the total area of all the observed FA.

Data were analysed by the program Statistica CZ 12 (Statsoft CR) using a general linear model with fixed effects of breed, parity, month of lactation and feeding ration:

$$Y_{ijkl} = \mu + B_i + P_j + MOL_k + F_l + \varepsilon_{ijkl}$$

where:

Y_{ijkl} = dependent variables: milk yield (kg/d); fat, protein and lactose content (g/100 g); proportion of individual milk FA (g/100g of total FA); and groups of FA (g/100 g of total FA),
μ = mean,
B_i = breed (i = Czech Fleckvieh, Holstein),
P_j = parity (j = 1, 2, ≥ 3),
MOL_k = months of lactation (k = < 30 days in milk (1); 31-60 (2); 61-90 (3); 91-120 (4); 121-150 (5); 151-180 (6); 181-210 (7); 211-240 (8); 241-270 (9); > 271 (10),
F_l = feeding ration (l = March, June, September and December),
ε_{ijkl} = residual error.

Tukey HSD test for unequal N was used for group comparisons (*post hoc* test).

Total explained variation (R^2, coefficient of determination) and variation explained by

III: *Parameters of chromatographic analysis of fatty acids.*

Parameter		Value
Column		CP-Select CB for FAME, 50 m × 0.25 mm, 0.25 µm thickness
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		1 µl, split 10

individual effects (factors variation) were calculated using sum of squares and were expressed as a percentage. R^2 was defined as [(1–(residual sum of squares/total sum of squares)).100]. Factor variation was defined as [(sum of squares of individual effects/total sum of squares).100].

RESULTS AND DISCUSSION

A general linear model, comprising breed, parity, month of lactation and feeding ration, was used to evaluate the variability of selected FA of bovine milk fat, groups of FA and their mutual relations. Using this model, the highest levels of R^2 were 65% and 52% for vaccenic acid and RA, respectively (Tab. IV). Within the observed groups, the value of R^2 for branched-chain FA was 54%. The high R^2 of selected FA were due, in particular, to high variability in feeding ration, with the exception of branched chain FA, where the value of R^2 was primarily affected by the variability in month of lactation. The major contribution of month of lactation to the R^2 in animal factors was previously reported by Kelsey *et al.* (2003).

Even though our work was carried out under common farm management conditions, we observed a significant effect of feeding ration on the R^2. A comparable effect was reported from exact feeding experiments (e.g. Leiber *et al.*, 2005; Wiking *et al.*, 2010).

The high explained variation in vaccenic acid and RA, as affected by the feeding ration, is probably due to feeding with fresh forage, lucerne and maize. However, previous studies found that, compared to grazing (Elgersma, 2015) or organic farming conditions (O'Donnell *et al.*, 2010), partial feeding of housed dairy cows with fresh forage had only a limited effect on the proportion of nutritionally desirable FA in milk fat (Leiber *et al.*, 2005). This could explain the low level of variation in oleic acid (6%) and the group of monounsaturated FA (5%). The higher level of explained variation in n-3 polyunsaturated FA (32%), compared to the n-6 polyunsaturated FA group (25%), was probably

IV: *Distribution of total explained variation (R2) for milk yield (kg/d), fat, protein and lactose content (g/100 g), fat corrected milk (FCM 4.0) and energy corrected milk (ECM) (kg/d), individual fatty acids (FA), and groups of FA (g/100 g of FA) in general linear model involving animal and feed factors.*

	Factors variation[1] Breed		Parity		Month of lactation		Feeding ration		R^2	
	%	P	%	P	%	P	%	P	%	P
Milk yield and composition										
Milk yield	5	***	2	†	37	***	1	ns	45	***
Fat content	1	ns	0#	ns	12	*	22	***	35	***
Protein content	3	*	1	ns	32	***	4	†	39	***
Lactose content	0#	ns	3	†	31	***	1	ns	35	***
FCM 4.0	4	**	2	ns	33	***	2	ns	41	***
ECM	4	**	1	ns	32	***	2	ns	39	***
Individual FA										
16:0	2	†	3	†	15	**	10	***	30	***
18:1 *cis*-9	0#	ns	3	†	21	***	6	*	30	***
18:1 *trans*-11	0#	ns	1	ns	9	***	55	***	65	***
18:2 n-6	0#	ns	0#	ns	10	†	15	***	25	***
18:3 n-3	0#	ns	1	ns	6	ns	27	***	34	***
18:2 *cis*-9, *trans*-11	2	*	3	*	7	*	40	***	52	***
Group of FA[2]										
C4-14	0#	ns	3	ns	13	**	12	***	28	***
MUFA	0#	ns	2	ns	21	***	5	*	30	***
BCFA	0#	ns	4	**	33	***	17	***	54	***
PUFA n-6	0#	ns	0#	ns	6	ns	25	***	32	***
PUFA n-3	0#	ns	0#	ns	6	ns	32	***	39	***

P = probability, ns = not significant, † = P < 0.10, * = P < 0.05, ** = P < 0.01, *** = P < 0.001, # =% of factors variation < 0.5;1 Factors variation = proportion (%) of individual factors variation accounted for by R2 (coefficient of determination);
2 C4-14 = saturated FA, even, MUFA = monounsaturated FA, cis isomers, BCFA = branched chain FA, PUFA n-6 = polyunsaturated FA n-6, PUFA n-3 = polyunsaturated FA n-3.

caused by feeding with fresh lucerne. According to Wiking *et al.* (2010) legumes have a greater effect on the n-3 group than the n-6 group.

It is also evident that different FA formation pathways have a considerable effect on factors variation. According to Bauman and Griinari (2003), FA with 4 to 14 carbons (C4-14) and about 50% of palmitic acid (16:0) are synthesised *de novo* in the mammary gland, whereas FA with ≥ 18 carbons are transported to the mammary gland from feed as unesterified FA or as preformed FA. Microbial processes in the rumen are major factors affecting odd- and branched-chain FA (Vlaeminck *et al.*, 2006).

In our study, the contribution of feeding ration to the R^2 in most of the preformed FA was greater than that of the three animal factors combined. For example, 18:2 n-6 15% (feeding ration) vs. 10% (animal factors), 18:3 n-3 27% vs. 7%, n-6 polyunsaturated FA 25% vs. 6%, and n-3 polyunsaturated FA 32% vs. 6%. This contrasts with FA groups formed *de novo*, where animal factors contributed more to the R^2 than feeding ration: for example, C4-14 group 16% (animal factors) vs.12% (feeding ration). This is consistent with previous works showing that coefficients of heritability are affected by the number of carbons in FA chains (Stoop *et al.*, 2008).

In agreement with previous work, we found interbreed variability of FA in milk fat. Significant differences ($P < 0.05$) were observed in palmitic acid (30.5 and 31.7% for Czech Fleckvieh and Holstein, respectively), or RA (0.42% and 0.38%) – Tab. V. The differences between the breeds could be partially caused by different fat corrected milk (FCM) 19.3 and 22.0 kg for Czech Fleckvieh and Holstein, respectively ($P < 0.05$). Performance probably also affects differences caused by parity. Lake *et al.* (2007) reported differences in FA proportion particularly between multiparous and primiparous cows. Primiparous cows have a nutritionally more desirable FA composition. This was observed also

V: *Milk yield, fat corrected milk (FCM 4.0) and energy corrected milk (ECM) (kg/d), fat, protein, and lactose content (g/100 g), individual fatty acids (FA), and groups of FA (g/100 g of FA) depending on breed, parity and feeding ration.*

Number of milk samples	Breed		Parity			Feeding ration				Total			
	CF	H	1	2	> 3	III	VI	IX	XII	Mean	Min.-Max.	SEM	RSD
	73	72	44	57	44	36	38	35	36				
Milk yield and composition													
Milk yield	18.7^{a}	22.2^{b}	18.8^{a}	19.9^{ab}	22.6^{b}	22.1^{b}	20.4^{ab}	18.0^{a}	21.0^{ab}	20.4	4.5-43.3	0.61	36
Fat content	4.22	4.02	4.11	4.20	4.03	3.58^{a}	4.30^{bc}	4.80^{c}	3.77^{ab}	4.12	2.17-6.60	0.07	21
Protein content	3.62^{b}	3.49^{a}	3.57	3.56	3.53	3.36^{a}	3.61^{b}	3.63^{b}	3.62^{b}	3.55	2.51-4.74	0.03	11
Lactose content	4.78	4.75	4.84	4.75	4.73	4.84	4.76	4.74	4.73	4.77	3.30-5.40	0.03	7
FCM 4.0	19.3^{a}	22.0^{b}	19.1^{a}	20.1^{ab}	22.8^{b}	20.5	21.3	19.9	20.8	20.6	3.7-42.0	0.58	33
ECM	21.2^{a}	24.1^{b}	21.0^{a}	22.0^{ab}	25.0^{b}	22.6	23.2	21.4	23.3	22.6	4.1-47.0	0.62	33
Individual FA													
16:0	30.5^{a}	31.7^{b}	30.1	31.4	31.6	31.3^{b}	32.1^{b}	32.0^{b}	29.0^{a}	31.0	23.2-39.0	0.30	11
18:1 *cis*-9	19.2	18.6	19.8	18.6	18.4	18.7^{ab}	17.7^{a}	20.1^{b}	19.2^{ab}	18.9	13.1-30.8	0.29	18
18:1 *trans*-11	1.55	1.49	1.57	1.54	1.45	1.20^{a}	1.71^{b}	1.94^{c}	1.23^{a}	1.52	0.89-2.97	0.04	28
18:2 n-6	1.69	1.66	1.68	1.67	1.68	1.58^{ab}	1.80^{bc}	1.51^{a}	1.81^{c}	1.68	0.67-3.20	0.03	20
18:3 n-3	0.44	0.43	0.45	0.45	0.40	0.46^{bc}	0.52^{c}	0.43^{b}	0.33^{a}	0.43	0.16-0.91	0.01	30
18:2 *cis*-9, *trans*-11	0.42^{b}	0.38^{a}	0.44^{b}	0.36^{a}	0.41^{ab}	0.40^{b}	0.41^{b}	0.26^{a}	0.51^{c}	0.40	0.10-0.80	0.01	35
Group of FA[1]													
C4-14	25.6	25.6	24.8^{a}	25.5^{ab}	26.5^{b}	25.9^{ab}	25.7^{ab}	23.5^{a}	27.0^{b}	25.6	14.8-34.7	0.31	14
MUFA	22.5	22.3	23.1	22.2	21.9	22.3^{ab}	21.2^{a}	23.6^{b}	22.6^{ab}	22.4	16.7-34.8	0.28	15
BCFA	2.49	2.44	2.53^{b}	2.54^{b}	2.32^{a}	2.47^{b}	2.60^{b}	2.58^{b}	2.22^{a}	2.47	1.45-3.31	0.03	13
PUFA n-6	1.98	1.91	1.94	1.94	1.95	1.90^{b}	2.12^{b}	1.60^{a}	2.13^{b}	1.94	0.69-3.91	0.04	22
PUFA n-3	0.56	0.53	0.56	0.55	0.52	0.59^{b}	0.66^{b}	0.45^{a}	0.46^{a}	0.54	0.16-1.10	0.01	30

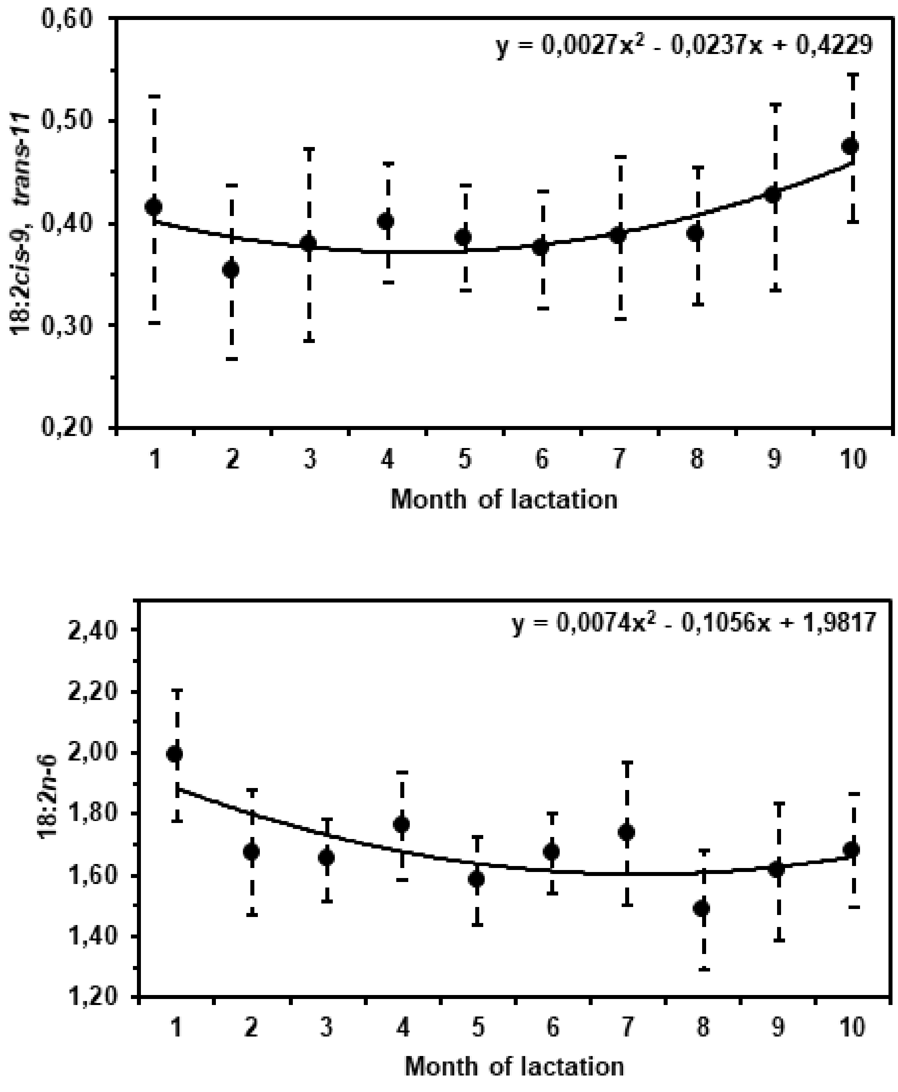
$^{a, b, c, d}$ = means in breed and parity groups with different superscripts within a row differ at $P < 0.05$, in feeding ration groups at $P < 0.01$, CF = Czech Fleckvieh, H = Holstein, III, VI, IX, XII = feeding rations in March, June, September, and December – see Tab. II, SEM = standard error of the mean, RSD = relative standard deviation (%) = [standard deviation/mean].100;

[1] C4-14 = saturated FA, even, MUFA = monounsaturated FA, *cis* isomers, BCFA = branched chain FA, PUFA n-6 = polyunsaturated FA n-6, PUFA n-3 = polyunsaturated FA n-3.

in our work. However, the differences were mostly statistically insignificant.

A more desirable FA composition was observed in cows at the beginning of lactation, i.e. between days 10 and 30. The proportions of palmitic acid and the C4-14 group were lower (27.7 % and 22.6 %, respectively) than during the late period of lactation; for instance, between days 211 and 240 (33.3 and 26.6 %, respectively). In contrast, the proportions of oleic acid (18:1 *cis*-9), linoleic acid (18:2 n-6), monounsaturated FA and n-6 polyunsaturated FA were higher between days 10 and 30 (23.4, 1.99, 26.5 and 2.27 %, respectively) than between days 211 and 240 (17.2, 1.48, 21.0 and 1.75 %, respectively) – data not given in the table. The proportion of linoleic acid is elevated during the early lactation, while that of RA is maximal at the end of lactation (Fig. 1).

As mentioned above, some differences in FA composition could be affected by breed, cow individuality, parity or month of lactation. Nevertheless, composition of feeding ration is the main factor determining the content of saturated and unsaturated FA (Kalac and Samkova, 2010). Within the common management of cow feeding, farmers strive to prepare balanced feeding rations (Slots *et al.*, 2009) and only adopt changes in feed components that give rise to changes in milk fat FA composition. In our work, the lowest proportion of palmitic acid (29.0 %) was associated with the highest proportion of rape seed in the feeding ration, oleic acid proportion increased during feeding of fresh maize (up to 20.1 %), and the highest proportion of alpha-linolenic acid (0.52 %) and n-3 polyunsaturated FA (0.66 %) was observed after feeding of fresh lucerne.



1: *Proportion of 18:2 n-6, and 18:2 cis-9, trans-11 (y-axis: g/100 g of fatty acids) depending on month of lactation (x-axis); vertical bars denote 0.95 confidence intervals.*

CONCLUSION

In conclusion, the fatty acid composition of milk fat in milked cows under common farm management conditions, particularly the presence of nutritionally desirable polyunsaturated fatty acids, is mainly affected by changes in feeding ration. The four tested factors – breed, parity, month of lactation and feeding ration – explained only a limited part of total variation. We therefore suppose that the unexplained variability is due to the individuality of cows (genetic predisposition) in their reaction to changes in feed composition. It therefore appears necessary to continue to study this topic.

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