

Comparison of different sorting of fatty acids in bovine milk in relation to body condition of Czech Fleckvieh dairy cows

Porovnání rozdílného třídění mastných kyselin v mléce ve vztahu k tělesné kondici dojníc českého strakatého plemene

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Abstract

Fatty acids in milk are one of the most important components of milk. The aim of this study was to determine relationships between groups of fatty acids and body condition score change in Czech Fleckvieh cows. Fatty acids were classified along its length of chain and its origin. A total of 50 Czech Fleckvieh cows with different parity were included to observation. During the first 4 weeks of lactation, milk samples were collected at a weekly interval and body condition score was assessed. Statistical analyses were performed using Microsoft Office Excel and the procedures MEANS and GLM of SAS 9.1. During the first four week of lactation, the proportions of short- and medium-chain fatty acids as well as *de novo* synthesised fatty acids increased. Moreover the cows with a greater body condition score change mobilized storage depot fat more intensively, which resulted in higher proportions of dietary and fatty acids originating in depot fat since week 1 of lactation, and long-chain fatty acids since week 2 of lactation. On the contrary, the animals with only a small body condition score change exhibited high proportions of short- and medium-fatty acids as well as *de novo* synthesised fatty acids in the most part of the period analysed. This indicates negative energy balance in early part of lactation and its compensation during observed period. So, the results confirm the relationships between different groups of milk fatty acids proportion, body condition score and negative energy balance. Both methods of FA sorting were confirmed as appropriate for evaluation of negative energy balance intensity. Further, results emphasize importance the monitoring of body condition, milk composition and good level of herd management in first part of lactation.

KEYWORDS: lactation, fatty acids, body condition score, dairy cows, Czech Fleckvieh, milk

Abstrakt

Mastné kyseliny v mléce jsou jedny z nejdůležitějších složek mléka. Cílem této práce bylo určit vztahy mezi skupinami mastných kyselin a změnou tělesné kondice u krav českého strakatého skotu. Mastné kyseliny byly tříděny podle délky uhlíkového řetězce a původu jejich vzniku. Do analýzy bylo zařazeno celkem 50 krav českého strakatého skotu s různým pořadím laktace. Během prvních 4 týdnů laktace byly odebírány vzorky mléka v týdenním intervalu a hodnocena tělesná kondice. Statistická analýza byla provedena za využití Microsoft Office Excel a procedur MEANS a GLM programu SAS 9.1. Během prvních čtyř týdnů laktace stoupal podíl mastných kyselin s krátkým, středně dlouhým řetězcem a syntetizovaných *de novo*.

Navíc krávy s výraznějším poklesem tělesné kondice mobilizovaly depotní tuk intenzivněji, což se projevilo vyšším zastoupením mastných kyselin pocházejících z výživy a depotního tuku, respektive s dlouhým řetězcem v 1. a 2. týdnu laktace. Oproti tomu, zvířata s malou změnou tělesné kondice měla po většinu sledovaného období vysoký podíl mastných kyselin s krátkým a středně dlouhým řetězcem, stejně jako mastných kyselin syntetizovaných *de novo*. Tato skutečnost svědčí o negativní energetické bilanci na začátku laktace a jejím vyrovnávání během sledovaného období. Výsledky potvrzují vztah mezi různými skupinami mastných kyselin, tělesnou kondicí a negativní energetickou bilancí. Pro hodnocení intenzity negativní energetické bilance se potvrdilo jako vhodné využití obou způsobů třídění mastných kyselin. Výsledky dále zdůrazňují důležitost monitorování tělesné kondice, složek mléka a dobrou úroveň managementu stáda v první části laktace.

Klíčová slova: laktace, mastné kyseliny, skóre tělesné kondice, dojnice, český strakatý skot

Detail Abstract

Začátek laktace je pro dojnice jedním z klíčových období celého mezidobí. Krávy v první fázi laktace nejsou schopny přijmout dostatečné množství energie prostřednictvím krmiva a upadají do negativní energetické bilance (NEB). Tento stav se projevuje změnou tělesné kondice (BCS), ale i složek mléka. Jednou z klíčových složek mléka je mléčný tuk skládající se z mastných kyselin (MK). MK v mléce ovšem řada autorů rozděluje podle různých hledisek. Do našeho příspěvku bylo použito třídění podle délky řetězce a podle původu vzniku mastných kyselin v mléce. Cílem našeho příspěvku bylo porovnat zastoupení MK při jejich rozdílném třídění ve vztahu ke změnám BCS, jako hlavnímu indikátoru NEB u dojeného skotu. Do hodnocení bylo zařazeno celkem 50 dojnic českého strakatého skotu, kdy 10 dojnic bylo v první, 16 dojnic ve druhé a 24 dojnic ve třetí a další laktaci. Hodnocení probíhalo po dobu prvních čtyř týdnů laktace. Obsah mastných kyselin byl stanoven na plynovém chromatografu za použití metody podle Rösse-Gottlieba (vážková metoda), která je upravena EN ISO 1211 (ČSN 57 0534). Analýzou byl stanoven obsah jednotlivých MK (34) v mléce ($\text{mg} \cdot 100\text{g}^{-1}$ a jejich procentické zastoupení) a poté byl hodnocen procentický podíl jednotlivých skupin MK podle dvojího způsobu třídění. MK podle délky řetězce byly rozděleny do skupin s krátkým, středně dlouhým a dlouhým řetězcem a dle způsobu syntézy do mléka byly rozděleny na *de novo* (denovo), mikrobiálního původu (mikrob) z bacheru a původu z krmiva, resp. depotního tuku (dietlip). Statistická analýza byla provedena v programu Microsoft Office Excel a statistickém programu SAS 9.1., s využitím procedur MEANS a GLM. Průměrný obsah mléčného tuku se ve sledovaném období pohyboval od 5,63 % v první týdnu laktace do 4,42 % ve čtvrtém týdnu laktace. Ve sledovaném období docházelo k nárůstu MK s krátkým a středně dlouhým řetězcem na úkor MK s dlouhým řetězcem. Podobně také docházelo k nárůstu MK *de novo* syntetizovaných na úkor MK pocházejících z krmiva a depotního tuku. Dále také krávy s větší změnou BCS během prvních čtyř týdnů laktace mobilizovaly více zásobního tuku, což se v mléce projevilo ve vyšším obsahu MK pocházejících z krmiva a depotního tuku v prvním týdnu laktace a vyšším obsahem MK s dlouhým řetězcem ve druhém týdnu laktace. Oproti tomu zvířata s malou změnou BCS měla v mléce vyšší obsah MK s krátkým, středně dlouhým řetězcem a *de novo* syntetizovaných ve většině hodnocených týdnů laktace. Pro hodnocení intenzity negativní energetické bilance se potvrdilo jako vhodné využití obou způsobů třídění mastných kyselin.

Introduction

Milk fat is mostly (97 to 98%) composed of glycerol and fatty acid esters and is considered as one of the most important milk components (Jensen, 2002). Milk fatty acids (FA) are classified into different types according to various aspects, for instance according to the carbon chain length. Milk fat is typically composed (53 – 72%) of FA with even carbon numbers $C_{4:0}$ – $C_{20:0}$ (Samková, et al., 2008). The milk fat from ruminants is unique with its high proportion of short- and medium-chain FA (4, 6, 8, and 10 C) (McGuire and Bauman, 2003; Samková, et al., 2008). However, the classification according to the carbon chain length used in the literature may differ substantially. According to Topping and Clifton (2001), only $C_{2:0}$ and $C_{4:0}$ belong to short chain FA, whereas also $C_{6:0}$ (Månsson, 2008), $C_{8:0}$ to $C_{11:0}$ (Hanuš et al., 2010) and $C_{12:0}$ and $C_{13:0}$ (Pešek, et al., 2006) were included in this group. Medium-chain FA were comprised of $C_{8:0}$ to $C_{10:0}$ (Takeuchi, et al., 2008), $C_{12:0}$ to $C_{16:0}$ (Hanuš, et al., 2010), or $C_{14:0}$ to $C_{17:1}$ (Pešek, et al., 2006). Long-chain FA were then represented by more than 12, 16 and 18 carbons in the chain as reported by Takeuchi, et al. (2008), Hanuš, et al. (2010), and Pešek, et al. (2006), respectively. The concentrations of milk short-chain FA were reported 6.8% (Månsson, 2008), 9.16% (Hanuš, et al., 2010), or 12.25% (Pešek, et al., 2006), whereas medium-chain FA comprised 1 to 3% (Takeuchi, et al., 2008), 53.36% (Hanuš, et al., 2010), or 46.49% (Pešek, et al., 2006) of the total FA. According to these authors, the remaining FA were then the long-chain FA.

There are three main metabolic sources of milk FA. The first source is volatile FA produced in the rumen from the feed, which are used for the *de novo* synthesis of FA as a product of microbial fermentation of carbohydrates and proteins in the rumen (Dijkstra, et al., 1993). These FA are re-absorbed directly from the rumen (Reece, 1998). The second source is free FA from the feed which pass the rumen intact or which are mobilized from adipose depots containing primarily longer-chain FA, especially oleic acid ($C_{18:1}$), palmitic acid ($C_{16:0}$) and (in ruminants) stearic acid ($C_{18:0}$) (Zeman, et al., 2006). The third source of milk FA is the products of lipolysis and biohydrogenation of dietary fats (synthesis of microbial lipids). Approximately half of milk FA from ruminants ($C_{4:0}$ to $C_{14:0}$ and half of $C_{16:0}$) is synthesised *de novo* in the mammary gland from short-chain FA with two-carbon units (acetyl CoA) (Kaylegian and Lindsay, 1995). The second half of FA (half of $C_{16:0}$ and $C_{18:0}$ and longer chain FA) is transported to the mammary gland by blood, especially by its highly labile β -lipoprotein fraction, in the form of non-esterified FA (NEFA) derived directly from the diet (Harvatine, et al., 2009) or released from the adipose tissue (Bauman, et al., 2006; Samková, et al., 2008). The proportions of individual FA and FA groups vary during lactation (Toušová et al., 2013) due to the variable synthesis of milk fat and the different absorption of FA from blood (Kaylegian and Lindsay, 1995; Jelínek, et al., 2003). The greatest changes in the content and composition of milk FA occur in the early lactation when cows may suffer from negative energy balance (NEB) (Ducháček, et al., 2012a, 2012b). As a result, the adipose tissue is degraded and the body condition score (BCS) is reduced (Roche, et al., 2009). No individual studies (Hanuš, et al., 2010; Takeuchi, et al., 2008; Pešek, et al., 2006) evaluated NEB intensity in relation to content of FA in milk, however many of them reported different proportions of milk FA groups caused by different sorting based on the carbon chain length. This fact evokes confusions using FA groups proportion as NEB indicator. Unification of sorting FA by carbon chain length in relation to NEB is complicated due to significant cows' individual differences in metabolism intensity. Most of published papers evaluated NEB according to FA division to saturated and unsaturated FA

proportions only (Gross, et al., 2011). The second point of view is sorting FA based on the biological background of depot fat degradation and subsequent biochemical processes within metabolism. Therefore, we can hypothesise that the proportions of FA groups according to their origin and type of synthesis (Kaylegian and Lindsay, 1995; Samková, et al., 2008) may be used as another applicable NEB indicator. Therefore, the objective of this study was to compare both types of milk FA classification and to evaluate the relationship between different FA groups and the changes in BCS in terms of the indication NEB intensity.

Material and methods

A total of 50 Czech Fleckvieh cows were included in the analysis – 10, 16, 15, and 9 in the first, second, third, and fourth and later lactation, respectively. The average daily milk yield of the whole data set ranged from 25.7 to 28.61 l of milk with the standard deviation ranging from 6.26 to 6.64. The cows were loose housed in a cubicle straw-bedded barn and fed a total mixed ratio (TMR). The ingredient composition of the diet was uniform for all cows observed during the first four weeks of lactation. BCS was evaluated weekly (totally 5 times; BCS0, BCS1, BCS2, BCS3, BCS4) on a 5-point scale with 0.25 point increments (Parker, 1989). Aliquot milk samples from each cow were collected during the afternoon milking every week in accordance with the official methodology of the milk performance recording system. The analysis of milk samples comprised the determination of % content of fat using Milkoscan 133B (N. Foss Electric; Denmark) and FA as well as FA group proportions. When determining milk FA composition, at first the milk fat was extracted using the standard Röss-Gottlieb method (gravimetric) in accordance with EN ISO 1211 (ČSN 57 0534, 2010). The extract was obtained using a water-based-solution of ammonia, ethanol, diethylether and petrolether. FA methyl esters were prepared by the potassium hydroxide catalysed methylation and extracted into heptane. Gas chromatography of FA methyl esters was performed using the Master GC (DANI Instruments S.p.A.; Italy) (split regime, FID detector) on a column with polyethylene glycol stationary phase (FameWax – 30 mm x 0.32 mm x 0.25 µm). Helium was used as the carrier gas at a flow rate of 5 ml/min. The temperature programme used for GC was as follows: 50 °C (2 min), after which the temperature was increased to 230 °C at 10 °C/min (8 min), the temperature of the detector being 220 °C. Gravimetric contents (mg/100 g of milk) and proportions of the total FA were determined in 34 individual FA. Two methods were used to classify FA into FA groups. First, FA were classified according to the carbon chain length into short- ($C_{4:0}$ – $C_{10:0}$), medium- ($C_{11:0}$ – $C_{14:0}$), and long- ($C_{15:0}$ – $C_{24:1}$), chain FA (Takeuchi, et al., 2008). Second, FA were classified according to their origin into *de novo* synthesised (de novo - $C_{4:0}$ - $C_{14:0}$ and half $C_{16:0}$), synthesised by rumen microorganisms (mikrob - $C_{17:0}$ - $C_{17:1}$), and originating from the diet or released from the adipose tissue (dietlip - half $C_{16:0}$ and $C_{18:0}$ and with more C) in accordance with Kaylegian and Lindsay (1995) and Harvatine, et al. (2009). A total of 200 milk samples were analysed. The data were evaluated with Microsoft Office Excel and the statistical software SAS 9.1. (SAS/STAT® 9.1., 2004) using the MEANS and GLM procedures. The cows used in the experiment were assigned to groups A, B, and C according to their BCS change between calving and week 4 of lactation (BCS_{4-0}) (Table 1).

Table 1. Average BCS in different animal groups according to the week of lactation
 Tabulka 1. Průměrná BCS u skupin zvířata podle týdnů laktace

Group	N	BCS 0	BCS 1	BCS 2	BCS 3	BCS 4	BCS ₄₋₀
A (≥ -1 bod)	20	4.37	3.77	3.69	3.5	3.15	-1.21
B (-0.25 až -0.75)	15	4.15	3.7	3.63	3.56	3.49	-0.67
C (≤ -0.25)	15	3.94	3.92	3.83	3.7	3.91	-0.03

BCS 0 – body condition score at calving;

BCS 1-4 – body condition score at weeks 1, 2, 3, and 4 of lactation;

BCS₄₋₀ – difference between BCS 0 and BCS 4.

The model equation used for the evaluation of the relationship between BCS and FA groups was as follows:

$$Y_{ijk} = \mu + BCS_{4-0i} + porlak_j + e_{ijk} \quad \text{where:}$$

Y_{ijk} = dependent variable (proportion of FA group – short, medium, long, de novo, mikrob, dietlip),

μ = mean value,

BCS_{4-0i} – fixed effect of the ith group in accordance to the BCS change between calving and week 4 of lactation (i=A, BCS₄₋₀ ≥ -1, n=20; B, BCS₄₋₀ = -0.25 to -0.75, n=15; C, BCS₄₋₀ ≤ -0.25, n=15),

porlak_j = fixed effect of lactation (first, n = 10; second, n = 16; third and other, n = 24),
 e_{ijk} – random residual error.

The significance of differences was assessed in detail using the method of GLM and the probability level $P < 0.05$ was considered statistically significant.

Results and discussion

The average content of milk fat was from 5.63% in the first week, 5.14% in the second, 4.80% in the third, and 4.42% in the fourth week of lactation. In accordance with McGuire and Baumann (2003), the milk from Czech Fleckvieh cows was found to contain relatively high proportions of short- and medium-chain FA compared to Holstein dairy cows. The average proportion of short-chain FA ranged from 10.39 to 11.62% (Table 2).

Table 2. Basic statistics for milk FA groups
 Tabulka 2. Základní statistiky pro skupiny MK v mléce

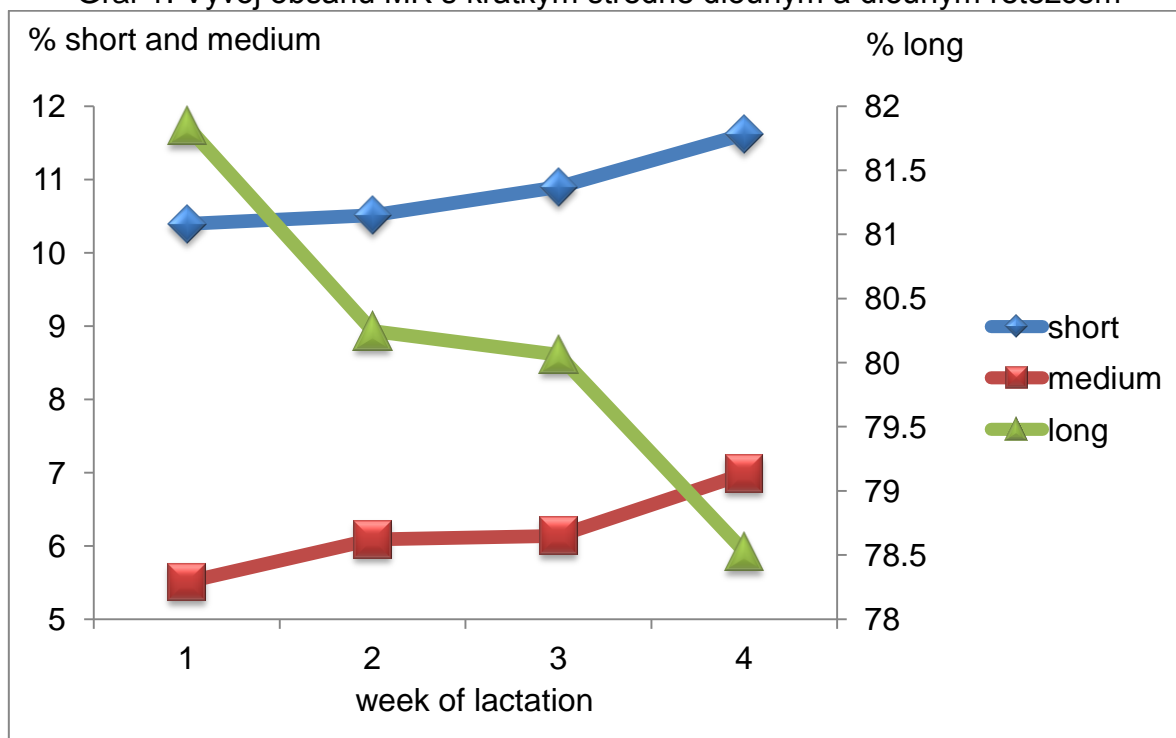
Sorting	FA group	Lactation week	N	\bar{x}	s_d	min.	max.	e.	V (%)
carbon chain	short	1	50	10.4	3.7	4.5	19.7	0.5	35.2
	medium		50	5.5	2.1	2.3	12.6	0.3	39.1
	long		50	81.9	5.1	67.4	90.9	0.7	6.2
	short	2	50	10.5	4.6	2.0	28.0	0.7	43.9
	medium		50	6.0	2.5	1.5	14.2	0.4	42.0
	long		50	80.3	7.7	55.9	93.5	1.1	9.6
	short	3	50	10.9	3.3	3.8	19.5	0.5	30.5
	medium		50	6.1	2.2	1.2	10.7	0.3	35.9
	long		50	80.0	6.3	60.3	93.1	0.9	7.9
	short	4	50	11.6	4.3	4.2	26.5	0.6	36.9
	medium		50	6.9	2.0	2.8	12.1	0.3	29.6
	long		50	78.5	6.5	60.1	89.4	0.9	8.2
origin	de-novo	1	50	42.1	6.3	28.4	61.2	0.9	15.1
	mikrob		50	1.8	0.4	1.3	3.0	0.1	23.4
	dietlip		50	53.8	6.3	35.4	67.7	0.9	11.7
	de-novo	2	50	43.5	7.7	25.1	64.6	1.1	17.8
	mikrob		50	1.8	0.3	1.1	2.32	0.0	15.1
	dietlip		50	51.6	8.3	30.8	71.0	1.2	16.2
	de-novo	3	50	43.7	6.6	26.7	58.1	0.9	15.0
	mikrob		50	1.7	0.3	1.2	2.6	0.0	17.3
	dietlip		50	51.7	7.7	30.1	69.1	1.1	15.0
	de-novo	4	50	46.9	6.4	31.7	63.4	0.9	13.6
	mikrob		50	1.8	0.4	1.0	2.7	0.1	20.3
	dietlip		50	48.5	7.1	29.0	63.0	1.0	14.6

short – short chain fatty acids ($C_{4:0} - C_{10:0}$); medium – medium chain fatty acids ($C_{11:0} - C_{14:0}$); long – long chain fatty acids ($C_{15:0} - C_{24:1}$); *de novo* – fatty acids synthesised de novo ($C_{4:0} - C_{14:0}$ and half $C_{16:0}$); mikrob – fatty acids synthesised by rumen microorganisms ($C_{17:0} - C_{17:1}$); dietlip – fatty acids originating from the diet or released from the adipose tissue (half $C_{16:0}$ and $C_{18:0}$ and with more C); \bar{x} - means; s_d – standard deviation; min. – minimal value; max. – maximal value; e – error of the means; V (%) – coefficient of variance.

Somewhat different proportions of milk short-chain FA ranging from 6.1 to 8% were reported for Holstein cows (Månsson, 2008). Similarly, whereas the average proportion of medium-chain FA ranged from 5.51 to 6.98% in our study, it was 3.6 to 5.4% in the study by Månsson (2008). The proportion of long-chain FA ranged from 78.53 to 81.85%. The observed results indicate the existence of differences between breeds or specific genotypes. This finding agrees with those of Back and Thomson (2005). However, mentioned differences can be evoked mainly by different type and/or composition of diet as well. Quite dissimilar results in terms of FA group proportions were reported by Pešek, et al. (2006) and Hanuš, et al. (2010). However, a different classification of FA according to the carbon chain length was used in these studies. This demonstrates the ambiguity in the FA classification systems used resulting in the inaccuracy of the evaluation, which further supports our original hypothesis.

During the lactation period observed, the proportions of short- and medium-chain FA increased and long-chain FA decreased (Fig. 1).

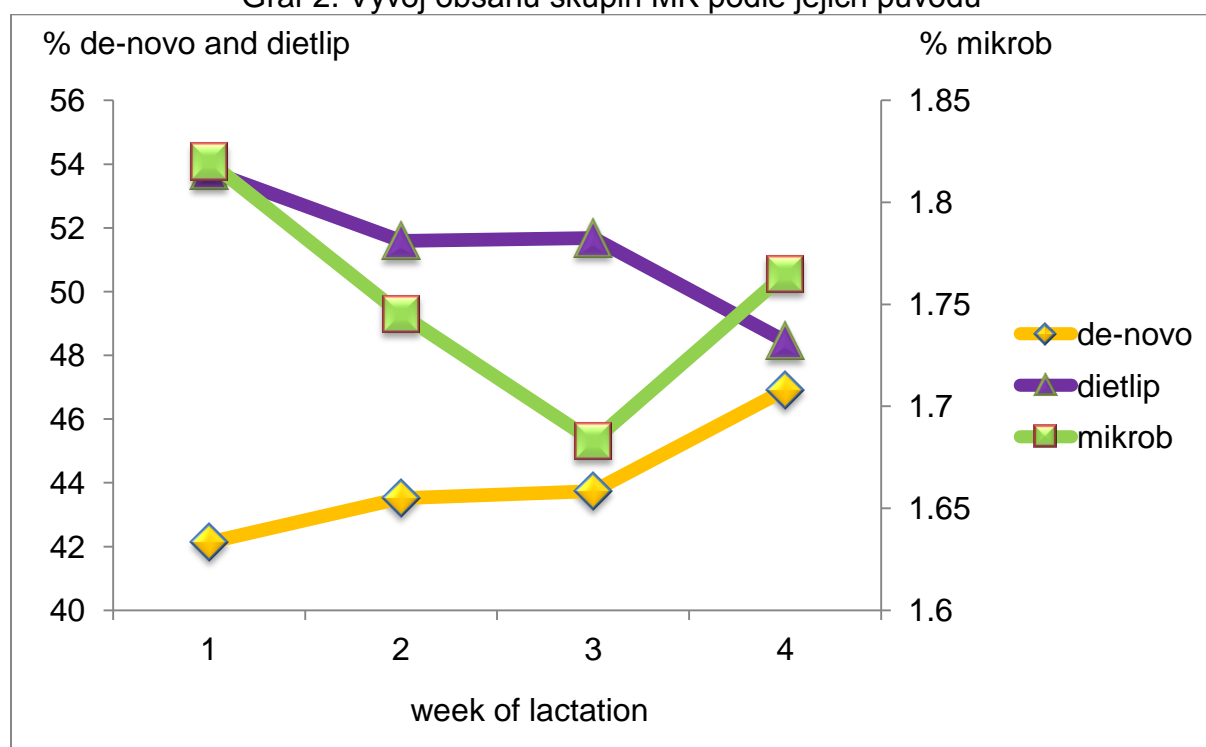
Figure 1. Changes in short-, medium- and long-chain FA
 Graf 1. Vývoj obsahu MK s krátkým středně dlouhým a dlouhým řetězcem



short – short chain fatty acids ($C_{4:0} - C_{10:0}$); medium – medium chain fatty acids ($C_{11:0} - C_{14:0}$); long – long chain fatty acids ($C_{15:0} - C_{24:1}$).

As long-chain FA are mostly released from the diet compared to depot fat degradation whereas short- and medium-chain FA are mostly synthesised *de novo*, these changes may be explained by the recovery of animals from NEB during this period. Decline in long-chain FA document decrease of body fat degradation, it means lower proportion represents mainly long-chain FA originated from the diet. The average proportion of *de novo* synthesised, microbial, and dietary and depot FA ranged from 42.13 to 46.91, 1.68 to 1.82, and 50.21 to 55.61%, respectively. The changes in *de novo* synthesised, synthesised by rumen microorganisms and dietary plus depot FA (Fig. 2) are in agreement with the results of Kaylegian and Lindsay (1995).

Figure 2. Changes in FA groups according to their origin
 Graf 2. Vývoj obsahu skupin MK podle jejich původu



de novo – fatty acids synthesised *de novo* (C_{4:0} - C_{14:0} and half C_{16:0}); mikrob – fatty acids synthesised by rumen microorganisms (C_{17:0} - C_{17:1}); dietlip – fatty acids originating from the diet or released from the adipose tissue (half C_{16:0} and C_{18:0} and with more C).

During the first 4 weeks of lactation, the proportion of *de novo* synthesised FA increased, whereas the proportion of dietary plus depot FA were reduced. In addition, the relationship between BCS changes and FA group proportions was analysed. In the first 3 weeks of lactation, the proportions of short-chain and *de novo* synthesised FA were higher in the animals with the smallest change of BCS. This tendency corresponds with the biological principles of the FA synthesis and metabolic pathways during the period of NEB. Significant differences ($P < 0.05$) were found between the animals with the greatest and smallest BCS change in the week 1 and 3 of the lactation. For *de novo* synthesised FA, this applied also for week 4 (Table 3). The greatest BCS change was associated with the reduced proportion of short-chain as well as *de novo* synthesised FA. Compared to the animals with the smallest BCS change, the differences ranged from 0.48 to 2.73% depending on the carbon number in short-chain FA, and from 3.13 to 6.77% in *de novo* synthesised FA. Similarly, the proportions of medium-chain and *de novo* synthesised FA were also higher ($P < 0.05$) in the animals with the smallest change of BCS. Furthermore, since week 2 of the lactation, long-chain FA were higher ($P < 0.05$) in the greatest BCS change animal group (81.67 to 83.44%). The milk from these animals with the greatest BCS change also contained more dietary plus depot FA compared to the group with the smallest BCS change. Significant differences were detected in weeks 1, 3 and 4 ($P < 0.05$) and ranged from 4.15 to 7.98%. Generally, the differences in *de novo* synthesised and dietary plus depot FA tended to increase with raising changes of BCS. No significant differences were observed in microbial FA.

Conclusion and recommendations

During the first four weeks of lactation analysed, the proportions of short- and medium-chain FA as well as *de novo* synthesised FA increased. In contrast, the proportions of long-chain FA, dietary and depot FA decreased, which may be explained by the occurrence of NEB and the resulting degradation of adipose tissue. Although the influence of the BCS change after calving on FA group proportions was rather inconsistent, certain relations to the level of NEB could be detected. The cows with a greater BCS change mobilized storage depot fat more intensively, which resulted in higher proportions of dietary and depot FA since week 1 of lactation, and long-chain FA since week 2 of lactation. On the contrary, the animals with only a small BCS change exhibited high proportions of short- and medium-FA as well as *de novo* synthesised FA in the most part of the period analysed. It is concluded that the classification of milk FA based on their source is another applicable indicator for the NEB detection and that provides very similar findings compared to sorting based on the length of their carbon chain.

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Table 3. The effect of BCS change between calving and week 4 of lactation on milk FA group proportions during the first 4 weeks of lactation

Tabulka 3. Vliv změny BCS mezi otelením a 4 týdnem laktace na obsah skupin MK v prvních 4 týdnech laktace

Sorting	FA group	BCS ₄₋₀	N	Lactation week			
				I.	II.	III.	IV.
carbon chain	short	-1.21	20	10.3 ± 0.8	10.5 ± 10.4	9.6 ± 0.7 ^A	10.5 ± 1.0
		-0.67	15	8.7 ± 0.9 ^A	10.0 ± 1.2	12.1 ± 0.8 ^B	13.3 ± 1.1
		-0.03	15	12.5 ± 1.3 ^B	12.4 ± 1.2	12.3 ± 0.8 ^B	11.0 ± 1.1
	medium	-1.21	20	5.3 ± 0.5 ^A	6.1 ± 0.6	5.1 ± 0.5 ^A	6.2 ± 0.5 ^A
		-0.67	15	4.8 ± 0.5 ^A	5.4 ± 0.6 ^A	6.7 ± 0.5 ^B	7.0 ± 0.5
		-0.03	15	6.8 ± 0.5 ^B	7.3 ± 0.7 ^B	7.2 ± 0.5 ^B	7.5 ± 0.5 ^B
	long	-1.21	20	81.9 ± 1.1	81.7 ± 1.7	83.4 ± 1.3 ^A	81.67 ± 1.4 ^A
		-0.67	15	84.0 ± 1.3 ^A	81.1 ± 2.0	77.9 ± 1.5 ^B	76.4 ± 1.6 ^B
		-0.03	15	79.0 ± 1.3 ^B	76.9 ± 20.0	77.4 ± 1.6 ^B	78.3 ± 1.6
origin	de-novo	-1.21	20	41.2 ± 1.4 ^A	43.9 ± 1.7	40.7 ± 1.4 ^A	44.5 ± 1.5 ^A
		-0.67	15	39.7 ± 1.6 ^B	41.8 ± 2.0	45.2 ± 1.6 ^B	48.0 ± 1.7
		-0.03	15	46.3 ± 1.6 ^A	47.0 ± 2.0	47.5 ± 1.6 ^B	48.8 ± 1.7 ^B
	mikrob	-1.21	20	1.9 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	1.8 ± 0.1
		-0.67	15	2.0 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.8 ± 0.1
		-0.03	15	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.9 ± 0.1
	dietlip	-1.21	20	54.5 ± 1.4 ^A	52.6 ± 1.9	55.7 ± 1.6 ^A	52.1 ± 1.5 ^A
		-0.67	15	55.9 ± 1.6 ^B	53.0 ± 2.2	49.8 ± 1.9 ^B	47.0 ± 1.8 ^B
		-0.03	15	50.3 ± 1.6 ^A	48.0 ± 2.2	47.8 ± 1.9 ^B	46.3 ± 1.8 ^B

short – short chain fatty acids (C_{4:0} – C_{10:0}); medium – medium chain fatty acids (C_{11:0} – C_{14:0}); long – long chain fatty acids (C_{15:0} – C_{24:1}); *de novo* – fatty acids synthesised *de novo* (C_{4:0} - C_{14:0} and half C_{16:0}); mikrob – fatty acids synthesised by rumen microorganisms (C_{17:0} - C_{17:1}); dietlip – fatty acids originating from the diet or released from the adipose tissue (half C_{16:0} and C_{18:0} and with more C); BCS₄₋₀ – body condition score change between calving and week 4 of lactation; A,B – different superscript letters mean significant differences (P<0.05) within the column.

REFERENCES

- Back, P. J., Thomson, N. A., (2005). Exploiting cow genotype to increased milk value through production of minor milk components. Proceedings of the New Zealand Society of Animal Production. New Zealand, January 2005, 65, 53-58.
- Bauman, D. E., Mather, I. H., Wall, R. J., Lock, A. L., (2006) Major advances associated with the biosynthesis of milk. *Journal of Dairy Science*, 89(4), 1235–1243.
- ČSN EN ISO 1211 (57 0534), (2010) Mléko – Stanovení obsahu tuku - Vážková metoda, (Referenční metoda). CEN. Evropský výbor pro normalizaci. Brusel, 24.
- Dijkstra, B. J., Boer, H., Van Bruchem, J., Bruining, M., Tamminga, S., (1993) Absorption of volatile acids from the rumen of lactating dairy cows as influenced by volatile fatty acid concentration, pH and rumen liquid volume. *The British Journal of Nutrition*, 69(2), 385-396.
- Ducháček, J., Vacek, M., Stádník, L., Beran, J., Okrouhlá, M., (2012a) Changes in milk fatty acid composition in relation to indicators of energy balance in Holstein cows. *Acta Universitatis Agriculturae et Silviculturae Mendeleianae Brunensis*, 60(1), 29-38.
- Ducháček, J., Stádník, L., Beran, J., Okrouhlá, M., (2012b) The relationship between fatty acid and citric acid contents in milk from Holstein cows during the period of negative energy balance. *Journal of Central European Agriculture*, 13(4), 615-630.
- Gross, J., van Dorland, H. A., Bruckmaier, R. M., Schwarz, F. J., (2011) Milk fatty acid profile related to energy balance in dairy cows. *The Journal of Dairy Research*, 78 (4), 479-488.
- Hanuš, O., Samková, E., Špička, J., Sojková, K., Hanušová, K., Kopec, T., Vyletělová, M., Jedelská, R., (2010) Vztah koncentrace zdravotně významných skupin mastných kyselin ke složení a technologickým vlastnostem kravského mléka. *Acta Universitatis Agriculturae et Silviculturae Mendeliana Brunensis*, 58(5), 137–154.
- Harvatine, K. J., Boisclair, Y. R., Bauman, D. E., (2009) Recent advances in the regulation of milk fat synthesis. *Animal*, 3(1), 40–54.
- Jelínek, P., Koudela, K., Doskočil, J., Illek J., Kotrbáček, V., Kovářů, F., Kroupová, V., Kučera, M., Kudláč, E., Trávníček, J., Valent, M., (2003) Fyziologie hospodářských zvířat. 1 rd ed. Mendelova zemědělská a lesnická univerzita, Brno.
- Jensen, R. G., (2002) The composition of bovine milk lipids: January 1995 to December 2000. *Journal of Dairy Science*, 85(2), 295–350.
- Kaylegian, K. E., Lindsay, R. C., (1995) Handbook of Milkfat Fractionation Technology and Applications. Champaign, Illinois.
- Månsson, H. L., (2008) Fatty acids in bovine milk fat. *Food & Nutrition Research*, 52. 1-3.
- McGuire, M. A., Bauman, D. E., (2003) Milk biosynthesis and secretion: milk fat. In: H., Roginsky, J. W., Fuquay, P. F., Fox, 2 nd ed. (2011) Encyclopedia of dairy sciences. Elsevier Science Ltd. New York, Academic Press, p.p. 1828-1834.
- Parker, R., (1989) Using Body Condition Scoring in Dairy Herd Management, Ministry of Agriculture, Food & Rural Affairs, Ontario, [Online] Available

at:<http://www.omafra.gov.on.ca/english/livestock/dairy/facts/94-053.htm#top>
[Accessed 20 November 2012].

- Pešek, M., Samková, E., Špička, J., (2006) Fatty acids and composition of their important groups in milk fat of Czech Pied cattle. *Czech Journal of Animal Science*, 51(5), 181–188.
- Reece, O. W., (1998) *Fyziologie domácích zvířat*. 1 st ed. Grada Publishing spol s.r.o., Praha.
- Roche, J. R., Friggens, N. C., Kay, J. K., Fisher, M. W., Stafford, K. J., Berry, D. P., (2009) Body condition score and its association with dairy cow productivity, health, and welfare. *Journal of Dairy Science*, 92(12), 5769–5801.
- SAS, (2009) *SAS/STAT® 9.1. User's Guide*. Cary, NC: SAS Institute Inc. 5121 pp..
- Samková, E., Pešek, M., Špička, J., (2008) *Mastné kyseliny mléčného tuku skotu a faktory ovlivňující jejich zastoupení*. 1 st ed. Jihočeská univerzita v Českých Budějovicích, Zemědělská fakulta.
- Takeuchi, H., Sekine, S., Kojima, K., Aoyama, T., (2008) The Application of medium-chain fatty acids: edible oil with a suppressing effect on body fat accumulation. *Asia Pacific Journal of Clinical Nutrition*, 17(S1), 320–323.
- Topping, D. L., Clifton, P. M., (2001) Short-chain fatty acids and human colonic function: Roles of resistant starch and nonstarch polysaccharides. *Physiological Reviews*, 81(3), 1031-1064.
- Toušová, R., Stádník, L., Ducháček, J., (2013) Effect of season and the time of milking on spontaneous and induced lipolysis in bovine milk fat. *Czech Journal of Food Science*, 31(1), 20-26.
- Zeman, L., Doležal, P., Kopřiva, A., Mrkvicová, E., Procházková, J., Ryant, P., Skládanka, J., Straková, E., Suchý, P., Veselý, P., Zelenka, J., (2006) *Výživa a krmení hospodářských zvířat*. 1 st ed. Profi Press, Praha.