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CHANGES IN MILK FATTY ACID COMPOSITION IN RELATION TO INDICATORS OF ENERGY BALANCE IN HOLSTEIN COWS

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Abstract

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The contents of protein (P), fat (F), a F:P ratio, and content of individual fatty acids (FA) and groups of FA, including ratio of SFA:UFA (saturated FA-to-unsaturated FA) and MUFA:PUFA (ratio monounsaturated FA-to-polyunsaturated FA) in milk during the first 17 weeks of lactation with respect to the development of NEB demonstrated by the change in cows' body condition. The differences in fat content were more pronounced than those in protein content. The resulting changes of the fat-to-protein ratio over time are associated with adipose tissue mobilization due to NEB, which was also confirmed by the changes in the BCS during the experimental period. The results indicate the possibility of using both fat content and fat-to-protein ratio as indicators of NEB in early lactation cows. The highest concentrations of saturated FA (SFA) were observed for $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, and also for the SFA with low carbon numbers. The highest concentrations of unsaturated FA (UFA) were determined for $C_{18:1}$, $C_{18:2}$, and $C_{16:1}$. The significant difference in SFA:UFA between cows with decreasing of the BCS and cows without big change in the BCS after calving were not found. The changes in MUFA:PUFA in milk during the first 5 weeks of lactation differed between the cows with low and high decrease of BCS one month after calving. In cows with a greater NEB effect was observed ratio of PUFA:MUFA in milk with a value above 6.5. The results suggest the possibility of using MUFA:PUFA ratios as suitable indicators of NEB in cows after calving.

NEB, body condition, fat-to-protein ratio, fatty acids in milk, PUFA:MUFA ratio

In early lactation, most dairy cows suffer from negative energy balance (NEB), when the energy intake is lower than energy requirements for milk production and body maintenance. The level of NEB is closely associated with the parameters of milk production, fertility and health of dairy cows (Stádník *et al.*, 2007). The energy status of cows can be evaluated on the basis of the balance of energy intake and output (Rukkwamsuk *et al.*, 1999) or in accordance to the development of the body condition score (BCS) (Vacek and Stádník, 2007). The onset of NEB is mainly associated with a decreased BCS (Stádník *et al.*, 2002), changes in the contents of milk solids (Ducháček *et al.*, 2010), and according to several authors also with modified milk fatty acid composition (Hanuš *et al.*, 2010). Cows with a more extensive loss of BCS produced more milk with a higher fat-to-protein ratio (Berry *et al.*, 2007). Increased lipomobilization in cows leads to the elevation of the fat-to-protein ratio from 1.20 to 1.35 (Bergk and Swalve, 2011). Milk fat composition is also changed during lactation (Samková *et al.*, 2008). Milk fat synthesized in the mammary gland contains as many as 400 different FA, but only approximately 70 can be identified by common analytical methods (Collomb *et al.*, 2002; Samková *et al.*, 2008). Many of them are present in low concentrations, and therefore only 20 to 30 of the most important milk FA are determined in most studies (Pešek *et al.*, 2005). Milk fat is mainly composed (53–72 %) of FA

with even carbon numbers $C_{4:0}$-$C_{20:0}$ (Samková *et al*., 2008). FA $C_{4:0}$-$C_{10:0}$ comprise approximately 10%, whereas $C_{12:0}$ and C14:0 represent 10 to 20% of the total FA (Gibson, 1991; Kaylegian and Lindsay, 1995). According to Samková *et al*. (2008), these short-chain saturated FA (SFA) are typical for bovine milk. Monounsaturated FA (MUFA) $C_{14:1}$-$C_{18:1}$ comprise 26 to 42% of the total FA, with the most abundant MUFA – oleic acid – representing 20 to 30% of total FA. The milk polyunsaturated FA (PUFA) represented in highest concentrations are those with the carbon number C_{16} to $C_{18,}$ which comprise 2 to 6% of the total FA (Samková *et al*., 2008). The low concentrations of milk fat PUFA are due to the biohydrogenation of FA in the rumen (Jenson, 1995; Welch *et al*., 1997). Approximately half of milk fat from ruminants (FA C_4 to C_{14} and half of C_{16}) is synthesized *de novo* in the mammary gland from short-chain FA. The second half of FA (half of C_{16} and C_{18} 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) originating directly from the diet or from adipose tissue (Kaylegian and Lindsay, 1995; Bauman and Griinari, 2003; Samková *et al*., 2008). According to Christie (1981), as much as 60% of $C_{16:0}$ is *de novo* synthesized.

The composition of milk fat and thus the content of FA are not constant and are affected by a number of factors. Diet composition (Veselý *et al*., 2009; Cieslak *et al*., 2010), nutrient contents in different diet components (Kalač and Samková, 2010), nutrient utilization (Perdrix *et al*., 1996; Třináctý *et al*., 2009), rumen fermentation (Jalč *et al*., 2009) and the breed of cows (Samková *et al*., 2008) are considered as the most important. The composition of FA is also influenced by season and lactation stage (Thomson *et al*., 2000; Garnsworthy *et al*., 2006; Frelich *et al*., 2009) as well as the NEB or a decline of body condition of cows after calving (Bastin *et al*., 2001; Key *et al*., 2005; Lake *et al*., 2007; Stoop *et al*., 2009). Most of works describe increasing of SFA (mainly FA $C_{16:0}$ and $C_{18:0}$) during NEB, which suggests mobilization of body fat reserves.

The objective of this study was to evaluate the contents of fat, protein and individual FA and groups of FA, including ratio of SFA to UFA, in milk during the first 17 weeks of lactation with respect to the development of NEB demonstrated by the change in cows' body condition.

MATERIAL AND METHODS

A total of 27 Holstein cows were included in the analysis – 11, 8 and 8 in the first, second, and third and later lactation, respectively. The average daily milk yield ranged from 19.2 to 31.3 litres of milk with the standard deviation ranging from 7.59 to 12.62. The cows were loose-housed in a cubicle straw-bedded barn and fed a total mixed ratio (TMR) consisting of maize and alfalfa silage, straw, grass and alfalfa hay, brewery draff, bakery waste, molasses, commercial concentrates, and mineral supplements. BCS was evaluated monthly on a 5-point scale with 0.25 point increments (Parker, 1989). From day 7 to 119 of lactation, two proportional milk samples were collected from the morning milking every week in accordance with the methodology of milk performance recording. The first sample preserved using Broad Spectrum Microtabs II (D&F Control Systems – Advanced Instruments, Inc.; USA) was analysed for the contents of protein (P), fat (F), lactose (L), dry matter (DM) and solids non fat (SNF) using Milko Scan 133B (N. Foss Electric; Denmark). Fat and protein contents were used to calculate the fat-to-protein ratio. The second sample (non-preserved) was analysed for the total content and composition of FA. The milk fat was extracted using the standard Röss-Gottlieb method (gravimetric) in accordance with EN ISO 1211 (2002). The extract was obtained using a water-based-solution of ammonia, ethanol, diethylether and petrolether. FA methyl esters were prepared by potassium hydroxide catalysed methylation and extracted into heptane. Gas chromatography (GC) of FA methyl esters was performed using the Master GC (split regime, FID detector – made by DANI Instruments S.p.A.; Italy) 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. FA were expressed as gravimetric contents (mg/100 g of milk) and percentages of the total FA were determined. Individual FA, SFA, and unsaturated fatty acids (UFA; UFA = MUFA + PUFA) were evaluated. A total of 413 pairs of milk samples were included in the analysis with the average number of samples per cow 15.3. The differences in the numbers of samples analysed were due to culling of several cows during the experimental period.

The data were evaluated with the statistical software SAS 9.1. (SAS/STAT® 9.1., 2004). The effect of the BCS of cows at calving and its changes after parturition in the contents of protein, fat, lactose, dry matter, and fat-free dry matter as well as the fat-to-protein ratio in milk was analysed using the linear model with the fixed effects of lactation number and the breeding value for protein yield (BVP). The same model was applied to analyse the changes in milk FA composition and SFA:UFA or MUFA:PUFA ratios. Differences between groups of cows with a maximum BCS reduction to 0.5 points (n = 12) and groups of cows with a BCS reduction more than 0.5 points (n = 15) during the first month of the 1st lactation were tested. Statistical significance was tested at the levels $P<0.05$, $P<0.01$ and $P<0.001$. Polynomial trend functions were used to demonstrate graphically the development of the values analysed.

RESULTS AND DISCUSSION

Basic statistics of the variables evaluated are given in Tab I.

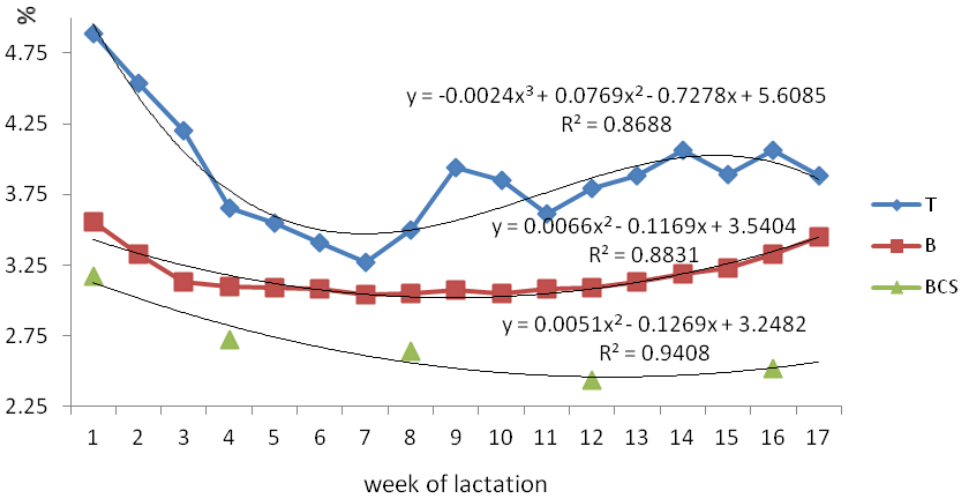
The changes in milk fat and protein contents as well as the development of the BCS in the post-parturition period are demonstrated in Fig. 1. The content of milk fat decreased from 4.89% at the beginning of lactation to 3.27% in week 7, and then it increased to 4.06% in weeks 14 and 16 of lactation. The used 3rd order polynomial trend function explained 86.88% of fat content variability. The protein content tended to decrease slightly until week 7, and then it increased until the end of the period observed. The average protein content ranged from 3.04 to 3.56%. The 2nd order polynomial trend function explained 88.3% of variability. The average BCS of cows continually decreased from 3.18 points at calving to the third month of lactation (2.48 points). The changes in BCS were characterised by the 2nd polynomial trend function explaining 94.08% of BCS variability. These results were generally in agreement with those reported in similar studies (Berry *et al.*, 2007; Kubešová *et al.*, 2009; Vacek and Kubešová, 2009; Ducháček *et al.*, 2010).

Fig. 2 demonstrates the development of the fat-to-protein ratio used as an indicator of NEB in relationship to the changes in BCS. The maximum value of this ratio, 1.62, was observed in the first week of lactation. Later it decreased to 1.08 in week 7, and then it slightly increased and became stabilized around the value of 1.2. The 3rd polynomial trend function explained 86.78% of the fat-to-protein ratio variability. Fig. 2 also shows the changes in BCS including the 2nd polynomial trend function explaining 94.08% of its variability. The average BCS decreased from 3.18 points at the beginning of lactation to 2.48 points in week 12. Subsequently the BCS was increased. Similarly, the average decrease in BCS in the first six months of lactation from 3.59 to 2.43 points, with the most rapid reduction observed in the first three months, was reported previously (Stádník *et al.*, 2002; Maršálek *et al.*, 2008). Also, the BCS of cows can be reduced as low as 2.5 points until the fourth month of lactation with the loss

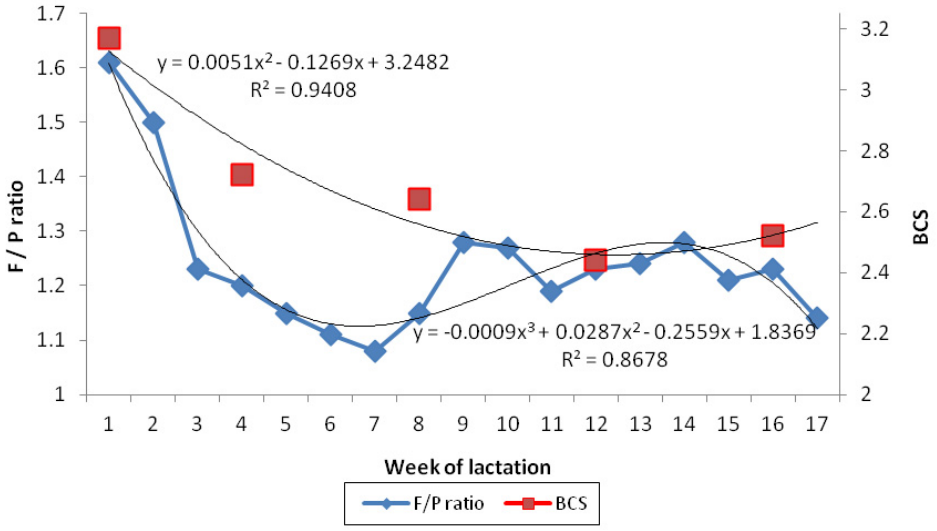
I: *Basic statistics of analysed variables*

Variable	n		S_d	V
BCS at calving [pts]	27	3.18	0.318	10.01
BCS 1 month after calving [pts]	27	2.75	0.304	11.07
BCS 2 months after calving [pts]	25	2.68	0.371	13.84
BCS 3 months after calving [pts]	25	2.48	0.339	11.43
Fat content in milk [%]	400	3.83	0.882	22.91
Protein content in milk [%]	408	3.16	0.303	9.62
Lactose content in milk [%]	408	4.84	0.368	6.45
Dry matter content in milk [%]	405	12.51	0.928	7.41
Fat-free dry matter content [%]	408	8.66	0.365	4.23
Fat % to Protein % ratio	406	1.23	0.377	30.65
Average daily milk yield [l]	408	27.6	10.36	37.55

n = number of observations, x = mean, s_x = standard deviation, V= coefficient of variation in %



1: *Development of fat (F) and protein (P) content in milk and the BCS of cows during 1st 17 weeks after calving*



2: *Development of F to P ratio and BCS after calving*

of 1.5 kg of fat tissue per day (Parker, 2009). In our study, the BCS decreased only until the third month of lactation, the increased fat and protein ratio in milk was observed in the whole group of cows only in the first two weeks after calving.

Basic statistics of 27 FA determined in milk samples (mg/100 g of milk; percentage of total FA) are given in Tab. II. The SFA with highest concentrations were $C_{16:0}$ (palmitic acid; 27.09 %), $C_{14:0}$ (myristic acid; 11.45 %), and $C_{18:0}$ (stearic acid; 10.56 %). The percentages of these three FA reported for Holstein cows in the study by Pešek *et al.* (2005) were 32.99, 12.55 and 4.45 %, respectively. A different rank order of the most abundant SFA ($C_{16:0} > C_{18:0} > C_{14:0}$) was found by Kay *et al.* (2005). In our study, relatively high concentrations were also observed for the FA $C_{4:0}$ – $C_{10:0}$ (butyric, capronic, caprylic and capric acid; in all 16.66 %) and $C_{12:0}$ (lauric acid; 4.08 %). Similar results were reported by Kaylegien and Lindsay (1995) and Gibson (1991), whereas Garnsworthy *et al.* (2006) observed a high concentration of $C_{16:0}$ followed by $C_{4:0}$ (butyric acid) in early lactation. The other SFA were determined only in low concentrations.

The UFA were mainly represented by $C_{18:1}$ (oleic acid; 20.22 %) followed by $C_{18:2}$ (linoleic acid; 2.69 %) and $C_{16:1}$ (palmitoleic acid; 2.17 %). Similar percentages of UFA and oleic acid were reported by Samková *et al.* (2008). In the study by Pešek *et al.* (2005), over the entire lactation the most abundant UFA were oleic (21.68 %), palmitoleic (2.20 %), linoleic (3.64 %), and myristoleic acids (1.06 %). Oleic acid as the UFA with the highest concentration was also reported by Kay *et al.* (2005). Most FA determined were highly variable between animals, which may reflect their metabolic status and other inter-individual differences. Therefore, one of the objectives of this study was to evaluate the changes in the concentrations of different FA groups and especially their ratios during the first 17 weeks of lactation when the metabolism of cows is influenced by NEB.

Our results are in agreement with those of Kay *et al.* (2005) and Garnsworthy *et al.* (2006), who concluded that at the beginning of lactation the synthesis of $C_{4:0}$ (butyric acid) and $C_{12:0}$ (lauric acid) increased. The highest concentration of butyric acid in milk fat was in the first month of lactation, whereas $C_{6:0}$-$C_{14:0}$ increased during the first 8 weeks of lactation. Similarly, in agreement with Kay *et al.* (2005), the concentrations of myristic ($C_{14:0}$) and palmitic acid ($C_{16:0}$) tended to increase as lactation continued. On the contrary, the concentration of stearic acid ($C_{18:0}$) decreased, which is probably associated with the hydrolysis of depot fat (Bauman and Griinari, 2003). We also demonstrated the increase of total SFA.

The MUFA with the highest concentrations determined were oleic acid ($C_{18:1}$), palmitoleic acid ($C_{16:1}$), and myristoleic acid ($C_{14:1}$). During the 17 weeks of the experimental period, oleic and palmitoleic acids tended to decrease, whereas the concentration of myristoleic acid slightly increased. The decrease of $C_{18:1}$ during lactation was also reported by Kay *et al.* (2005) and Stoop *et al.* (2009). Linoleic ($C_{18:2}$) and α linolenic ($C_{18:3(9)}$) acids were the most abundant PUFA. Both markedly decreased during the 17 weeks of lactation. The changes in MUFA and PUFA found in this study were in agreement with those reported by Pešek *et al.* (2005) for the entire lactation.

The tendency of changes in SFA, MUFA and PUFA is demonstrated in Fig. 3. It is evident that during the experimental period the concentration of SFA increased, whereas that of MUFA and PUFA decreased, which may be associated with the compensation of negative energy balance (Ducháček *et al.*, 2011) consistent with Stoop *et al.* (2009).

Therefore, we also investigated the changes in SFA:UFA and MUFA:UFA ratios as related to the

II: *Basic statistics of fatty acid composition*

FA	x	s_x	V
$C_{4:0}$	6.60	3.479	52.7
$C_{6:0}$	5.43	2.242	41.3
$C_{8:0}$	2.88	1.246	43.3
$C_{10:0}$	5.25	2.286	43.5
$C_{11:0}$	0.09	0.069	81.0
$C_{12:0}$	4.44	1.746	39.3
$C_{13:0}$	0.15	0.068	46.4
$C_{14:0}$	11.94	2.469	20.7
$C_{14:1}$	1.13	0.532	47.2
$C_{15:0}$	1.18	0.283	24.0
$C_{16:0}$	26.50	3.596	13.6
$C_{16:1}$	1.91	0.722	37.9
$C_{17:0}$	0.67	0.235	35.2
$C_{17:1}$	0.37	0.237	64.5
$C_{18:0}$	9.49	3.035	32.0
$C_{18:1}$	18.28	5.415	29.6
$C_{18:2}$	2.42	0.620	25.6
$C_{18:3}$	0.09	0.043	46.7
$C_{18:3(9)}$	0.53	0.135	25.6
$C_{20:0}$	0.14	0.079	55.1
$C_{20:1}$	0.09	0.057	66.9
$C_{20:2}$	0.02	0.022	120.9
$C_{20:3}$	0.07	0.051	67.5
$C_{20:4}$	0.15	0.061	39.5
$C_{21:0}$	0.02	0.031	134.4
$C_{22:0}$	0.04	0.040	103.9
$C_{22:2}$	0.11	0.097	90.0
$C_{24:0}$	0.02	0.025	139.0
SFA	74.83	6.245	8.3
MUFA	21.77	5.769	26.5
PUFA	3.40	0.782	23.0

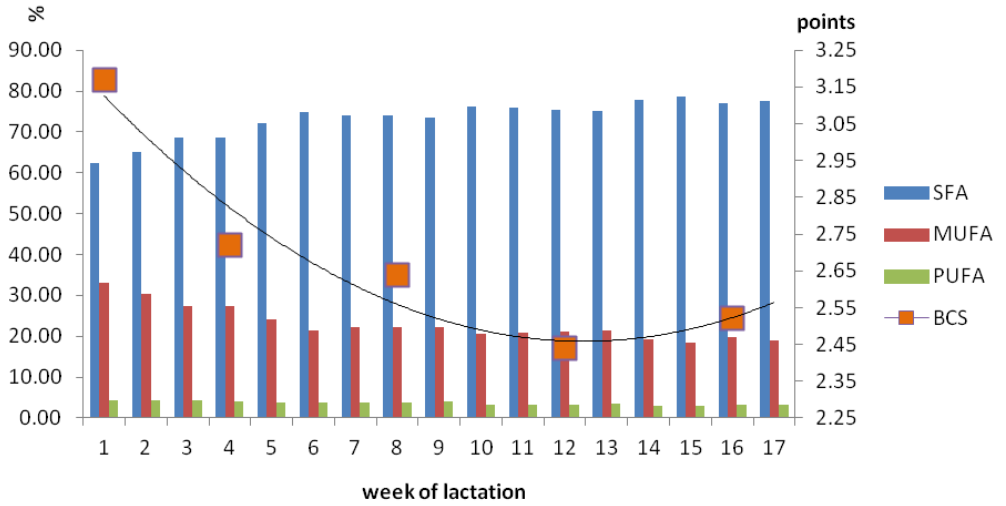
X = mean, s_x = standard deviation, V= coefficient of variation

development of NEB. The change in BCS after calving was considered the most reliable indicator of NEB, as a close relationship was observed between the BCS and milk fat-to-protein ratio. Fig. 4 shows the changes in milk SFA:UFA ratio separately for cows with different patterns in the change of BCS (small = BCS decrease < 0.5 point; large = BCS decrease ≥ 0.5 point = more severe NEB).

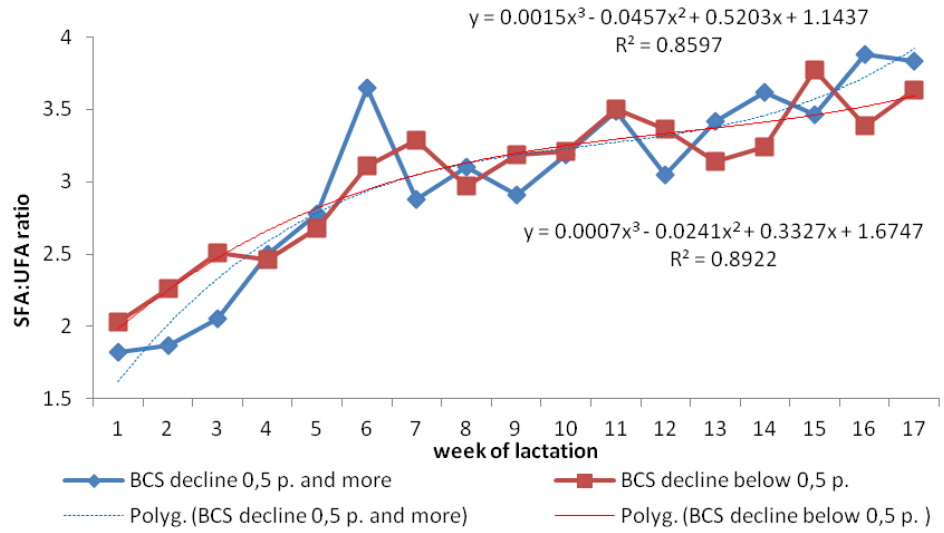
It is clear from Fig. 4 that there was no difference between mild and severe NEB cows in the SFA:UFA ratio throughout the entire experimental period. When the cows were assigned to one of two groups according to the change in BCS evaluated two months after calving, the SFA:UFA ratio was also similar between these groups. Changes in the SFA:UFA ratio during lactation similar to those in the present study were reported previously (Soyeurt *et al.*, 2008), but the authors did not investigate the effect of different BCS or the severity of NEB on this trait. The results of later published works (Stoop *et al.*, 2009) suggest increasing of SFA and decreasing of UFA in cows with NEB.

Because of the identified changes in the content of individual groups of UFA, we examined the ratio between MUFA and PUFA. Fig. 5 shows the changes in MUFA:PUFA ratios. In early lactation, this ratio was markedly lower in the cows with mild NEB due to a decreased concentration of MUFA in milk. Later in lactation, the magnitude of differences decreased and from the week 7 the MUFA:UFA ratio was similar between mild and severe NEB cows. It is apparent that the recovery of energy balance is associated with a decrease of the concentration of MUFA and increase of PUFA within UFA. Similarly, reduction MUFA associated with elevated fat content in milk was reported earlier (Soyeurt *et al.*, 2008).

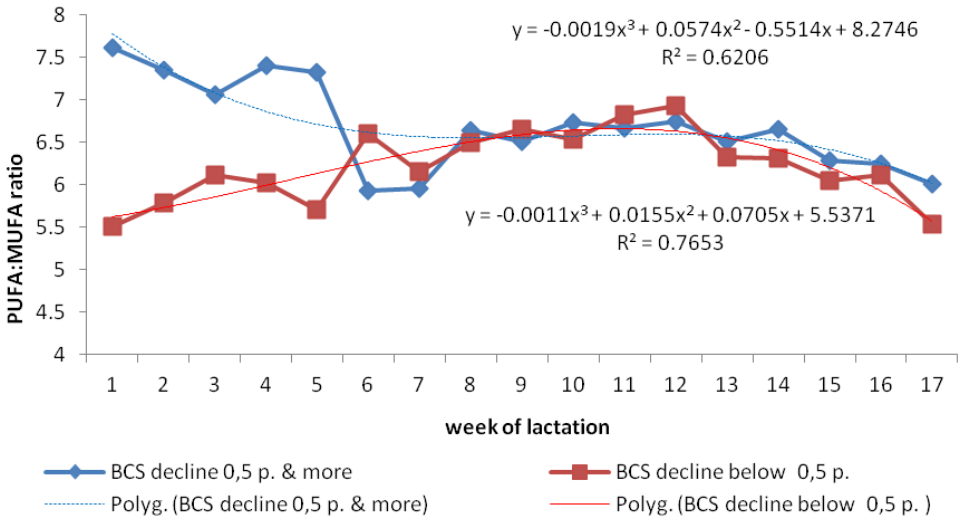
BCS at calving is positively correlated with the reduction of BCS after calving and is thus associated with the extent of NEB in early lactation (Vacek and Stádník, 2007; Berry *et al.*, 2007; Roche *et al.*,



3: *Development of FA groups content in milk and the cows BCS after calving*



4: *Development of SFA:UFA ratios in milk in accordance to the change in BCS during 1st mo. after calving*

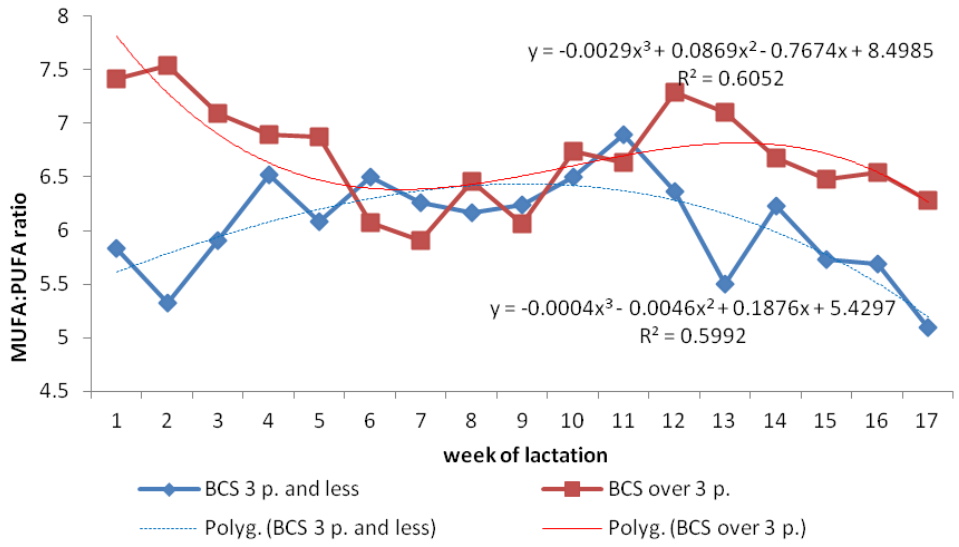


5: *Development of MUFA:PUFA ratios in milk in accordance to the change in BCS during 1st mo. after calving*

2008; Vacek and Kubešová, 2010). Therefore, we also investigated the effect of the BCS at calving on MUFA:PUFA ratios. The changes of MUFA:PUFA in the cows with different BCS at calving (BCS ≤ 3 *vs.* BCS > 3) are demonstrated in Fig. 6. Overconditioned cows at calving exhibited a higher concentration of MUFA ($P < 0.01$) and therefore also a higher ratio MUFA:UFA (7.54) in the second week of lactations compared to cows with the BCS below 3 points (MUFA:UFA = 5.32). The magnitude of differences between MUFA:UFA ratios decreased in later lactation similarly to that between the mild and severe NEB decline groups mentioned above (Fig. 5). From week 12, however, the magnitude of differences increased again, which was not observed between groups sorted by the NEB decline after calving. This observed trend is consistent with the results of Baer (1991) who has published that the content of stearic and oleic acids are highest in early lactation, falling in the middle lactation and rise again in the end of lactation. This course, especially a high content of oleic acid in early lactation, is attributed by some authors (Palmquist *et al.*, 1993; Bauman and Griinari, 2003) to increased uptake of fatty acids arising from adipose tissue due to the NEB.

CONCLUSIONS

During the first 17 weeks of lactation, the differences in fat content were more pronounced than those in protein content. The resulting changes of the fat-to-protein ratio over time are associated with adipose tissue mobilization due to NEB, which was also confirmed by the changes in the BCS during the experimental period. The results indicate the possibility of using both fat content and fat-to-protein ratio as indicators of NEB in early lactation cows.



6: *Development of MUFA:PUFA ratios in milk in accordance with the BCS at calving*

Our results are also in agreement with the published results of FA composition during the first stage of lactation. The highest concentrations of SFA were observed for $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, and also for the SFA with low carbon numbers. The highest concentrations of UFA were determined for $C_{18:1}$, $C_{18:2}$, and $C_{16:1}$. The changes in ratio MUFA:PUFA in milk during the first 5 weeks of lactation differed between the cows with low and high decrease of BCS one month after calving. In cows with a greater NEB effect was observed ratio of PUFA:MUFA in milk with a value above 6.5. The results suggest the possibility of using MUFA:PUFA ratios as suitable indicators of NEB in cows after calving.

SUMMARY

The contents of protein (P), fat (F), a F:P ratio, and content of individual fatty acids (FA) and groups of FA, including ratio of SFA:UFA (saturated FA-to-unsaturated FA) and MUFA:PUFA (ratio monounsaturated FA-to-polyunsaturated FA) in milk during the first 17 weeks of lactation with respect to the development of NEB demonstrated by the change in cows' body condition.

BCS was evaluated monthly on a 5-point scale with 0.25 point increments, two proportional milk samples were collected from the morning milking every week from day 7 to 119 of lactation. The first sample was analysed for the contents of protein (P), fat (F), lactose (L), dry matter (DM) and solids non fat (SNF) using Milko Scan 133B. The second sample (non-preserved) was analysed for the total content and composition of FA using a gas chromatography (GC). FA were expressed as gravimetric contents (mg/100 g of milk) and percentages of the total FA were determined. Individual FA, SFA, and unsaturated fatty acids (UFA; UFA = MUFA + PUFA) were evaluated. The data were evaluated with the statistical software SAS 9.1. (SAS/STAT® 9.1., 2004 using the linear model with the fixed effects of lactation number and the breeding value for protein yield (BVP).

The differences in fat content were more pronounced than those in protein content. The resulting changes of the fat-to-protein ratio over time are associated with adipose tissue mobilization due to NEB, which was also confirmed by the changes in the BCS during the experimental period. The results indicate the possibility of using both fat content and fat-to-protein ratio as indicators of NEB in early lactation cows. The highest concentrations of saturated FA (SFA) were observed for $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, and also for the SFA with low carbon numbers. The highest concentrations of unsaturated FA (UFA) were determined for $C_{18:1}$, $C_{18:2}$, and $C_{16:1}$. The significant difference in SFA:UFA between cows with decreasing of the BCS and cows without big change in the BCS after calving were not found. The changes in MUFA:PUFA in milk during the first 5 weeks of lactation differed between the cows with low and high decrease of BCS one month after calving. In cows with a greater NEB effect was observed ratio of PUFA:MUFA in milk with a value above 6.5.

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