

## The relationship between fatty acid and citric acid concentrations in milk from Holstein cows during the period of negative energy balance

Vztah mezi obsahem mastných kyselin a kyseliny citronové v mléce dojnic holštýnského skotu v průběhu období záporné energetické laktace

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### Abstract

The objective of this study was to determine the relationship between body condition score changes and the dynamics of energy balance indicators - fatty acid and citric acid contents - in milk during a early part of lactation. In addition, the relationship between these two indicators was also evaluated. A total of 27 Holstein cows that calved within three consecutive weeks were included in the analysis. During the first 17 weeks of lactation, milk samples were collected at a weekly interval and body condition score was assessed once a month. Statistical analyses were performed using Microsoft Office Excel and the procedures MEANS and CORR of SAS 9.1. Trend functions describing the development of fatty acid and citric acid contents explained 67.67 to 92.19 % of their variability. Similar relationships between fatty acid and citric acid contents and the changes in body condition score during the first three months of lactation were observed. In addition, a similar decreasing tendency was also determined for the contents of both the dependent variables in this period. Significant correlations ( $P < 0.01 - 0.001$ ) were calculated ( $r = 0.51 - 0.74$ ) for lactation weeks 6 and 7, thus before the subsequent decrease of body condition score by 0.2 points between weeks 8 and 12 after parturition. The results indicate the possibility of using the contents of fatty acids and citric acid as indicators of energy balance in dairy cows. The results also confirm the relationships between these indicators and emphasise the importance of proper herd management with respect to body condition score changes and the contents of fatty acids and citric acid in milk.

**KEYWORDS:** fatty acid, citric acid, milk fat, BCS, Holstein cattle

### Abstrakt

Cílem této práce bylo vyhodnotit vztah mezi změnami tělesné kondice a vývojem indikátorů energetické bilance – obsah mastných kyselin a kyseliny citronové – v mléce v průběhu laktace. Navíc byl také hodnocen vztah mezi těmito dvěma

indikátory. Do hodnocení bylo zařazeno 27 holštýnských dojnic otelených během tří po sobě jdoucích týdnů. Během prvních 17 týdnů laktace byly v týdenních intervalech odebrány vzorky mléka. Tělesná kondice byla hodnocena v měsíčním intervalu. Statistické vyhodnocení bylo provedeno za použití programu Microsoft Office Excel a SAS 9.1., procedury MEANS a CORR. Trendové funkce popisující vývoj obsahu mastných kyselin a kyseliny citronové vysvětlovali 67,67 až 92,19 % jejich variability. Podobně také byly sledovány vztahy mezi obsahem mastných kyselin, kyseliny citronové a změnami tělesné kondice během prvních tří měsíců laktace. Pro obě sledované proměnné byly v tomto období zjištěny stejně klesající tendence. Průkazné korelace ( $P < 0,01 - 0,001$ ) byly vypočteny ( $r = 0,51 - 0,74$ ) pro 6. a 7. týden laktace, tudíž před následným poklesem tělesné kondice o 0,2 bodů mezi 8. a 12. týdnem po otelení. Výsledky naznačují možnost využití obsahu mastných kyselin a kyseliny citronové jako indikátory energetické bilance u dojnic. Výsledky také potvrzují vztah mezi těmito indikátory a zdůrazňují důležitost správného managementu stáda s ohledem na změny tělesné kondice a obsah mastných kyselin a kyseliny citronové v mléce.

**Klíčová slova:** mastné kyseliny, kyselina citronová, mléčný tuk, tělesná kondice, holštýnský skot

## Detailed Abstract

Dojnice se na začátku laktace dostávají do záporné energetické bilance, což se projevuje změnami tělesné kondice, obsahu mléčného tuku (nebo poměrem tuk/bílkoviny v mléce) a jiných indikátorů záporné energetické bilance jako jsou složení mastných kyselin a obsah kyseliny citronové v mléce.

Cílem našeho příspěvku bylo vyhodnotit vztah mezi vývojem obsahu skupin mastných kyselin a obsahem kyseliny citronové. Do hodnocení vstupovalo 27 dojnic holštýnského plemene skotu, kdy 11 dojnic bylo na první, 8 na druhé a 8 na třetí a další laktaci. Hodnocení probíhalo po dobu prvních sedmnácti týdnů laktace. Obsah mastných kyselin byl stanoven za použití metody podle Rösse-Gottlieba (vážková metoda), která je upravena EN ISO 1211 (ČSN 570534). Analýzou na plynovém chromatografu byl stanoven obsah jednotlivých mastných kyselin (35) v mléce ( $\text{mg} \cdot 100\text{g}^{-1}$  mléka a jejich procentické zastoupení) a poté byl hodnocen procentický podíl jednotlivých skupin mastných kyselin: nasycené (SFA) a nenasycených (UFA). V rámci UFA bylo také hodnoceno zastoupení mono (MUFA), resp. polynenasycených mastných kyselin (PUFA). Pro stanovení obsahu kyseliny citronové ( $\text{mmol} \cdot \text{l}^{-1}$ ) byla použita spektrofotometrická metoda. Ze stanovených obsahů mastných kyselin a kyseliny citronové byla sestavena databáze pro vlastní statistické vyhodnocení.

Statistická analýza byla provedena v programu Microsoft Office Excel a statistickém programu SAS 9.1., procedury MEANS a CORR. Průměrný obsah tuku v mléce se ve sledovaném období pohyboval od 5,01 do 2,56 %. V průběhu sledovaných 17 týdnů laktace docházelo k nárůstu obsahu SFA a poklesu obsahu UFA (a to především MUFA). Trendové funkce popisující vývoj obsahu mastných kyselin a kyseliny citronové vysvětlovali 67,67 až 92,19 % jejich variability. Podobně také byly sledovány vztahy mezi obsahem mastných kyselin, kyseliny citronové a změnami

tělesné kondice během prvních tří měsíců laktace. Pro obě sledované proměnné byly v tomto období zjištěny stejně klesající tendence. Průkazné korelace ( $P < 0,01 - 0,001$ ) byly vypočteny ( $r = 0,51 - 0,74$ ) pro 6. a 7. týden laktace, tudíž před následným poklesem tělesné kondice o 0,2 bodů mezi 8. a 12. týdnem po otelení. Výsledky naznačují možnost využití obsahu mastných kyselin a kyseliny citronové jako indikátory energetické bilance u dojníc. Výsledky také potvrzují vztah mezi těmito indikátory a zdůrazňují důležitost správného managementu stáda s ohledem na změny tělesné kondice a obsah mastných kyselin a kyseliny citronové v mléce.

## Introduction

Cows in early lactation fall into negative energy balance (NEB), which results in the development of the lipomobilization syndrome (González, et al., 2011) followed by a decrease in body condition score (BCS) (Maršálek, et al., 2008; Stádník, et al., 2007). NEB affects the intensity of a cow's metabolism (ten Napel, et al., 2009) and as a consequence also the composition of milk, especially the contents of protein (Jankowska, et al., 2010), fat (Hanuš, et al., 2011a), urea (Janušík, 2009), and acetone (Hanuš, et al., 2011b). The most frequently used method of assessing the development of NEB is the determination of milk fat content and fat-to-protein ratio (Čejna and Chládek, 2005; Ducháček, et al., 2012) due to marked changes in milk fat percentage in early lactation (Ducháček, et al., 2010). The composition of milk fat is different from that of blood plasma. Milk fat is synthesised in the mammary gland from its precursors and fatty acids (FA) originating either from the diet or from the neutral fat (triacylglycerols – TAG) from the liver and the adipose tissue (Bauman, et al., 2006). Approximately half of the milk fat from ruminants (FA C<sub>4</sub> to C<sub>14</sub> and half of C<sub>16</sub>) is synthesised *de novo* in the mammary gland from short-chain FA (Kaylegian and Lindsay, 1995; Bauman and Griinari, 2003). The second half of FA (half of C<sub>16</sub> and C<sub>18</sub> and longer chain FA) is transported to the mammary gland by blood, especially by its highly labile  $\beta$ -lipoprotein fraction, in the form of non-esterified FA (NEFA) originating directly from the diet or from the adipose tissue (Samková, et al., 2008). Milk fat is mainly composed of saturated FA (SFA) (Ducháček, et al., 2011). C<sub>16:0</sub> comprises 30% and short carbon chain (C<sub>4:0</sub> – C<sub>12:0</sub>) above 15 % of the total FA. Monounsaturated FA (MUFA) (C<sub>14:1</sub> – C<sub>18:1</sub>) comprise 26 to 42 % of the total FA. Polyunsaturated FA (PUFA) in milk usually comprise only 2 – 6 % of the total FA (Samková, et al., 2008) due to extensive rumen biohydrogenation (Jensen, 1995; Welch et al., 1997). The composition of milk fat and also FA is quite variable and is influenced by a number of factors (Welch et al., 1997). Diet composition as well as nutrient content and utilization are considered the most important (Perdrix, et al., 1996; Samková, et al., 2009; Hanuš, et al., 2011a). Milk FA composition is also affected by the season and lactation stage (Thomson, et al., 2000; Kay, et al., 2005; Garnsworthy, et al., 2006; Stoop, et al., 2009) as well as by intra-individual variability (Soyeurt, et al., 2006; Pešek, et al., 2009). FA composition also influences the technological properties of milk as evidenced by the relationship found between the content of lactose and that of unsaturated FA (Hanuš, et al., 2010), and also the nutritive quality and health characteristics of milk (Butler et al., 2008).

The content of citric acid (CA) is another indicator of energy balance which is being currently studied. Citrates are part of Krebs cycle and their concentration is about 0.2 %. Most citrates in milk are in the form of free CA which is synthesised from pyruvic acid in the mammary gland cells. CA is an important part of the citrate cycle and

influences the regulation of acetyl-CoA metabolism in liver cell mitochondria (Bremer, et al., 1974). The content of CA in milk may be a good indicator of energy balance in dairy cows. Several studies have been recently published on this topic (Baticz, et al., 2002). For example, Kubešová, et al. (2009) analysed the relationship between the content of CA in milk and reproduction parameters in Holstein and Czech Fleckvieh cows. The effect of CA content on health was also studied. The concentration of CA is higher in healthy than in sick cows in early lactation and it decreases in later lactation (Khaled, et al., 1999). It is concluded that the content of CA in milk is assumed to be a good indicator of a cow's organism functioning and health.

Due to metabolic interactions of biochemical pathways in cows, it is assumed that the changes in milk contents of FA and CA in early lactation can be used as NEB indicators. It is also possible that a relationship exists between the contents of FA and CA. Therefore, the objective of this study was to examine the relationship between the change in BCS and milk FA and CA contents as well as to evaluate the relationship between FA (sum of FA, SFA, UFA, MUFA, PUFA) and CA contents during the first 17 weeks of lactation.

## Material and methods

A total of 27 Holstein cows calved within 3 weeks in June were included in the analysis - 11, 8 and 8 in the first, second, and third and later lactation, respectively. A total of 413 milk samples collected during the first 17 weeks of lactation were included in the evaluation. The average daily milk yield ranged from 19.16 to 31.29 l of milk with the standard deviation ranging from 7.59 to 12.62. The fat content in milk was in range 5.01 to 2.56 %. 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. The content of different components in the diet was adjusted according to the daily milk yield. BCS was evaluated first time directly in calving day and subsequently monthly on a 5-point scale with 0.25 point increments (Parker, 1989). The average BCS was ranged from 3.18 at calving to 2.48 point in the 3rd month after calving. From day 7 to 119 of lactation, proportional milk samples were collected from the morning milking every week in accordance with the methodology of milk performance recording. The total FA and CA milk contents were determined.

For the FA analysis, the milk fat was extracted using the standard Röss-Gottlieb method (gravimetric) in accordance with EN ISO 1211 (ČSN 570534) (2010). 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 (DANI Instruments S.p.A., Italy) (split regime, FID detector) on a column with polyethylene glycol stationary phase (FameWax – 30 m 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. Milk FA (35) were expressed as gravimetric contents (mg.100g<sup>-1</sup> of milk) and percentages of the total FA. The contents and percentages of different FA groups – SFA and unsaturated (UFA; UFA = MUFA + PUFA) were also determined.

The analysis of CA content was performed using the spectrophotometric method (Genčurová, 2008; Marier and Boulet, 1958). A 3 ml milk sample was placed in a 25 ml volumetric flask and 5 ml of trichloroacetic acid was added. Then distilled water was added to the solution up to a volume of 25 ml. The mixture was quantitatively transferred to a beaker, left to incubate for 30 min at room temperature and then filtrated. An 8-point concentration series was prepared from trisodic citrate and trichloroacetic acid. The concentration series and 5 ml of filtrated mixture were pipetted to test tubes and then 0.65 ml of pyridine and 2.85 ml of acetic anhydride were added. The mixture was thoroughly stirred and placed in a water bath at a temperature of 32°C for 30 min. The reaction of citric acid with pyridine and acetic anhydride produced a yellow colour whose intensity was measured against a blind sample as a reference by the spectrophotometer Genessys 10VIS (Thermo Electron Scientific Instruments LLC, USA) at a wave length of 428 nm. The concentration of citric acid ( $\text{mmol.l}^{-1}$ ) was calculated according to the concentration series. A total of 413 samples were analysed with an average of 15.3 samples per cow, minimum 6 samples per cow (due to their culling during the experimental period), and maximum 17 samples per cow. The database was analysed using Microsoft Office Excel and the procedures of MEANS and CORR of SAS 9.1. (SAS/STAT® 9.1., 2004). The values  $P < 0.05$ ,  $P < 0.01$ , and  $P < 0.001$  were used as levels of statistical significance.

## Results and discussion

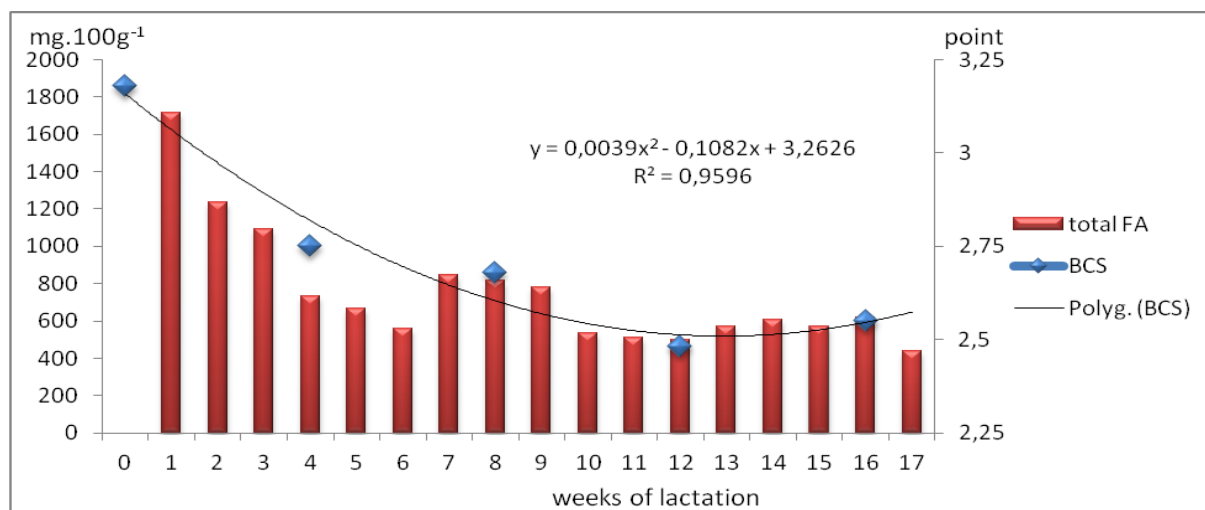
Most cows are in NEB in this period, which is demonstrated by the change (loss) of BCS. The BCS was declined by 0.7 points until the 3rd month of lactation in our study and subsequently it increased (Fig. 1 and 2). These figures are shown polynomial trend function curves, which explain 95.96 % of BCS variability, thus providing evidence for BCS as an indicator of the energy balance of cows. Similarly, the most marked changes in BCS were observed up to the third month of lactation in a report by Maršálek, et al. (2008).

The contents of total FA and 35 individual FA as well as the content and percentages of four FA groups (SFA, UFA, MUFA, and PUFA) were determined. The average milk fat content ranged from 5.01 % in week 1 to 2.56 % in week 17 of lactation. The most extensive decrease in milk fat content as well as the most extensive FA content changes were observed between weeks 1 and 7 of lactation. As demonstrated in Fig. 1, high FA contents were determined simultaneously with the marked reduction of BCS (week 0 – week 3), and from weeks 7 to 9 prior to another BCS decline. This might indicate a relationship between the development of NEB and milk FA content.



Figure 1. Changes of body condition score (BCS) and total fatty acids (FA) contents (mg.100g<sup>-1</sup> of milk)

Graf 1. Změny tělesné kondice (BCS) a celkového obsahu mastných kyselin (total FA) (mg.100g<sup>-1</sup> mléka)



The development of different FA groups content (Table 1; mg/100 g) and shares (Fig. 2; %) over period observed was evaluated as well. The contents of FA groups were also highly variable and were affected by lactation week. The contents of FA groups decreased until approx. week 6 of lactation. The highest values were observed during the first 3 weeks of lactation. From weeks 7 to 9, the contents of FA groups slightly increased as the result of an intensive hydrolysis of subcutaneous fat followed by a decrease in BCS (Ducháček, et al., 2010). In the subsequent period, the content of UFA decreased and the ratio between different FA groups became stabile, which indicates the compensation of NEB (Ducháček, et al., 2011). The highest content of MUFA during the first month of lactation is in agreement with results reported previously (Kay, et al., 2005; Garnsworthy, et al., 2006). In our study, however, the content of UFA and, to a lesser extent, MUFA and PUFA, recurrently increased from weeks 7 to 9, simultaneously with reduced BCS. Not only the beginning of lactation but also other factors may result in the repeated onset or deepening of NEB as well as BCS reduction in a given lactation stage, which are associated with changes in FA group contents. One of them is social stress resulting from the transfer of animals between different production groups during lactation, which is usually associated with changes in diet composition (ten Napel, et al., 2009). In later stages of lactation, the composition of milk continues to slightly change, but no repeated onset or deepening of NEB usually occurs after day 80 to 90 of lactation (Stádník, et al., 2007; Maršálek, et al., 2008).

Table 1. Contents of fatty acid groups in different lactation weeks (mg.100g<sup>-1</sup> of milk)

Tabulka 1. Obsah mastných kyselin ve sledovaných týdnech laktace (mg.100g<sup>-1</sup> mléka)

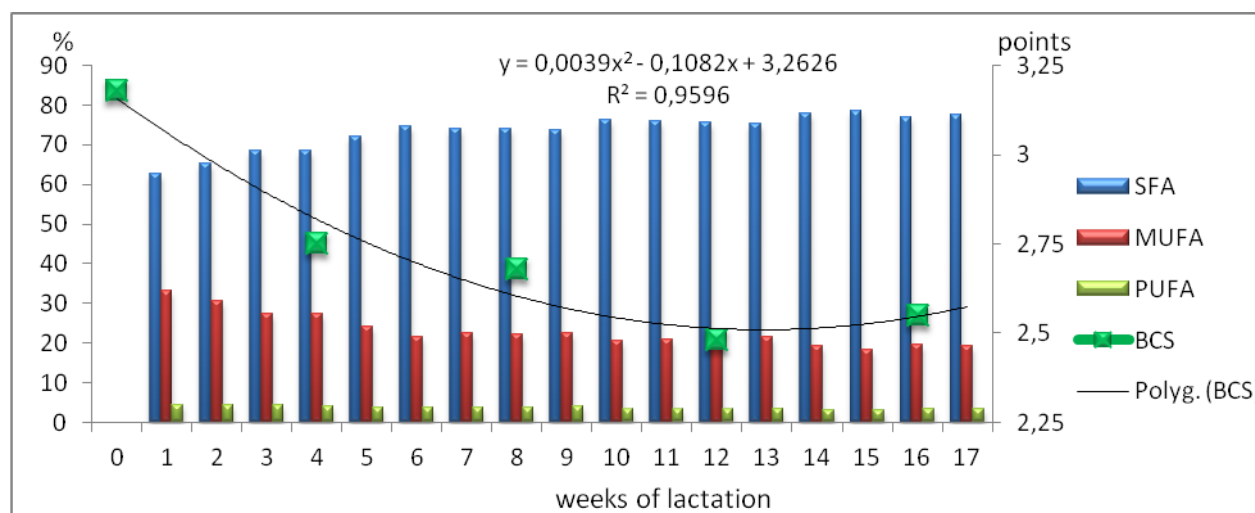
Week	N	SFA	UFA	MUFA	PUFA
1	15	1072.53 ± 848.26	643.31 ± 554.61	567.26 ± 503.4	76.05 ± 56.95
2	17	804.26 ± 332.62	430.36 ± 240.42	376.28 ± 216.09	54.09 ± 28.3
3	27	749.37 ± 513.4	344.3 ± 265.37	298.41 ± 231.1	45.88 ± 36.35
4	27	502.22 ± 322.39	229.52 ± 205.18	199.41 ± 179.21	30.1 ± 26.76
5	27	478.59 ± 314.63	185.64 ± 138.03	160.38 ± 119.4	25.26 ± 20.46
6	27	418.1 ± 564.66	141.01 ± 211.21	120.42 ± 178.11	20.6 ± 33.59
7	25	624.33 ± 607.57	220.06 ± 238.17	188.48 ± 203.84	31.58 ± 34.72
8	26	603.41 ± 613.87	210.81 ± 226	180.63 ± 191.24	30.18 ± 35.5
9	23	575.21 ± 605.77	207.02 ± 257.1	174.56 ± 220.57	32.46 ± 40.67
10	25	407.69 ± 297.02	127.64 ± 90.24	110.3 ± 77.37	17.33 ± 13.11
11	24	387.38 ± 250.71	122.51 ± 107.53	106.14 ± 93.89	16.37 ± 14.06
12	24	375.2 ± 316.8	121.57 ± 129.74	105.57 ± 112.91	16 ± 17.02
13	25	430.09 ± 284.14	141.99 ± 120.05	122.62 ± 103.49	19.37 ± 16.99
14	25	473.16 ± 396.97	135.09 ± 114.08	116.36 ± 97.2	18.73 ± 17.01
15	24	447.35 ± 330.06	121.23 ± 85.57	104.22 ± 74.33	17.01 ± 11.53
16	25	476.99 ± 339.51	142.26 ± 115.94	121.95 ± 99.21	20.31 ± 17.21
17	24	341.59 ± 235.73	98.77 ± 86.75	84.1 ± 74.18	14.66 ± 12.7

Week - week of lactation; SFA – saturated fatty acids; UFA – unsaturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids.

It is evident that the % share of SFA increased, whereas that of MUFA and PUFA decreased, which may be associated with the compensation of NEB (Ducháček, et al., 2011; Stoop, et al., 2009). This fact also corresponds with the significant decline of BCS during the first and also during the third month of lactation.

Figure 2. Development of body condition score (BCS) and fatty acids (FA) group percentages (mg.100g<sup>-1</sup> of milk)

Graf 2. Vývoj tělesné kondice (BCS) a procentického obsahu skupin mastných kyselin (FA) (mg.100g<sup>-1</sup> mléka)



The content of CA in milk was used as second evaluated indicator of NEB. The CA content was higher in the first 4 weeks of lactation and also in week 7 (Table 2), which corresponds to the milk total content and groups of FA. Similarly, significantly higher concentrations of CA in early lactation were reported in a study by Garnsworthy, et al. (2006). Also, Baticz, et al. (2002) observed higher concentrations of CA in the first 10 to 20 days postpartum. The CA content varied considerably during the period analysed and tended to decrease in a wave pattern as demonstrated in Fig. 3. Variation coefficients (V) indicate that this variable has quite a homogeneous character.

Table 2. Descriptive statistics of the content of citric acid in different lactation weeks (mmol.l<sup>-1</sup>)

Tabulka 2. Popisné statistiky obsahu kyseliny citronové ve sledovaných týdnech laktace (mmol.l<sup>-1</sup>)

Week	N	$\bar{x}$	s <sub>d</sub>	min.	max.	e	V (%)
1	10	10.62	2.03	7.49	14.16	0.64	19.11
2	16	11.83	5.49	6.14	29.22	1.37	46.42
3	25	13.52	6.08	5.38	26.59	1.22	44.95
4	26	12.80	4.55	6.18	22.46	0.89	35.55
5	27	9.52	3.10	4.49	16.27	0.60	32.61
6	27	9.85	3.74	4.73	25.83	0.72	38.00
7	26	12.38	5.29	4.40	25.72	1.04	42.71
8	26	9.26	3.79	3.84	23.58	0.74	40.98
9	25	9.02	3.47	3.97	21.34	0.69	38.48
10	23	9.03	2.44	4.84	12.52	0.51	27.06
11	25	9.55	3.18	3.98	19.92	0.64	33.32
12	24	9.77	3.30	4.28	15.72	0.67	33.76
13	25	8.94	3.27	3.86	13.47	0.65	36.58
14	25	8.29	3.35	4.16	14.47	0.67	40.37
15	25	9.13	3.67	3.72	21.38	0.73	40.14
16	25	8.44	2.91	4.29	17.27	0.58	34.48
17	25	8.00	3.66	3.96	17.99	0.73	45.67

Week - week of lactation;  $\bar{x}$  - arithmetic means; s<sub>d</sub> – standard deviation; min. – minimal value; max. – maximal value; e – means error; V (%) – coefficient of variance.

The changes of total FA and FA group contents tended to be similar to that of the content of CA (Fig. 3 – 7). The proportions of variability explained by polynomial trend functions were 85.73, 79.56, and 91.8 % for total FA, SFA, and UFA, respectively. The highest proportion of variability was explained by the polynomial function for MUFA (92.19 %), which corresponds to the above-discussed relationship between the content of MUFA and BCS changes in early lactation (Kay et al., 2005; Garnsworthy et al., 2006). The polynomial trend function explained 67.67 % of milk CA content variability in a given lactation period. Therefore, total FA and FA group contents appear to be more accurate indicators of energy balance than the content of CA.

A similar pattern was detected for the developments of total FA and FA groups (SFA, UFA, MUFA, and PUFA). The highest values were observed between weeks 1 and 3



of lactation (Fig. 3 – 7). The content of FA then decreased between weeks 4 and 6 and increased again between weeks 7 and 9 of lactation. Similarly, the highest concentrations of CA were determined up to week 4 postpartum, then they decreased (weeks 5 to 6) and markedly increased in week 7 of lactation.

The values of FA and CA contents in milk between weeks 1 and 3 of lactation are related to severe NEB and correspond to a -0.43 point decline in BCS during the first month of lactation. In the following period (weeks 4 to 6), FA and CA contents decreased, whereas BCS declined by only -0.07 points until week 8 of lactation. In accordance with the management practice used in the herd participating in this study, cows were usually transferred to another production group in week 7 of lactation with respect to current housing capacity and daily milk yield. This was associated with changes in diet composition and sometimes also with the disruption of social relationships among animals. We suggest that these stress factors resulted in increased FA contents between weeks 7 and 9 and increased CA contents in week 7 of lactation. The changes in FA and CA contents are related to the hydrolysis of subcutaneous fat and the resulting reduction of BCS by -0.2 points between weeks 8 and 12, when cows strive to recover from the previous transfer and diet modification. These relationships are described in Fig. 3 – 7 and prove the importance of herd management measures, which influence the function of a cow's organism and its adaptability under specific production conditions (ten Napel, et al., 2009).

Figure 3. Changes of citric acid (CA) (mmol.l<sup>-1</sup>) and total fatty acids (total FA) contents (mg.100g<sup>-1</sup> of milk)

Graf 3. Změny obsahu kyseliny citronové (CA) (mmol.l<sup>-1</sup>) a celkového obsahu mastných kyselin (total FA) (mg.100g<sup>-1</sup> mléka)

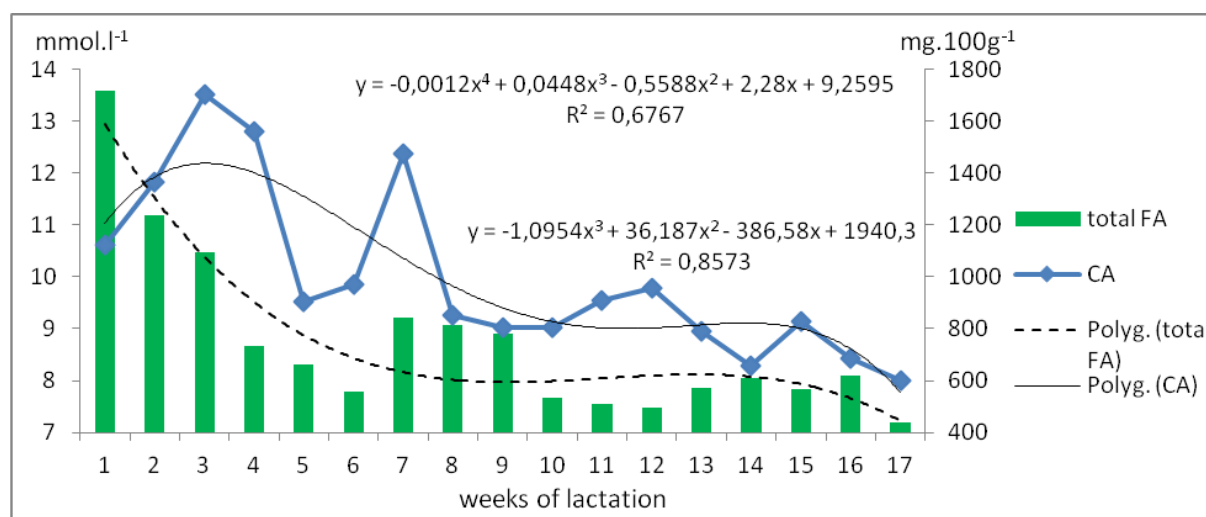


Figure 4. Changes of citric acid (CA) (mmol.l<sup>-1</sup>) and saturated fatty acids (SFA) contents (mg.100g<sup>-1</sup> of milk)

Graf 4. Změny obsahu kyseliny citronové (CA) (mmol.l<sup>-1</sup>) a nasycených mastných kyselin (SFA) (mg.100g<sup>-1</sup> mléka)

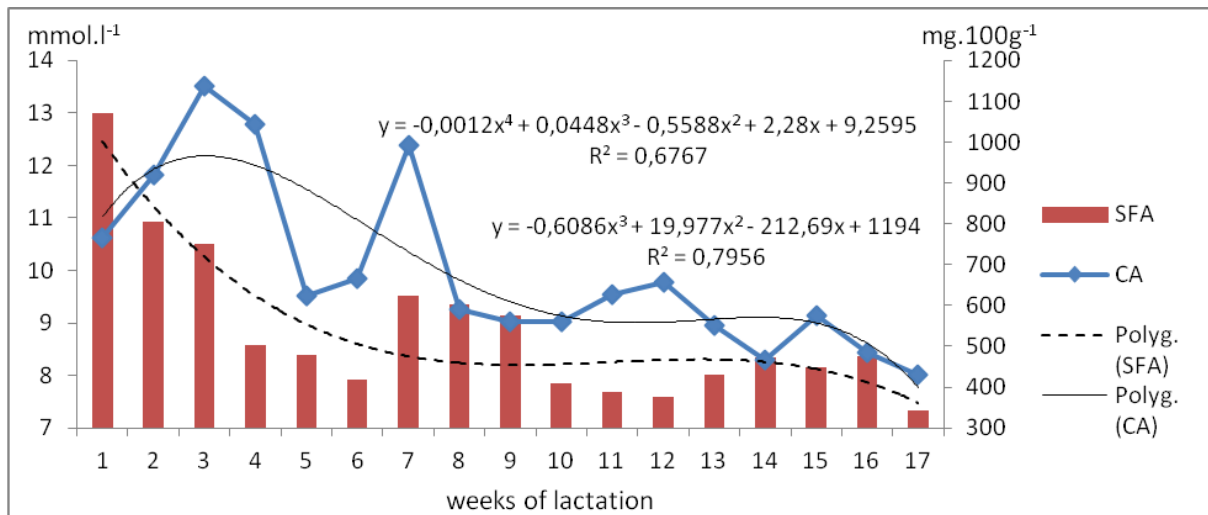


Figure 5. Changes of citric acid (CA) (mmol.l<sup>-1</sup>) and unsaturated fatty acids (UFA) contents (mg.100g<sup>-1</sup> of milk)

Graf 5. Změny obsahu kyseliny citronové (CA) (mmol.l<sup>-1</sup>) a nenasycených mastných kyselin (UFA) (mg.100g<sup>-1</sup> mléka)

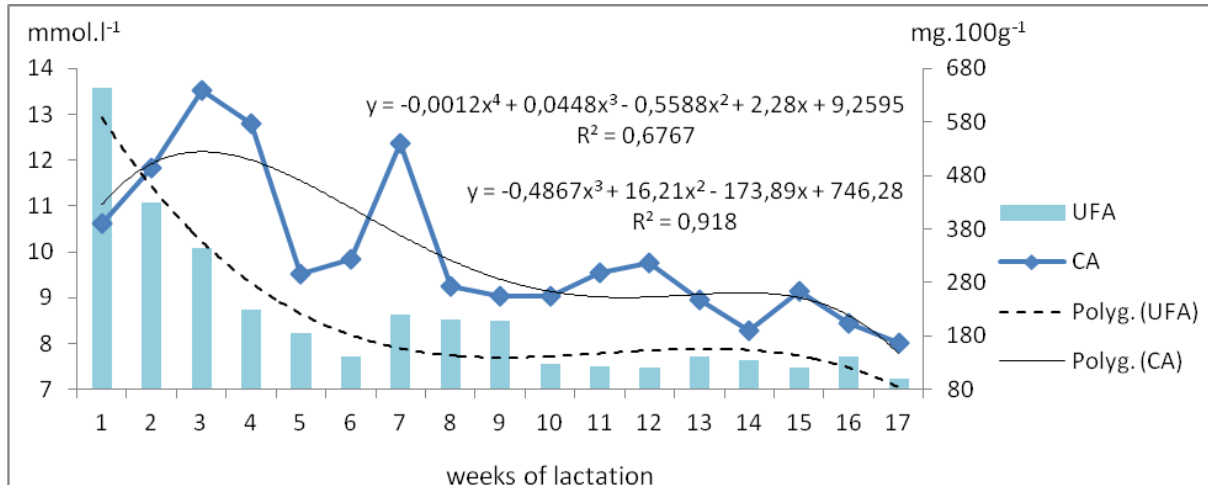


Figure 6. Changes of citric acid (CA) (mmol.l<sup>-1</sup>) and monounsaturated fatty acids (MUFA) contents (mg.100g<sup>-1</sup> of milk)

Graf 6. Změny obsahu kyseliny citronové (CA) (mmol.l<sup>-1</sup>) a mononenasyčených mastných kyselin (MUFA) (mg.100g<sup>-1</sup> mléka)

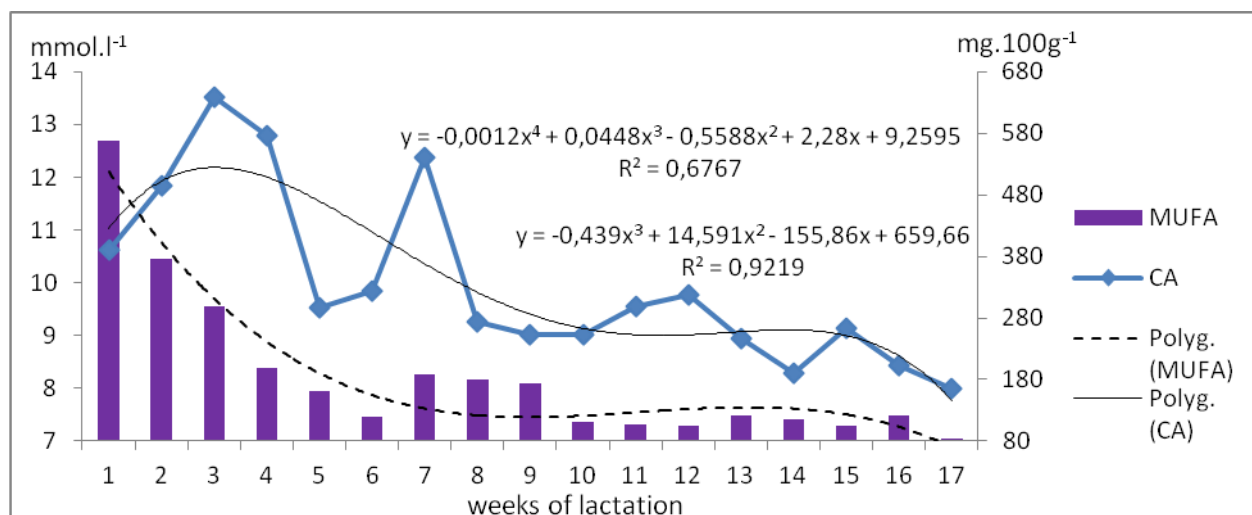
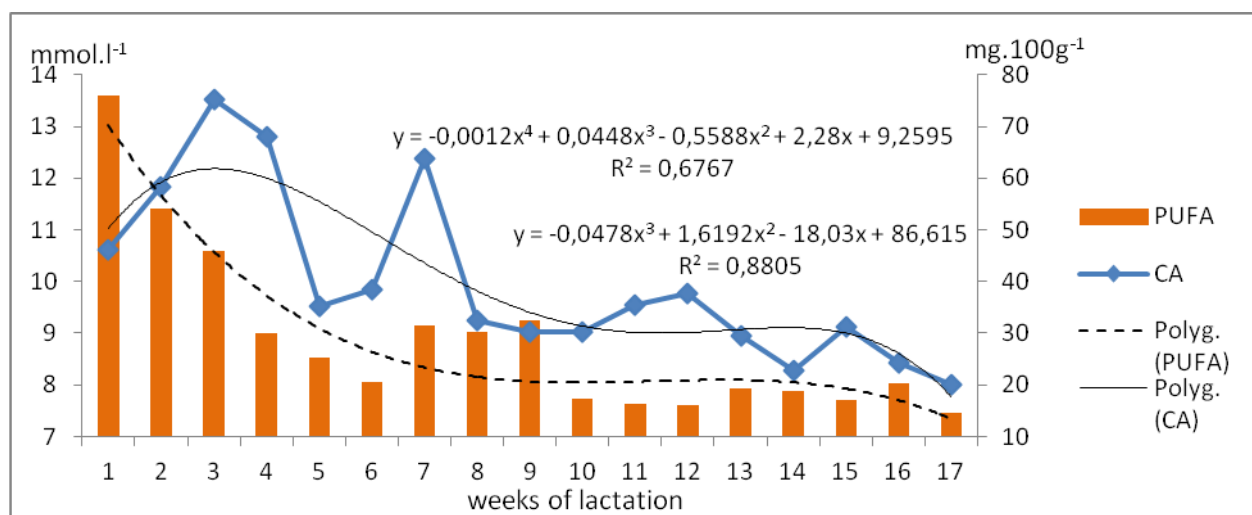


Figure 7. Changes of citric acid (CA) (mmol.l<sup>-1</sup>) and polyunsaturated fatty acids (PUFA) contents (mg.100g<sup>-1</sup> of milk)

Graf 7. Změny obsahu kyseliny citronové (CA) (mmol.l<sup>-1</sup>) a polynenasycených mastných kyselin (PUFA) (mg.100g<sup>-1</sup> mléka)



The results are also confirmed by Pearson correlation coefficients calculated for the relationships between FA groups (total FA, SFA, MUFA, PUFA, and UFA) and CA contents. Close correlations ranging from  $r=0.66$  to  $r=0.74$  ( $P<0.001$ ) were found between CA content and total FA as well as FA group contents in week 6 of lactation, whereas moderate correlations between these traits ranging from  $r=0.51$  to  $r=0.66$  ( $P<0.01$ ) were found in week 7 of lactation. The significance of these correlation coefficients confirmed the importance of dairy herd management with respect to the above-mentioned relationships between FA and CA contents in milk, in case that, simple and chipper methods for FA and CA analysis would develop.

## Conclusion and recommendations

Based on the results of this study we conclude that total FA, FA group and CA contents in milk can be used as NEB level indicators. The changes in FA and CA milk contents were found to be associated with the changes in BCS in the first 17 weeks of lactation in Holstein cows. Both FA and CA contents in milk tended to decrease except for weeks 6 and 7, when they temporally increased, and subsequently BCS repeatedly decreased in the period between weeks 8 and 12 of lactation. The percentage of SFA slightly increased in the observed lactation period, whereas the percentage of UFA tended to decrease, which is closely associated with recovery from NEB. The existence of relationships between total FA (FA group) and CA contents was confirmed by strong to moderate correlation coefficient. The results demonstrated relationships between the severity of NEB in cows and milk components, which can be used as NEB indicators. In addition, they also influence the nutritive quality of milk as food. Repeated onset of NEB due to inadequate management measures may negatively affect cow herd efficiency. Therefore, the importance of optimum dairy herd management as well was highlighted in this study.

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