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Relationships between milk ketone bodies and selected milk indicators during conventional and extended lactation

Vztahy mezi ketolátkami a vybranými ukazateli mléka v průběhu normované a prodloužené laktace

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ABSTRACT

During lactation, dairy cows undergo various metabolic changes that are reflected not only in milk yield but also in milk composition. This study aimed to determine the milk performance and composition depending on the stage of lactation and describe relationships between energy metabolism indicators (ketone bodies: β-hydroxybutyrate (BHB), acetone) in milk and selected indicators of milk performance and composition. The study was conducted on 1,909 dairy cows (Holstein, Czech Fleckvieh, and their crossbreds), which were divided into four groups according to the stage of lactation (early (6-100 days in milk), mid (101-200 days), late (201-305 days), and extended (>305 days)). The obligatory dynamics of the main milk components were found during the entire lactation, i.e., an increase in the protein content (from 3.30 to 3.76 g/100 g) and fat content (from 4.25 to 4.42 g/100 g) and a decrease in the lactose content (from 5.06 to 4.91 g/100 g). Significantly higher ($P < 0.001$) contents of ketone bodies were found in early and extended lactation than in mid and late lactation. The effect of lipomobilization on milk fat composition is documented by positive correlation coefficients between ketone bodies and long-chain fatty acids (FAs) and medium-chain FAs and negative correlation coefficients between ketone bodies and short-chain FAs. Positive correlation coefficients were calculated between ketone bodies and milk fat, citric acid, fat-to-protein ratio, and somatic cell score, and negative correlation coefficients were calculated between ketone bodies and milk protein, lactose, and free FAs. Our results demonstrate that currently often realized extended lactation in high-yielding dairy cows is similarly demanding as early lactation regarding energy metabolism and, thus, milk composition changes.

Keywords: dairy cows, milk samples, β-hydroxybutyrate, acetone, milk composition, fatty acids

ABSTRAKT

V průběhu laktace dochází u dojnic k různým metabolickým změnám, které se odrážejí nejen v mléčné užitkovosti, ale také ve složení mléka. Cílem této studie bylo stanovit užitkovost a složení mléka v závislosti na stadiu laktace a popsat vztahy mezi ukazateli energetického metabolismu v mléce (kyselina β-hydroxymáselná (BHB) a aceton) a vybranými ukazateli užitkovosti a složení mléka. Studie byla provedena na 1 909 dojnicích (plemena holštýnské, české strakaté a jejich kříženci), které byly rozděleny do čtyř skupin podle stadia laktace (raná, 6-100 dní; střední, 101-200 dní; pozdní, 201-305 dní a prodloužená, >305 dní). V průběhu celé laktace byla zjištěna obvyklá dynamika hlavních složek mléka, tj. zvýšení obsahu bílkovin (z 3,30 na 3,76 g/100 g) a tuku (z 4,25 na 4,42 g/100 g) a snížení obsahu laktózy (z 5,06 na 4,91 g/100 g). V období rané a prodloužené laktace byl zaznamenán statisticky významně vyšší ($P < 0,001$) obsah BHB a acetonu. Vliv lipomobilizace na složení mléčného tuku dokumentují pozitivní korelační koeficienty mezi BHB, resp. acetonem a mastnými kyselinami (MK) s dlouhým a středním uhlíkovým řetězcem a negativní korelační koeficienty mezi BHB, resp. acetonem a MK s krátkým uhlíkovým řetězcem. Pozitivní korelační koeficienty byly zjištěny mezi BHB, resp. acetonem a tukem, kyselinou citronovou, poměrem tuk/bílkoviny a somatickými buňkami a záporné korelační koeficienty byly zjištěny mezi BHB, resp. acetonem a bílkovinami, laktózou a volnými MK. Naše výsledky dokládají, že u vysokoprodukčních dojnic je nezbytné sledovat rizika vzniku kétozy a změn složek mléka nejen v období časné laktace, ale také v období prodloužené laktace.

Klíčová slova: dojnice, vzorky mléka, kyselina β-hydroxymáselná, aceton, složení mléka, mastné kyseliny

INTRODUCTION

In recent decades, with increasing milk productivity, the attention of breeders, veterinarians, and nutritional consultants has been mainly focused on successfully managing the transition period as the most demanding period of the entire lactation (Wankhade et al., 2017).

Due to the increased energy requirements at the beginning of lactation, dairy cows practically physiologically enter a negative energy balance (NEB), which can last for several (approximately 6-8) weeks (Gross et al., 2011b; Bruckmaier and Gross, 2017). Although NEB is most common at the beginning of lactation, it can also appear later during lactation due to health disorders associated with decreased appetite (Gross et al., 2011b). The efficiency with which this metabolic discomfort is balanced depends on several factors. Additionally, individual dairy cows differ tremendously in their adaptive success (Sundrum, 2015). Changes in body reserves, i.e., cyclically alternating lipolysis and lipogenesis of adipose tissue, in this regard, certainly belong to the main adaptation mechanisms. These events allow dairy cows to balance energy demands, particularly during the sudden onset of high milk production, conception, and development of the foetus, as well as all other situations associated with a lack of energy (Arfuso et al., 2016). On the other hand, the enormous lipolysis of adipose tissue, followed by ketogenesis, burdens the dairy cow's organism with adverse effects such as impaired immunity, a tendency towards inflammatory processes, and a decrease in both milk production and reproductive performance (Sordillo et al., 2009; Bradford et al., 2015).

As documented in numerous studies, milk yield and composition are closely related to metabolic status (Stoop et al., 2009; Jorjong et al., 2014; Gross and Bruckmaier, 2019). While at the beginning of lactation, despite NEB, milk production rises almost steeply up to the peak of lactation, if NEB was experimentally induced in later lactation, this energy deficiency caused an almost immediate decrease in milk production (Gross et al., 2011b). In other words, the mammary gland (i.e., milk secretion) is metabolically prioritized over the maternal organism itself at the beginning of lactation to ensure the survival of offspring (Useni et al., 2018).

Milk is a suitable medium for assessing the physiological state because the alterations in the blood are closely mirrored by the composition of milk (Gross and Bruckmaier, 2019). In addition, milk compared to blood can be easily and noninvasively obtained at virtually any time during lactation. It is well known that

the relationships between milk components can be used as markers indicating deficiencies in nutrition or risks of various metabolic diseases. For instance, changes in the ratio of milk fat to protein (F/P) may indicate an increased risk for rumen acidosis and ketosis (Gross and Bruckmaier, 2019). An interesting thing in this regard is also the changes in the fatty acid (FA) composition of milk fat at the beginning and later during lactation, which was described in detail in the review by Hanuš et al. (2018). Milk thus represents a suitable tool for obtaining relevant information not only at the level of the organism (i.e., metabolic/energy status) but also of the entire herd.

One of the phenomena related to the increase in milk yield is extended lactation. Although 305-day lactation is considered economically optimal (Yamazaki et al., 2018), for dairy cows with high peak yield, an extended lactation can be justified. Some previous studies have shown that animals with extended lactation have, for instance, greater average milk solid production than animals with conventional 305-day lactation (Kolver et al., 2007; Sorensen et al., 2008). A higher cheese yield production per 100 kg of milk is also reported in connection with increased protein concentration in the milk of dairy cows with an extended lactation (Auldist et al., 2010). The various effects of extended lactation in high-yielding dairy cows on milk production, udder health, body and reproductive measurements have been recently studied (Niozas et al., 2019a; Niozas et al., 2019b).

Energy status and milk composition are subject to profound changes during the course of lactation (Bruckmaier and Gross, 2017). It is essential to realize that thorough knowledge of these contexts in such a sophisticated and overloaded system as the dairy cow's organism is a prerequisite for optimal management at the herd level.

Therefore, the present study aimed *i)* to evaluate selected indicators of milk performance and composition both in conventional (305-day) and extended lactation (over 305 days) depending on the stage of lactation and *ii)* to describe relationships between ketone bodies in milk and selected indicators of milk performance and composition.

MATERIALS AND METODS

Milk sampling (animals)

All sampling was performed following relevant guidelines and regulations recommended by the Ministry of Agriculture of the Czech Republic, applying methodological demands for animal health protection. In our work, cow milk samples were taken exclusively during the regular testing of milk performance and quality, where approval by a properly constituted research ethics committee was not needed.

Milk sample collection was designed to obtain most of the main factors (farm, season, breed, parity, and stage of lactation) that affect milk composition, including milk FA composition.

Milk samples were taken in five commercial dairy herds, four times in each herd. The cows were kept in a free stall housing system with milking parlours. In all herds, cows were fed a total mixed ration. The main components of diets were silages widely used in Czech farming practices (i.e., maize and grass silages). The concentrates were fed according to milk yield and nutrition demand standards.

In total, 20 samplings were carried out in two consecutive years, and the number of samples ranged from 104 to 467 per sampling. The composite milk samples (i.e., milk from all four cow quarters) were taken from 1,909 cows, of which 36.1% cows were Holstein breed, 38.4% were Czech Fleckvieh breed (a dual-purpose breed on the Simmental basis), and 25.5% were crossbreeds of these breeds (of which 88.7% were dairy cows with a predominance of Czech Fleckvieh). The number of samples taken from individual dairy cows varied: 17.8% of dairy cows were sampled four times, 40.5% three times, 23.8% twice, and 18.0% once. Therefore, the dataset represents, in total, 4,158 milk samples obtained from various farms, different seasonal periods, different breeds, and different parities and stages of lactation (Table 1). Detailed information on average values of parity and days in milk according to the stage of lactation are also shown in Table 2 (see chapter Results).

Table 1. Characteristics of obtained milk samples (n = 4,158)

Factor	Group	Frequency (%)	Factor	Group	Frequency (%)
Farm	1	25.0	Season[2]	Summer	50.1
	2	35.8		Winter	49.9
	3	13.1	Parity	Primiparous	34.0
	4	17.3		Multiparous	66.0
	5	8.8	Stage of lactation[3]	Early	30.9
Breed[1]	H100	36.9		Mid	32.9
	C100	37.3		Late	29.1
	Crossbreds	25.8		Extended	7.1

[1] H100 – Holstein, C100 – Czech Fleckvieh
[2] Summer – June, July, August, September, October; Winter – November, December, February, March, April
[3] Early (6-100 days in milk), Mid (101-200 days), Late (201-305 days), Extended (>305 days)

Analysis of milk samples

Analyses of milk samples were carried out in the accredited laboratory according to CSN EN ISO/IEC 17025 (CNI, 2018) by the Czech Institute for Accreditation as national authority and by International Committee for Animal Recording) of the Czech-Moravian Breeders Corporation for milk recording in Buštěhrad on the CombiFoss FT+ device (FOSS - FOSS Electric A/S, Hillerød, Denmark). CombiFoss is based on infrared spectroscopy in the central region using a Michelson interferometer and data processing by Fourier transformation (FT-MIR) and flow cytometry (FC). Milk indicators were determined as follows: fat, protein, and lactose content; acetone; citric acid; β-hydroxybutyrate (BHB); urea; free FAs (FFAs); and somatic cell count (SCC).

The content of milk FAs was obtained by processing the values according to FOSS Application Note 64 (Samková et al., 2020). The units in g FA per 100 g milk were converted to g FA per 100 g total FAs by the following model:

$$FAs\ (g/100\ g\ of\ FAs) = FAs\ (in\ milk) \times 100/MF \times 0.95,$$

where FAs (in milk) = FAs and groups of FAs (g/100 g in milk) determined by the FT-MIR, MF = milk fat (g/100 g) determined by the FT-MIR, and 0.95 = conversion factor from total fat to total FAs.

Three groups of FAs, short-chain FAs (SCFAs), medium-chain FAs (MCFAs), and long-chain FAs (LCFAs), and three individual FAs (palmitic, stearic, and oleic acids) were obtained.

Statistical analysis

Statistica CZ, version 12 (StatSoft CR) software was used for statistical calculations. Indices F/P (fat to protein ratio) and F/L (fat to lactose ratio) were calculated. The number of somatic cell counts was converted to a log score to ensure the normal distribution of the trait according to the following formula:

$$SCS = \log_2 (SCC/100) + 3,$$

where SCS is somatic cell score, and SCC is the number of somatic cell counts (in thousands of units/ml).

One-way ANOVA and unequal N HSD Tukey post-hoc comparisons at level of significance $P < 0.001$ were used for statistical evaluation of the effect of the stage of lactation. Correlation analysis was used to assess the relationship between the indicators of cows' energy balance (contents of BHB and acetone in milk) and selected indicators of milk performance and composition. Pearson correlation coefficients (r) were used at the usual levels of significance (0.05; 0.01; 0.001).

RESULTS

Milk performance and composition depending on the stage of lactation

Table 2 shows indicators of milk performance (daily milk yield, content of basic milk components) and selected indicators of dairy cows' energy metabolism according to the stage of lactation. A high standard of milk performance is characterized by the average daily milk yield in early lactation (32.3 ± 9.7 kg) and the high contents of basic milk components, especially milk fat (4.25 ± 0.71 g/100 g). During the entire lactation, changes in milk indicators were observed. The relative decrease in daily milk yield was only -9.0% between early and mid-lactation and -16.7% between mid and late lactation. A further decrease (-16.7%) was found between late and extended lactation. The main milk components documented an obligatory dynamic, i.e., an increase in the protein content (from 3.30 to 3.76 g/100 g; $P < 0.001$) and fat content (from 4.25 to 4.42 g/100 g) and a decrease in the lactose content (from 5.06 to 4.91 g/100 g; $P < 0.001$). The highest dynamics were observed for protein, an increase of +13.9%, and the lowest for lactose, a decrease of -3.0%. In fat content, the increase was also low (+4.0%). The contents of milk fat, protein, and lactose in extended lactation did not statistically differ from these contents in the previous (late) stage of lactation, even with lower milk performance.

Table 2. Selected indicators of milk performance and energy metabolism of dairy cows according to the stage of lactation

Indicators[1]	Stage of lactation (days in milk)							
	Early (6-100)		Mid (101-200)		Late (201-305)		Extended (>305)	
	mean	SD	mean	SD	mean	SD	mean	SD
n	1296		1379		1207		276	
Parity	2.6^{b}	1.6	2.4^{ab}	1.5	2.3^{a}	1.4	2.0^{a}	1.4
Days in milk	52^{a}	26	150^{b}	29	249^{c}	30	353^{d}	57
Indicators of milk performance								
Milk yield (kg)	32.3^{d}	9.7	29.4^{c}	8.6	24.5^{b}	8.2	20.4^{a}	6.3
Fat (g/100 g)	4.25^{a}	0.71	4.21^{a}	0.61	4.40^{b}	0.63	4.42^{ab}	0.64
Protein (g/100 g)	3.30^{a}	0.32	3.51^{b}	0.32	3.68^{c}	0.34	3.76^{c}	0.36
Lactose (g/100 g)	5.06^{b}	0.22	5.02^{b}	0.21	4.96^{a}	0.27	4.91^{a}	0.29
Indicators of energy metabolism								
Fat/Protein	1.30^{b}	0.23	1.20^{a}	0.17	1.20^{a}	0.16	1.18^{a}	0.14
Fat/Lactose	0.84^{a}	0.15	0.84^{a}	0.13	0.89^{b}	0.14	0.90^{b}	0.15
BHB (mmol/l)	0.068^{b}	0.055	0.052^{a}	0.038	0.061^{b}	0.052	0.077^{b}	0.055
Acetone (mmol/l)	0.172^{b}	0.128	0.129^{a}	0.069	0.131^{a}	0.082	0.150^{a}	0.083
Urea (mmol/l)	4.55^{a}	1.19	4.85^{b}	1.29	4.69^{ab}	1.37	4.29^{a}	1.41
Citric acid (mmol/l)	10.50^{b}	1.39	10.14^{a}	1.28	10.15^{a}	1.32	9.77^{a}	1.58
FFA (mmol/100 g of fat)	0.811^{a}	0.392	0.923^{b}	0.419	0.977^{b}	0.400	0.902^{ab}	0.408
SCS	2.955^{a}	1.827	3.127^{a}	1.687	3.495^{b}	1.629	4.227^{c}	1.602

^{a,b,c,d} means within a row with different superscripts indicate statistical significance at $P < 0.001$

[1] BHB – β-hydroxybutyrate, FFA – free fatty acids, SCS – somatic cell score

Changes in the content of milk fat and protein during the entire lactation are also expressed by the F/P ratio. The highest F/P was found in early lactation (1.30 ± 0.23), and its subsequent slight decrease was related to the gradual increase in the milk protein content.

Compared to mid-lactation, the average content of ketone bodies in early lactation was significantly higher (+30.8% for BHB, and +33.3% for acetone). Increased values of ketone bodies were also observed in extended lactation. Compared to mid-lactation, the BHB value was higher by +48.1% and acetone by +16.3% in extended lactation.

The average urea content did not exceed 5.0 mmol/l. In early lactation, the urea content was 4.55 ± 1.19 mmol/l, and at the same time, the lowest protein content (3.30 g/100 g) was observed. The lowest urea content was in extended lactation (4.29 ± 1.41 mmol/l). The citric acid content gradually decreased during the entire lactation (from 10.50 to 9.77 mmol/l; $P < 0.001$).

The content of FFAs in milk fat showed a slight increase during the entire lactation. The lowest content was in early lactation (0.811 ± 0.392 mmol/100 g of fat), and the highest content was in late lactation (0.977 ± 0.400 mmol/100 g of fat). The relative difference between the mentioned stages reached +20.5%.

The average values of SCS gradually increased during the entire lactation. Statistically higher values ($P < 0.001$) were found in late and extended lactation (3.495 ± 1.629; 4.227 ± 1.602) than in early and mid-lactation (2.955 ± 1.827 and 3.127 ± 1.687).

The proportion of FAs in milk fat and their groups is shown in Table 3. The proportion of SCFAs gradually decreased during lactation (from 10.8 to 10.2%; $P < 0.001$). While the MCFA proportions were lower in early and extended lactation (45.1 ± 8.7 and 46.7 ± 9.4%), the LCFA proportions were higher (39.4 ± 7.1 and 37.4 ± 4.7%) than in the other two stages (mid and late lactation).

The proportion of palmitic acid, as the main representative of the MCFA group, was the lowest in early lactation (36.0 ± 4.5%) and the highest in mid-lactation (37.8 ± 3.8%). The proportion of stearic acid was the highest (15.1 ± 2.0%) in early lactation, and then a gradual decrease was noted. The dynamics of oleic acid were different. High proportions were found in both early and extended lactation (29.8 ± 5.0% and 29.3 ± 3.4%, respectively).

Relationship between milk ketone bodies and selected milk indicators

The relationships between ketone bodies (β-hydroxybutyrate, acetone) and selected indicators of milk performance respectively milk components at different stages of lactation are summarized in Table 4. The highest positive correlation coefficients were found between ketone bodies - BHB and acetone (from +0.7747 to +0.8422, $P < 0.001$).

During the entire lactation, the negative influence increased between ketone bodies (BHB and acetone) and milk yield. While in early lactation, a practically zero relationship was observed for BHB (-0.0041), in late and extended lactation, the relationship was closer (-0.2056 and -0.2195; $P < 0.001$). In the case of acetone, statistically significant correlation coefficients were found in early lactation (-0.1440, $P < 0.001$), with the fact that they were stronger again in late and extended lactation (-0.2456 and -0.2175, $P < 0.001$).

Among the basic milk components, the above mentioned ketone bodies affect lactose the most, negatively, and in all monitored stages of lactation (from -0.1947 to -0.5411, $P < 0.001$). The negative effect, given by positive correlation coefficients, was also found for SCS (from +0.0562, $P > 0.05$ to +0.4049, $P < 0.001$).

Between ketone bodies (BHB and acetone) and urea, the correlation coefficients were strongest in the final stages of lactation (i.e., late and extended) when an increase in BHB and acetone values was reflected in a decrease in urea content.

Table 3. Fatty acids (FAs) and their groups in milk (g/100 g of milk) and milk fat (g/100 g of FAs) according to the stage of lactation

Indicators[1]	Stage of lactation (days in milk)							
	Early (6-100)		Mid (101-200)		Late (201-305)		Extended (>305)	
	mean	SD	mean	SD	mean	SD	mean	SD
n	1296		1379		1207		276	
	g/100 g of FAs							
SCFA	10.8^{c}	1.5	10.7^{bc}	1.4	10.5^{ab}	1.4	10.2^{a}	1.4
MCFA	45.1^{a}	8.7	47.2^{b}	9.1	47.2^{b}	9.0	46.7^{ab}	9.4
LCFA	39.4^{c}	7.1	34.6^{a}	4.1	36.3^{b}	4.3	37.4^{b}	4.7
Palmitic acid	36.0^{a}	4.5	37.8^{c}	3.8	37.0^{b}	3.6	36.8^{abc}	3.2
Stearic acid	15.1^{b}	2.0	13.3^{a}	1.4	13.2^{a}	1.3	13.1^{a}	1.4
Oleic acid	29.8^{c}	5.0	26.8^{a}	3.2	28.1^{b}	3.4	29.3^{bc}	3.4
	g/100 g of milk							
SCFA	0.44	0.11	0.43	0.10	0.44	0.10	0.43	0.11
MCFA	1.84^{a}	0.53	1.90^{a}	0.52	1.99^{b}	0.53	1.98^{ab}	0.57
LCFA	1.60^{c}	0.46	1.38^{a}	0.27	1.52^{b}	0.29	1.58^{bc}	0.34
Palmitic acid	1.45^{a}	0.29	1.51^{b}	0.28	1.55^{b}	0.28	1.55^{b}	0.29
Stearic acid	0.61^{c}	0.14	0.53^{a}	0.09	0.55^{b}	0.09	0.55^{ab}	0.10
Oleic acid	1.21^{b}	0.32	1.07^{a}	0.19	1.17^{b}	0.21	1.23^{b}	0.24

^{a,b,c} means within a row with different superscripts indicate statistical significance at $P < 0.001$

[1] SCFAs – short-chain FAs, MCFAs – medium-chain FAs, LCFAs – long-chain FAs

The opposite trend was noted in the relationship between ketone bodies and citric acid. In all stages of lactation, correlations between ketone bodies (BHB and acetone) and FA groups sorted by carbon number were statistically significant, both when expressed in g/100 g FAs and g/100 g of milk. While negative correlation coefficients were found for SCFAs (except for early lactation, in g/100 g of milk), positive correlation coefficients were found for MCFAs and LCFAs.

The tightness of the relationship has changed during the entire lactation. For SCFAs, the highest correlation coefficient was found in the extended lactation (-0.4367 and -0.4375, $P < 0.001$, g/100 g FAs; -0.2109 and -0.2640, $P < 0.001$, g/100 g of milk). For LCFAs, the highest correlation coefficient was found in early lactation (+0.4319 and +0.4869, $P < 0.001$, g/100 g FAs; +0.4983 and +0.5042, $P < 0.001$, g/100 g of milk).

Table 4. The relationships (Pearson correlation coefficient) between β-hydroxybutyrate (BHB), acetone, and selected indicators of milk performance and composition in different stages of lactation

Indicators[1]	Ketone bodies							
	BHB (mmol/l)				Acetone (mmol/l)			
Stage of lactation	Early	Mid	Late	Extended	Early	Mid	Late	Extended
n	1277	1376	1202	270	1277	1376	1202	270
Milk yield (kg)	-0.0041	-0.0427	-0.2056***	-0.2195***	-0.1440***	-0.1416***	-0.2175***	-0.2456***
Fat (g/100 g)	0.3030***	0.0256	0.0265	0.0521	0.2348***	-0.0842**	-0.0954***	-0.0402
Protein (g/100 g)	-0.2200***	-0.1767***	-0.1307***	-0.0739	-0.1566***	-0.1220***	-0.1616***	-0.1470*
Lactose (g/100 g)	-0.3984***	-0.4295***	-0.5411***	-0.5388***	-0.2730***	-0.1947***	-0.3308***	-0.3439***
Fat/Protein	0.4198***	0.1470***	0.1153***	0.1320*	0.3284***	-0.0066	0.0082	0.0749
Fat/Lactose	0.3950***	0.1668***	0.2607***	0.2714***	0.2998***	-0.0094	0.0628*	0.1086
BHB (mmol/l)	x	x	x	x	0.8171***	0.7747***	0.8068***	0.8422***
Acetone (mmol/l)	0.8171***	0.7747***	0.8068***	0.8422***	x	x	x	x
Urea (mmol/l)	0.0191	-0.0103	-0.1028***	-0.2594***	0.0312	-0.0031	-0.1047***	-0.2606***
Citric acid (mmol/l)	0.4126***	0.1866***	0.0839**	0.0574	0.3525***	0.2372***	0.1878***	0.1481*
FFA (mmol/100 g of fat)	-0.1553***	-0.1869***	-0.2398***	-0.2614***	-0.1858***	-0.1765***	-0.2166***	-0.1980**
SCS	0.1876***	0.2073***	0.2826***	0.4049***	0.0836**	0.0562	0.1369***	0.2111***
	(g/100 g of FAs)							
SCFA	-0.1728***	-0.2226***	-0.3455***	-0.4367***	-0.0988***	-0.1397***	-0.2870***	-0.4375***
MCFA	0.1905***	0.2260***	0.2977***	0.3276***	0.1789***	0.2306***	0.2752***	0.2207***
LCFA	0.4319***	0.0588*	0.0947**	0.1553*	0.4869***	0.1810***	0.2388***	0.2567***
Palmitic acid	-0.1851***	0.1324***	0.1494***	0.1743**	-0.1665***	0.1574***	0.1927***	0.0707
Stearic acid	0.3385***	0.0466	0.0406	0.0740	0.4045***	0.1786***	0.2049***	0.1852**
Oleic acid	0.3448***	-0.0435	0.0135	0.0816	0.4201***	0.0522	0.1352***	0.2205***
	g/100 g of milk							
SCFA	0.0977***	-0.1033***	-0.1890***	-0.2109***	0.0953***	-0.1248***	-0.2267***	-0.2640***
MCFA	0.3097***	0.1721***	0.2269***	0.2515***	0.2653***	0.1165***	0.1463***	0.1319*
LCFA	0.4983***	0.0695**	0.0940**	0.1442*	0.5042***	0.0680*	0.0945**	0.1489*
Palmitic acid	0.1081***	0.0934***	0.1016***	0.1239*	0.0618*	0.0191	0.0270	0.0007
Stearic acid	0.4664***	0.0676*	0.0539	0.0922	0.4566***	0.0525	0.0446	0.0889
Oleic acid	0.4655***	0.0076	0.0473	0.1069	0.4818***	-0.0095	0.0338	0.1260*

*** significant at $P < 0.001$, ** significant at $P < 0.01$, * significant at = $P < 0.05$

[1] FFA – free fatty acids, SCS – somatic cell score (n = 1113, 1184, 1056, 259 for early, mid, late and extended lactation, respectively), SCFAs – short-chain FAs, MCFAs – medium-chain FAs, LCFAs – long-chain FAs

DISCUSSION

Modern dairy farming often results in forced milk production, giving rise to metabolic disorders in cows (Andjelić et al., 2022). The highest incidence of metabolic disorders in dairy cows is observed in the early postpartum period (Vergara et al., 2014) when sharply elevated energy requirements for milk production combined with a relatively low energy intake can result in NEB (de Vries and Veerkamp, 2000).

However, NEB can occur practically at any time during lactation if feed intake is restricted for some reason (Gross et al., 2011b; Billa et al., 2020). Ketone production in the liver in cows suffering from NEB causes an accumulation of ketone bodies in blood and in milk (Duffield, 2000; Enjalbert et al., 2001; Guliński, 2021; Song et al., 2021). In particular, BHB and acetone, both in blood and in milk, are helpful indicators for the early detection of energy metabolism disorders (de Roos et al., 2007; Klein et al., 2020; Xu et al., 2020).

However, the contents of the parallel milk components, F/P and F/L ratios, level of citric acid, FFAs and other compounds can also be used as energy metabolism indicators (Friggens et al., 2007; Manzenreiter et al., 2013; Weber et al., 2013; Larsen and Moyes, 2015; Cabezas-Garcia et al., 2021; Churakov et al., 2021). In addition, the deficiencies caused by NEB are reflected in the proportions of FA in milk fat (Gross et al., 2011a; Mann et al., 2016).

In our study, considerable changes, compared to mid and late lactation, were observed in most milk indicators not only in early but also in extended lactation, i.e., lactation over 305 days (Tables 2 and 3). Moreover, it is evident from the correlation coefficients that most of the monitored indicators are closely related (Table 4).

A metabolically highly demanding early lactation is characterized by high milk performance and high levels of ketone bodies, as documented Krnjaić et al. (2022). The high contents of BHB and acetone in milk (Santschi et al., 2016; Benedet et al., 2019) are related to increased body energy reserve mobilization (De Koster et al., 2018; Ha et al., 2023).

The relationship between NEB and lipomobilization reflects the positive correlations between BHB and acetone, as well as between both ketone bodies and fat content (+0.3030; +0.2348), the F/P ratio (+0.4198; +0.3284) and the F/L (+0.3950; +0.2998), which were the highest in early lactation. The energy intake and demand imbalance leading to NEB in early lactation is also confirmed by unused nitrogen in the form of a higher urea content (4.55 mmol/l) with a lower milk protein content (3.30 g/100 g). This is related to the low but negative correlation coefficients between ketone bodies and the milk protein content (-0.2200, -0.1566).

Osorio et al. (2016) reported that protein synthesis in the mammary gland is a highly energy-demanding process. Thus, protein synthesis might be restricted due to energy deficiency in early lactation. This is supported by other studies (Friggens et al., 2007; Bondan et al., 2018; Chandler et al., 2018).

A citric acid content of 10 mmol/l corresponds to the physiological state and is a prerequisite for sufficient buffering capacity of milk (Garnsworthy et al., 2006; Hanuš et al., 2010; Ducháček et al., 2012). The content of citric acid (10.50 mmol/l) in early lactation shows a relatively close connection to ketone bodies (+0.4126; +0.3525). However, this does not correspond to the data on the dynamics of citric acid, the content of which should decrease in connection with the energy deficit (Baticz et al., 2002; Ducháček et al., 2012; Xu et al., 2020). The relationship between energy deficiency and the level of ketogenesis is evident in all stages of lactation, which is also confirmed by the negative correlation coefficients between the level of BHB and lactose (-0.3984 to -0.5388 for BHB).

High levels of milk ketone bodies were also observed in extended lactation. It also reflects increased energy requirements in this group of dairy cows, which can negatively affect drying-off dairy cows and their regeneration for the next lactation. The highest value of SCS and the highest correlation coefficient between BHB or acetone and SCS (+0.4049; +0.2111) were also determined.

The composition of FAs sorted according to the chain length was consistent with general information on their content in bovine milk fat (Lindmark Månsson, 2008; Markiewicz-Kęszycka et al., 2013; Hanuš et al., 2018). The distribution was similar in all stages of lactation. However, differences were observed in their dynamics. During the entire lactation, the greatest changes in dynamics were observed for LCFAs (relative decrease between early and mid-lactation was -12,1%). The high proportion of LCFAs (39.4 g/100 g of FAs) in early lactation can be related to the aforementioned lipomobilization, subsequent increased nonesterified FA levels, and increased ketogenesis (Grummer, 2008; Kessel et al., 2008; van Dorland et al., 2009; Gross et al., 2013; Sun et al., 2016; Belić et al., 2018; Štolcová et al., 2020). This is supported by the statistically significant values of the correlation coefficients in early lactation between BHB or acetone and LCFAs (+0.4319; +0.4869) and the negative correlation between ketone bodies and SCFAs throughout the entire lactation (from -0.0988 to -0.4375). The subsequent decrease in LCFAs, pronounced in mid-lactation, and the associated slight increase in MCFAs corresponds to the data of Bilal et al. (2014). LCFAs derived from blood and incorporated into milk fat have also been shown to inhibit the synthesis of SCFAs and MCFAs. SCFAs and MCFAs, or FAs with 14 carbons, are synthesized *de novo* directly in the mammary gland (Štolcová et al., 2020).

CONCLUSION

The proportion of FAs in milk fat and the content of selected milk indicators were evaluated concerning energy metabolism, characterized by milk ketone bodies (BHB, acetone), in a large group of dairy cows during 305-day lactation and in extended lactation (over 305 days). The highest contents of BHB and acetone were found in the milk of dairy cows in early and extended lactation. The most favorable state regarding energy balance and risk of increased ketogenesis was in dairy cows in mid and late lactation. Our results show that it is necessary to focus on early lactation and extended lactation, both in terms of ketosis and milk composition, especially in high producing dairy cows.

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