DOI: /10.5513/JCEA01/24.1.3766

The effect of beta-hydroxybutyrate concentration in the blood on reproduction, production, and health of cows in the first weeks after calving

Efekt koncentrace beta-hydroxybutyrátu v krvi na reprodukci, produkci a zdraví krav v prvních týdnech po otelení

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Received: October 14, 2022; accepted: December 4, 2022

ABSTRACT

The constant monitoring of dairy cow metabolism is extremely important, especially in the period immediately after calving, when the animals face the consequences of negative energy balance. The concentration of beta-hydroxybutyrate is one of the reliable indicators of metabolic status. The aim of this work was to evaluate the effect of beta-hydroxybutyrate concentration in the blood on selected parameters of production, reproduction, and health of Holstein dairy cows. The limit for subclinical ketosis was set to >1.2 mmol/L of beta-hydroxybutyrate in blood. Furthermore, the effects of lactation number, season, and year of assessment were added to the model equation. The results showed that high beta-hydroxybutyrate concentration in blood is associated with poor reproduction parameters (worse insemination interval and service period) and lower milk production. However, the results were ambiguous in relation to the somatic cell count and the occurrence of selected diseases (mastitis and ovarian cysts). Moreover, the effect of seasonality on the evaluated parameters was also confirmed ($P<0.05$), while assessed years were similar in most parameters. It can be concluded that the assessment of beta-hydroxybutyrate concentration as an indicator of potential ketosis is important and can be a suitable parameter for routine monitoring as it influences milk production, reproduction, and cow's health.

Keywords: ketosis, ovarian cysts, service interval, milk production, somatic cells count

ABSTRAKT

Sledování úrovně metabolismu u dojnice je neustále důležité a to hlavně v období bezprostředně po otelení, kdy jsou zvířata ohrožena důsledky negativní energetické bilance. Jedním z dobrých ukazatelů úrovně metabolismu je koncentrace beta-hydroxybutyrátu. Cílem této práce bylo zhodnotit vliv koncentrace beta-hydroxybutyrátu v krvi na vybrané parametry produkce, reprodukce a zdraví dojnic holštýnského skotu. Jako hranice subklinické ketózy byla nastavena hodnota beta-hydroxybutyrátu v krvi 1.2 mmol/L. Dele bylo provedeno vyhodnocení s využitím efektů pořadí laktace, kalendářního měsíce a roku hodnocení Z výsledků vyplývá, že je vysoká úroveň beta-hydroxybutyrátu v krvi spojená se zhoršenými parametry plodnosti (horší inseminační interval a servis perioda) a mléčné produkce. Nicméně, ve vztahu k počtu somatických buněk a výskytu vybraných onemocnění (mastitidy a výskyt ovariálních cyst) byly výsledky nejednoznačné. Navíc byl také potvrzen vliv sezónnosti na hodnocené ukazatele ($P<0.05$) a to i přesto, že hodnocené roky byly ve většině parametrů podobné. Z výsledků lze konstatovat, že hodnocení obsahu beta-hydroxybutyrátu jako indikátoru potenciální ketózy je důležité a potvrzuje se jako vhodný parametr pro rutinní monitoring ovlivňující mléčnou produkci, reprodukci a zdraví krav.

Klíčová slova: ketóza, ovariální cysty, inseminační interval, mléčná produkce, počet somatických buněk

INTRODUCTION

Dairy farming with the goal of having healthy cows with high yields of high-quality milk and proper reproduction is not achievable without optimal breeding conditions and well-functioning management. Animal welfare is the result of many external and internal factors. Inadequate conditions may result in increased stress for the cow and behaviour changes, which disturb the metabolism. The end result is metabolic disorders, decreased milk production, and worse reproduction. The dry period followed by the transition into production is one of the most demanding periods for dairy cows. It is characterised by metabolic, hormonal, and morphological changes in the organism (Leroy et al., 2017). The preparation for parturition is difficult, and it is vital to allow dairy cows to have maximum rest, sufficient feeding, and regular health checks. Numerous health complications during this period, especially after parturition, are associated with insufficient nutrition and energetic disbalance.

Dairy cows are in negative energy balance during the postpartum period, which results in lipomobilization (Tóthová et al., 2014), and the development of liver steatosis and ketosis (Remppis et al., 2011). Clinical and subclinical ketosis is an energy metabolism disorder manifested by the increased production of ketones and their excessive amounts in urine, blood, and milk. Acetone (AC), acetoacetate, and beta-hydroxybutyrate (BHB) are the most commonly detected ketone bodies in the blood. Ketosis is also accompanied by an increase in non-esterified fatty acids (NEFA) and a decrease in glucose concentration (less than 2.2 mmol/L) in the blood (Overton et al., 2017; Djoković et al., 2017).

The highest risk for ketosis is immediately after calving and at the beginning of lactation (10 - 60 days). Several studies on the incidence of ketosis have been conducted around the world. Summary data from several countries indicate that an average of 11.2-36.6% of dairy cows develop subclinical ketosis (Suthar et al., 2013). The clinical form manifests itself mainly in high-yielding cows immediately after birth and concerns 0.4-11.1% (Suthar et al., 2013).

The metabolism of adipose tissue is a major factor that can affect cow energy metabolism and subsequently incidence of metabolic disorders (Contreras et al., 2018). A negative energy balance results in an energy deficit. The affected cows then use body fat in the form of non-esterified fatty acids (NEFA) to cover for this deficit, which can be transformed into glucose by gluconeogenesis in the liver. Fat mobilisation (NEFA) increases 5-10 times in the postpartum period compared to the period before the calving. Most fat is used as an energy source for dairy cow metabolism, but some can also be stored in the liver. Mobilised fat can also be used as an energy source for milk fat synthesis (Smith, 2017). Oxidation of triglycerides produces acetyl coenzyme A (acetyl CoA). However, the deficiency of oxalacetate for the normal function of the Krebs cycle during the negative energy balance will push a surplus of acetyl CoA towards the overproduction of ketones, resp. beta-hydroxybutyric acid (Xu et al., 2008). There are three types of ketosis that have different factors of origin (Marczuk et al., 2018). Production ketosis is usually caused by inadequate nutrition of dairy cows at the beginning of lactation, followed by rapid/excessive mobilisation of fat reserves and later by an accumulation of ketone bodies in the blood. Secondary ketosis occurs due to a lack of feed and poor health at the same time (Pechová et al., 2018). The third type, alimentary ketosis, is caused by low-quality silage, which contains an above-limit concentration of butyric acid that is later converted to beta-hydroxybutyrate (BHB) during rumen processing (Guliński, 2021). Different types of ketosis are always accompanied by a high content of BHB in the blood. They can cause a reduction in milk production, change in milk quality, reduced feed intake, acetone odour in breath and urine, and reproductive or even neurological problems. Clinical ketosis occurs when the concertation of BHB rises above 3 mmol/L, while subclinical ketosis can be defined as higher than 1.4 mmol/L (Smith et al., 2017). Although, Itle et al. (2015) set the limit for the occurrence of ketosis at the level of 1.2 mmol/L.

The literature shows that BHB is a crucial indicator of metabolic status. It can directly affect not only production but also the reproduction and health of dairy cows in the first weeks after calving. The study aimed to investigate the relationships between the concentration of BHB in blood and parameters of reproduction, production, and health, and also to identify parameters significantly affected by BHB concentration.

MATERIALS AND METHODS

This experiment was carried out in accordance with Czech legislation for the protection of animals against abuse (No. 246/1992) and with directive 2010/63/EU on the protection of animals used for scientific purposes.

Farm and Animals

The study was conducted in the production environment of a commercial dairy farm located in the Hradec Králové Region. The production herd consists of around 400 Holstein cows (red variant). Milk production for standardized lactation was 8,864 kg, the fat content was 4.59%, and the protein content was 3.55%. The dairy cows were housed in a free-stall barn, and beds were bedded twice per day with straw and added limestone. High-pregnancy and dry cows were housed in separate barns complemented with paved paddocks. The nutrition of production cows was based on a mixed feed ration (TMR), consisting of corn silage (18 kg/pc), grass silage (21 kg/pc), a mixture of cereals (9.5 kg/pc), and malt (6 kg/pc). Feed pushing was performed ten times a day. Prior to the calving, the cows received grass silage (12 kg/pc), corn silage (3 kg/pc), straw (2 kg/pc), hay (approx. 1 kg/pc), a mixture of concentrated grain feed (1 kg/pc) and a mixture of minerals.

Experimental Design

The aim of the experiment was to measure the concentration of beta-hydroxybutyrate (BHB) in the blood in relation to selected parameters of milk yield, reproduction, and health. After calving, cows always received the carbohydrate drink BoviFit (Sano Ltd. CZE, Domažlice, Czech Republic), and older cows on second and higher lactation were given a calcium bolus. All cows received an energy drink with the addition of propylene glycol on the second day after calving. Calcium and glucose were reapplied if postpartum paresis occurred. The blood was collected on the seventh day after calving from the tail vein using a standard single-use Hemos tube with a short needle (GAMA GROUP, a.s., Dalečín, Czech Republic). The blood was analysed by a FreeStyle Optimum instrument (Abbott Diabetes Care Ltd., Maidenhead, Berkshire, UK), and the concentration of BHB was determined. The dairy cows were again given glucose, vitamin B12 and other vitamins if the BHB value exceeded a concentration above 0.9 mmol/L.

Data Collection

Milking took place twice per day in a dovetail herringbone parlour (Farmtec a.s., Jistebnice, Czech Republic). Data about milk yield and somatic cell count were taken from in-line real-time milk analysers (Afilab; Afifarm; Afikim; Israel). Reproduction management was based on artificial insemination and visual oestrus detection by caretakers and farm managers (3 times per day). The insemination interval was 60 days on average. If the cow did not get pregnant after the third insemination dose, ID Extra with double sperm count was used. The age of heifers at the first insemination was around 14 months. Pregnancy detection was performed by a veterinarian 30 days after the last insemination with sonography. The experiment lasted 20 months and 339 dairy cows participated in the evaluation, from which 134 samples were taken from the cows on the first lactation, 88 samples from the cows on the second lactation, and 117 samples from the cows on the third and higher lactation. Complementary data were taken from farm evidence (lactation number, reproduction results) and veterinary records (cyst incidence, mastitis incidence).

Statistical Evaluation

SAS 9.4 (SAS Institute Inc., Cary, NC, USA) was used for statistical evaluation. The UNIVARIATE procedure was used to determine the basic statistics. The STEPWISE method in the REQ procedure was used to select a suitable model for the evaluation of selected reproductive (number of insemination doses, insemination interval, service period), production (kg of milk for the first 100 days of lactation – MY100, milk in the first week of lactation - MYw1, milk in the second week of lactation - MYw2), and health parameters (number of somatic cells in the first week after calving - SSCw1, number of somatic cells in the second week after calving - SSCw2, the incidence of mastitis % and incidence of cysts %). The effect of lactation order was adjusted to three levels due to the low frequency of higher lactation cows in the test (1st lactation, 2nd lactation, 3rd and higher lactation). Two groups (<1.2 and above 1.2 mmol/L) were created based on the evaluation of BHB concentration, as was also recommended in the study of Itle et al. (2015). The GLM procedure was used to evaluate statistically significant differences among monitored animals and groups, followed by a detailed evaluation by the Tukey-Kramer test.

Model equation:

$$y_{ijklm} = \mu + a_i + b_j + c_k + d_l + e_{ijklm}$$

where:

y_{ijklm} - values of the dependent variable (the number of insemination doses, insemination interval, service period, milk yield for the first 100 days in milk in kg – MY100, milk in the first week of lactation - MYw1, milk in the second week of lactation - MYw2, the number of somatic cells in the first week of lactation - SSCw1, the number of somatic cells in the second week of lactation - SSCw2, the incidence of mastitis as a %, the incidence of cysts as a %),

μ - a general value of the dependent variable,

a_i - fixed effect of lactation number (i = 1., n = 134; i = 2., n = 88; i = 3. and others, n = 117),

b_j - fixed effect of BHB (j = <1.2 mmol/L, n = 302; j = > 1.2 mmol/L, n = 37),

c_k - fixed effect of the season (k = March to May – spring period, n = 67; k = June to August – summer period, n = 73; k = September to November – autumn period, n = 110; k = December to February – winter period, n = 89),

d_l - fixed effect of the year (l = 2016, n = 136; l = 2017, n = 203),

e_{ijklm} - random residual error.

Significance levels *P*<0.01, and *P*<0.05 were used to evaluate statistical significance.

RESULTS AND DISCUSSION

The average BHB content for both observed years was 0.69 mmol/L with a standard deviation of 0.65 (minimum 0.1 and maximum 5.6 mmol/L). These results highlighted the large dispersion of BHB values within this study and confirmed existing differences in the intensity of metabolism of individual dairy cows. Many studies also observed similar results, such as De Vries and Veerkamp (2000) and Leroy et al. (2017). The MY100 in the monitored group of animals ranged from 1728 to 5565 kg. The average insemination interval was 72.94 days, with an average service period of 122.96 days. The greatest variance was observed for SCC when the values range from 7 to 6,528 thousand/mL with an average of 256,360 thousand/mL. This subsequently corresponds to the average occurrence of mastitis of 11.08% and the average occurrence of cysts of 15.04% during the experimental period within the tested herd. The different intensity of metabolism at the beginning of the lactation is reflected in the milk yields and the content of solid components in milk, such as fat and proteins, or their ratio (García et al., 2015; Poljak et al., 2022) but also in the SCC and occurrence of disease (Duffield et al., 2009).

For a better demonstration, the development of BHB in the context of SCC is presented in Figure 1, and the development in the context of milk yield is shown in Figure 2. These figures showed a relatively large fluctuation of values throughout the year, which may be the reflection of heat stress and other environmental factors influencing the farm. Research shows, that the BHB concentration is associated with lower milk yield, poorer reproduction and the worse health status of the animals and is one of the possible indicators for the assessment of dairy cow metabolism (McArt et al., 2013). At the beginning of lactation, dairy cows are usually in a state of negative energy balance (Macrae et al., 2019). This period is characterised by insufficient energy intake, which does not match the increasing milk production (Herdt, 2000).

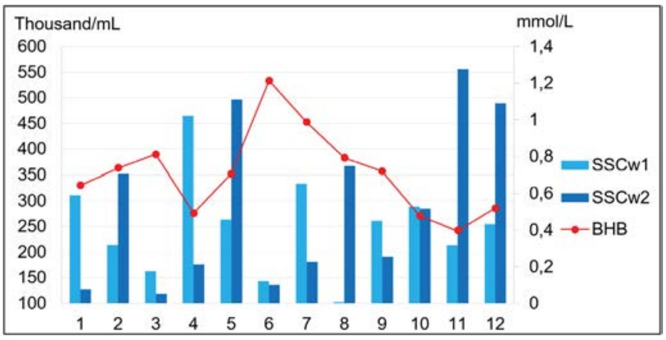

Figure 1. The development of BHB concentration (mmol/L) and SCC during the observed years

SSCw1 - the number of somatic cells in the first week of lactation in thousand/mL, SSCw2 - the number of somatic cells in the second week of lactation in thousand/mL, BHB – beta-hydroxybutyrate concentration in mmol/L

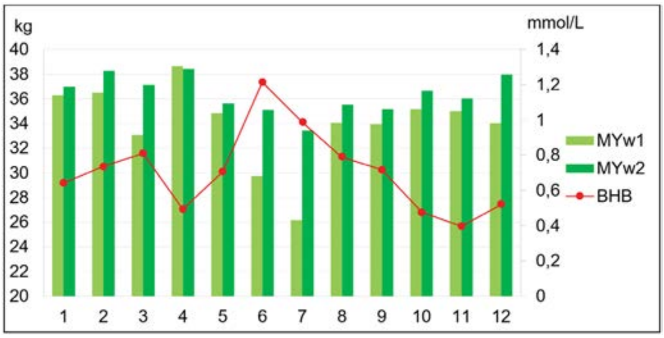

Figure 2. The development of BHB concentration (mmol/L) to the milk yield during the observed years

MYw1 - milk yield in the first week of lactation in kg, MYw2 - milk yield in the second week of lactation in kg, BHB – beta-hydroxybutyrate concentration in mmol/L

As seen from the research of Xu et al. (2018), several sub-indicators of metabolism (milk fat, glycin, choline and carnitine in milk) can be used for the evaluation of the level of negative energy balance.

The chosen model equation for evaluating monitored parameters was statistically significant (*P*<0.01) for the number of insemination doses, insemination interval, service period, MY100, MYw2 and occurrence of cysts. This model equation explained the variability of monitored parameters from 1.8% to 39.2%. The model equation was inconclusive for MYw1, SCCw1, SCCw2 and the occurrence of mastitis. The effect of the lactation number was significant (*P*<0.05) for the evaluation of MY100, MYw2, SSCw1 and the occurrence of cysts. The effect of BHB significantly influenced only the insemination interval and MY100 (*P*<0.05). Furthermore, the effect of the period of the year was significant (*P*<0.05) for the number of insemination doses, insemination interval, service period, and MY100. At last, the effect of the year proved to be statistically significant to the number of insemination doses, the service period and the incidence of cysts (%).

Table 1 and Table 2 show the results for the evaluated parameters of reproduction, health and production. The highest number of needed insemination doses was for cows on 3rd and higher lactation. The insemination interval was the highest for cows on the second lactation and the lowest for primiparous cows. The service period reached the highest values in the oldest cows, while the lowest was observed for the cows on the second lactation.

MY100 increased significantly (*P*<0.01) with increasing lactation number. MYw1 was significantly (*P*<0.01) highest for the cows on the third and higher lactation. In the second week of lactation, there was an increasing tendency for higher milk yield with increasing lactation number (*P*<0.01). The highest SSCw1 was observed for the cows on the second lactation (+155.8 to +227.5 thousand/mL). An increasing linear trend was observed for the SSCw2 with the order of lactation (from 179.67 to 345.66 thousand/mL). The highest incidence of mastitis was observed for the cows on the first lactation (13.61%), although the differences within the groups were not statistically significant. On the other hand, significant differences were found in the occurrence of cysts, and cows on the second lactation demonstrated the highest occurrence (25.42%).

The concentration of BHB was not associated with the number of insemination doses and did not show significant differences for the service period, although the service period for the higher BHB group had been extended by 19 days. Only the insemination interval from monitored reproduction parameters was significantly affected by higher BHB (+19.92 days for BHB above 1.2 mmol/L; *P*<0.01). These results could be influenced by the addition of glucose and vitamin B12 to cows with a level of BHB exceeding 0,9 mmol/L in the first week after calving. The concentration of NEFA and plasma glucose is related to the success of insemination (Garverick et al., 2013). Nevertheless, the results show that reproduction parameters worsen with a higher concentration of BHB as an indicator of a negative energy balance. This relation was observed mainly for a significant increase in the insemination interval, but also inconclusively for an increase in the insemination index and service period. Also, Rutherford et al. (2016) observed that more insemination doses are needed for conception and the insemination interval is prolonged at a higher level of BHB, resp. during subclinical ketosis. These authors also observed less noticeable oestrus symptoms in animals with subclinical ketosis. This relation might be partially explained by the effect of a negative energy balance on the quality of oocytes or embryos (Chaput and Sirard, 2020).

The lower BHB group also showed better results for the monitored milk production parameters. The cows with a BHB concentration below 1.2 mmol/L achieved higher values for MY100d (P<0.05), MYw1 (P<0.01) and MYw2. In the first week of lactation, lower BHB cows produced 5.61 kg more milk compared to the higher BHB group. A BHB concentration above 1.2 mmol/L is associated with hyperketonaemia, a metabolic disease often associated with high-yielding dairy cows (Benedet et al., 2019), which might negatively affect milk production. These metabolic problems might be the reason why the lower BHB group in this study achieved significantly higher MY100. Ospina et al. (2010) also reached similar results when they observed milk yields during normalised lactation. One of the explanations for this relationship can be genetic linkage. Belay et al. (2017) confirmed a positive relationship through genetic correlations between blood BHB content and milk production. On the other hand, some studies have confirmed the negative effect of a high level of BHB in blood or milk on milk yields (McArt et al., 2012; Santschi et al., 2016), although some studies observed the opposite (Vanholder et al., 2015; Ruoff et al., 2017). Moreover, Chapinal et al. (2012) and Santschi et al. (2016) found that the negative effect of hyperketonaemia or subclinical ketosis on milk production is more pronounced in older cows compared to primiparous cows. However, these differences might be due to differences in trial designs as well as breeds, nutrition and other factors.

On the contrary, results for SCCw1 and SCCw2 were in favour of the higher BHB group, when the differences between groups were +60.21 thousand cells/mL for the first week of lactation, and +107.3 thousand cells/mL for the second week (Table 2). Even though SCC was better for the higher BHB group, the occurrence of diseases increased. The higher BHB group showed a higher occurrence of mastitis (+1.65%; not significant) and a higher occurrence of cysts (+0.78%; not significant). The relationship between the concentration of metabolites such as BHB even before calving and the occurrence of various diseases was confirmed in the work of Chapinal et al. (2011) and Berge and Vertenten (2014). Similarly, Duffield et al. (2009) demonstrated that if the BHB level rises above 1.2 mmol/L in the first week of lactation, the risk of metabolic diseases (malignant dysplasia) and reproductive diseases (metritis) is increased. Even though different diseases were monitored in this study, it was found that a higher concentration of BHB caused an inconclusively higher incidence of mastitis and cysts. The higher SCC and increased occurrence of mastitis for animals with higher BHB were confirmed in the works of Berge and Vertenten (2014) and Santschi et al. (2016). However, the results of this study were inconclusive for monitored health parameters. Increased occurrence of diseases and SCC can also be put into context with the overall economics of milk production, which according to Kushwah et al. (2021) especially worsened during the clinical forms of ketosis. Therefore, many authors (Duffield, 2000; Herdt, 2000) confirm the importance of this metabolic indicator and the usefulness of its monitoring thanks to its chemical stability in collected samples. Nonetheless, BHB might not be a fully explanatory indicator, but in the context with complementary indicators, it can provide a full picture about the metabolic status of an animal.

Table 1. The GLM evaluation for the effect of parity, BHB, season and year on the reproduction, production and health parameters – 1. part

Effect	Level	n	Number of insemination doses	Insemination interval	Service period	MY100	MYw1
			LSM ± SELSM	LSM ± SELSM	LSM ± SELSM	LSM ± SELSM	LSM ± SELSM
Parity	1	134	2.33 ± 0.207	79.14 ± 3.111	136.56 ± 9.495	2946.44 ± 76.162[A]	29.56 ± 12.179
	2	88	2.29 ± 0.218	84.10 ± 3.300	124.26 ± 10.244	3605.65 ± 81.259[B]	46.23 ± 12.806
	3 +	117	2.61 ± 0.212	82.22 ± 3.341	137.75 ± 10.441	3794.92 ± 75.863[B]	32.66 ± 11.781
BHB	<1.2	302	2.40 ± 0.091	71.86 ± 1.331[A]	123.36 ± 4.326	3603.66 ± 34.428[a]	42.17 ± 5.309
	>1.2	37	2.43 ± 0.344	91.78 ± 5.288[B]	142.36 ± 15.687	3294.34 ± 122.780[b]	30.14 ± 19.945
Season	3.-5.	67	3.47 ± 0.251[A]	89.74 ± 3.766[A]	174.01 ± 11.706[A]	3596.94 ± 90.188[A]	49.90 ± 14.304
	6.-8.	73	1.91 ± 0.257[B]	78.31 ± 3.862	111.63 ± 11.784[B]	3207.45 ± 94.654[B,a]	28.20 ± 15.757
	9.-11.	110	1.99 ± 0.213[B]	84.05 ± 3.335[a]	119.25 ± 10.635[B]	3463.23 ± 79.118[b]	30.40 ± 12.454
	12.-2.	89	2.27 ± 0.237[B]	75.19 ± 3.529[B,b]	126.54 ± 11.080[B]	3528.40 ± 90.929[b]	36.12 ± 13.252
Year	2016	136	2.70 ± 0.204[A]	80.51 ± 3.113	146.12 ± 9.390[a]	3450.34 ± 75.397	33.97 ± 12.127
	2017	203	2.12 ± 0.207[B]	83.14 ± 3.164	119.60 ± 9.904[b]	3447.67 ± 74.966	38.34 ± 11.949

Different letters in columns within effects mean statistical significance A, B ... $P<0.01$; a, b... $P<0.05$. n - number of observations; MY100 - milk yield for the first 100 days in milk in kg; MYw1 - milk yield in the first week of lactation in kg; LSM – least squares mean; SELSM – standard error of least squares means; BHB – beta-hydroxybutyrate concentration in mmol/L

Table 2. The effect of parity, BHB, season and year on the reproduction, production and health parameters – 2 part

Effect	Level	MYw2	SSCw1	SSCw2	Mastitis occurrence %	Cysts occurrence %
		LSM ± SELSM	LSM ± SELSM	LSM ± SELSM	LSM ± SELSM	LSM ± SELSM
Parity	1	31.73 ± 0.947[A]	150.23 ± 90.897[a]	177.36 ± 118.480	13.61 ± 4.262	12.89 ± 4.486[a]
	2	37.72 ± 0.995[B]	377.73 ± 95.576[b]	192.12 ± 124.580	8.55 ± 4.640	25.42 ± 4.902[A,b]
	3 +	39.18 ± 0.916[B]	221.93 ± 87.930	347.40 ± 114.610	9.60 ± 4.186	8.23 ± 4.229[B]
BHB	< 1.2	36.50 ± 0.413	280.07 ± 39.623	289.41 ± 51.647	9.76 ± 1.966	15.12 ± 2.183
	> 1.2	35.92 ± 1.550	219.86 ± 148.860	188.52 ± 194.040	11.41 ± 6.922	15.90 ± 6.880
Season	3.-5.	36.97 ± 1.112	301.76 ± 106.750	208.56 ± 139.150	15.76 ± 5.134	20.61 ± 5.431[A]
	6.-8.	34.62 ± 1.225	211.76 ± 117.600	210.66 ± 153.290	7.89 ± 5.609	14.60 ± 5.825[B]
	9.-11.	36.12 ± 0.968	258.50 ± 92.953	260.09 ± 121.160	12.39 ± 4.534	16.61 ± 4.764[B]
	12.-2.	37.14 ± 1.030	227.83 ± 98.903	276.55 ± 128.920	6.32 ± 4.658	10.24 ± 5.077[B]
Year	2016	35.77 ± 0.943	260.62 ± 90.513	186.62 ± 117.980	9.97 ± 4.220	8.50 ± 4.443[A]
	2017	36.65 ± 0.929	239.31 ± 89.184	291.31 ± 116.250	11.20 ± 4.313	22.52 ± 4.435[B]

Different letters in columns within effects mean statistical significance A, B ... $P<0.01$; a, b ... $P<0.05$. n - number of observations; MYw2 - milk yield in the second week of lactation in kg; SSCw1 - the number of somatic cells in the first week of lactation in thousand/mL; SSCw2 - the number of somatic cells in the second week of lactation in thousand/mL; LSM – least squares mean; SELSM – standard error of least squares means; BHB – beta-hydroxybutyrate concentration in mmol/L

Milk yield, reproduction and health status during the early stage of lactation are further influenced by a number of factors, such as the genetic background and individuality of the animals (Knob et al., 2021). Moreover, these crucial parameters for dairy cattle farming are also influenced by the lactation number, the season of the year, the year itself and associated qualitative fluctuations in the nutrition and feeding of dairy cows. Previous research also pointed out these existing relationships (Sahin et al., 2012; Mellado et al., 2018). The highest number of insemination doses, together with the longest insemination interval and service period, could be observed during the spring period (P<0.01). Results for other seasons were surprisingly similar, although the summer period showed the worst results for reproduction parameters, but the differences were insignificant. The shortest insemination interval was observed during winter (Table 1). Regarding the MY100 (P<0.01), in the first week of lactation (P<0.01) and in the second week of lactation, the lowest values were recorded during the summer months. Milk production during other seasons was similar, numerically highest during spring (MY100, MYw1) and winter (MYw2). Significant differences based on the season of the year were not observed for the SCCw1 and SCCw2. Surprisingly, the numerically lowest SCC was observed during summer. The highest incidence of mastitis (15.76%; not significant) and cysts (20.61%; P<0.01) was recorded for the spring months. The effect of the season of the year and the lactation number on SCC and the occurrence of mastitis were also confirmed in the work of Olde Riekerink et al. (2007), and Sumon et al. (2020). Likewise, Mellado et al. (2018) observed seasonal differences in BHB content and thus in the occurrence of ketosis.

The differences between the observed years (2016 vs 2017) were significant for the number of insemination doses, service period and incidence of cysts. While reproduction parameters improved in 2017, the incidence of cysts increased from 8.50% to 22.52%. Milk production parameters were similar during both years, although it can be noticed slightly lower SCC and mastitis incidence in 2016 (not significant).

CONCLUSIONS

The results of this work indicate that monitoring BHB content in blood has some potential for identifying problematic cows in dairy herds. Although, it should be added that monitoring the content of BHB in the blood is somewhat more complicated compared to milk. On the other hand, blood analysis is more accurate and improves the estimation of the metabolic state of individual dairy cows. The use of BHB monitoring in the early diagnosis of negative energy balance, and furthermore in diagnostics of potential incidence of metabolic diseases, could help us reduce the resulting decrease of milk yield, worsened pregnancy outcomes and increased incidence of disease. Dairy cows with BHB concentration corresponding to the values reached during subclinical ketosis had impaired conception rate and reduced milk yields. However, there were rather opposite tendencies for the number of somatic cells. Furthermore, the effect of BHB concentration on the incidence of mastitis and ovarian cysts was inconclusive. The results further demonstrated a certain seasonality in reproduction, production and health, which might be caused by the differences in the composition and quality of the feed ration throughout the year, photoperiod, temperature and other environmental factors.

It should be noted that only a small proportion of tested cows had BHB above 1.2 mmol/L, therefore the experimental groups were imbalanced, which might have affected some parameters and their significance. More extensive research is needed to understand the relationship between individual metabolites and production-reproduction-health parameters. This research area will continue to be of vital importance for practice, as routine monitoring of metabolic status and energy balance is becoming more and more necessary for the correct management of dairy cows throughout their production lives.

ACKNOWLEDGEMENTS AND FUNDING

This work was supported by the Ministry of Agriculture of the Czech Republic (NAZV Project No. QK22010270) and the „S" grant by the Ministry of Education, Youth and Sports of the Czech Republic.

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