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Original article

Changes in the real-time registration of milk β-hydroxybutyrate according to stage and lactation number, milk yield, and status of reproduction in dairy cows

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Abstract

This study investigated changes in the real-time measured levels of milk β-hydroxybutyrate according milk yield, lactation number and status of reproduction in dairy cows.

A total of 378 cows were selected. According to their reproductive status the cows were classified as belonging to the following groups: Fresh (1 – 44 days after calving. n=43). Open (45 – 65 days after calving. n=78), Inseminated (1 – 35 days after insemination. n=133). Pregnant (35 – 60 days after insemination and pregnant (relatively pregnant) (n=124). The cows were milked with DeLaval milking robot (DeLaval Inc., Tumba, Sweden) in combination with a Herd Navigator (Lattec I/S. Hillerød. Denmark) analyser.

We observed that milk β-hydroxybutyrate (BHB) had a tendency of increasing with an increase of lactation number. The average BHB in multiparous cows was 11.111% higher in comparison with primiparous cows ($p<0.001$). We found higher BHB concentration in the multiparous cows in all reproduction status groups ($p<0.001$). A strong positive statistically significant ($p<0.001$) relationship has been found between BHB and the average milk yield within all groups of primiparous cows although we found a statistically unreliable coefficient of correlation (from -0.202 to 0.057) between highest milk yield and BHB in primiparous and multiparous cows.

Key words: cow, BHB, production, reproduction, herd navigator

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Introduction

When cows are unable to consume enough feed to support milk production, they often fall into severe negative energy balance. This leads to a weakened immune system and increases their susceptibility to infectious diseases. Reducing the milk production of cows subjected to acute nutritional stress decreases their energy deficit (Ollier et al., 2016). Poor production and reproduction performances are usually observed as an increased incidence of periparturient diseases in the herd (Suthar et al. 2013). Clinical ketosis is one of the most frequent metabolic diseases affecting dairy cattle (Belay et al. 2017). Milk β-hydroxybutyrate is a valuable tool for rapid diagnosis of hyperketonemia (Pinheiro et al. 2015). According to Chandler et al. (2017) milk ketone body thresholds diagnosed hyperketonemia with 64.0 to 92.9% accuracy in Holsteins and 59.1 to 86.6% accuracy in Jerseys. In addition to their routine availability, indicator traits (e.g., milk or blood β-hydroxybutyrate (BHB)) have moderate heritability (Jamrozik et al. 2016). With the use of Herd Navigator, inferior cows performance can easily be monitored on a real-time basis, and factors contributing to improper ketosis and reproductive management can be identified and corrected to improve farm performance (Blom et al. 2015). In 2008, an advanced milk analysis tool (Herd Navigator. DeLaval. Sweden) was developed for heat detection by measuring progesterone, mastitis detection by measuring lactate dehydrogenase (LDH) and ketosis detection by measuring beta-hydroxybutyrate (BHB). This system automatically takes representative milk samples from individual cattle from specific milking points during milking and automatically selects through a specific algorithm called 'biomodel' which cattle should be monitored and sampled at each milking session and which parameters should be measured when the animals arrive at the milking parlour (Mazeris 2010). In addition to progesterone this sensor provides real-time measurements of lactate dehydrogenase for the detection of (subclinical) mastitis, urea to assess the efficiency of protein feed rations and β-hydroxybutyrate to reveal (subclinical) ketosis and/or secondary metabolic disorders. This isn't truly 'in-line' as samples are taken away from the milkline and analysed by an adjoining instrument. Also it's an expensive system that can only be used with DeLaval milking systems (Dobson 2007). The analysis of reproductive performance revealed that ketosis cystic ovaries and postpartum anoestrus highly influence the conception rates and the length of the breeding period irrespective of the length of the voluntary waiting period. With the use of Herd Navigator, inferior cattle performance can be easily monitored on a real-time basis and factors contributing to improper ketosis and reproductive management can be identified and corrected to improve farm performance (Blom et al. 2015).

A fully automated real-time progesterone analyser called Herd Navigator (Lattec I/S. Hillerød. Denmark) that can be combined with a DeLaval milking robot (DeLaval Inc. Tumba. Sweden), has recently become commercially available. Although it requires a considerable investment during the installation stage which would be costly for small farms, the tool is profitable for large farms when compared to the frequent manual collection of progesterone information (Nyman et al. 2014). An inline sampler inside the milking robot automatically takes a representative sample of several millilitres of milk from an individual cow during milking. The sample is then submitted into the Herd Navigator. Additional assays (urea nitrogen. lactate dehydrogenase. and β-hydroxybutyrate) supplied by the analyser can be applied in real time to identify metabolic disorders and mastitis of the animal for more comprehensive control (Saint-Dizier and Chastant-Maillard 2012). The Herd Navigator has resulted in a reasonably accurate system although more work is needed to validate the system (Berkema et al. 2015).

According to Ježek et al. (2017) beta-hydroxybutyrate in milk is a good indicator of subclinical ketosis in dairy cows. Additional validation of predictions equations in commercial farms and integration of reference data from other populations were needed, nevertheless first results showed value of these non-invasive biomarkers for routine monitoring and for breeding. The aim of our study was to identify the changes in the real-time measurement of milk β-hydroxybutyrate according milk yield, lactation number and status of reproduction in dairy cows.

Materials and Methods

Location and experimental design

The experiment was carried out on a dairy farm in the eastern region of Europe at 56 00 N. 24 00 E. 378 Lithuanian Black and White fresh dairy cow were selected according to those fitting a profile of having had a second or more lactations and being clinically healthy (an average rectal temperature of +38.8°C, rumen motility five–six times per three minutes without signs of mastitis, lamenesis or metritis). The animals were kept in a loose housing system and were fed a feed ration throughout the year at the same time balanced according to their physiological needs. Feeding took place every day at 06:00 and 18:00. The cow were milked two times per day at 06:00 and 18:00.

Table 1. Milk β-hydroxybutyrate (BHB) based on status of reproduction.

Reproduction status	BHB (mmol/l)	95% CI
Fresh	0.060±0.001[a]	0.057-0.063
Open	0.058±0.001[b]	0.056-0.060
Inseminated	0.058±0.001[b]	0.056-0.059
Pregnant	0.057±0.001[c]	0.056-0.059

[abc] Row means with different superscripts differ significantly at $p<0.05$

Measurements

For milk BHB detection the fully automated real-time analyser Herd Navigator (Lattec I/S. Hillerød. Denmark) was used in combination with a DeLaval milking robot (DeLaval Inc. Tumba. Sweden). An in-line sampler in the milking robot automatically took a representative sample of several millilitres of milk from an individual cow during milking. The sample was then submitted into the Herd Navigator.

Animals and experimental conditionin

A total of 378 cows were selected. According to their reproductive status the cows were classified as belonging to the following groups: Fresh dairy cows (1 – 44 days after calving, n=43). Open (45 – 65 days after calving, n=78) Inseminated (1 – 35 days after insemination, n=133). Pregnant (35 – 60 days after insemination and pregnant (relatively pregnant) (n=124). The cows were milked with a DeLaval milking robot (DeLaval Inc. Tumba. Sweden) in combination with Herd Navigator (Lattec I/S. Hillerød. Denmark) analyser.

Data analysis and statistics

The statistical analysis of data was performed using the SPSS 20.0 (SPSS Inc., Chicago, IL, USA) software package. Using descriptive statistics, normal distributions were assessed for all variables by means of the Kolmogorov-Smirnov test. The results were expressed as the mean (M) ±standard error of the mean (SEM) and 95% confidence interval (CI). The Pearson correlation was calculated to define the statistical relationship between investigated traits. The differences in the mean values of normally distributed variables were analysed by the Student's t-test. A probability of less than 0.05 was considered significant (p-value<0.05).

Results

The cows included into the study had an average of 2.18±0.065 lactations, 179.45±5.560 lactation days, where the average milk BHB was 0.0578±0.0004 mmol/l. The average and highest milk yield per day was 28.87 ± 0.407 kg (26.96±0.644 kg - in primiparous and 30.07±0.509 kg – in multiparous cows; $p<0.001$) and 38.47± 0.369 kg (33.72±0.545 kg – in primiparous and 41.25± 0.431 kg – in multiparous cows; $p<0.001$), respectively.

Changes in milk BHB of dairy cows with different levels of reproduction status

We found that BHB level was higher (5.263%) in the fresh cows than of the pregnant ones ($p<0.05$) (Table 1). The study showed that BHB of open and inseminated cows was at the same level.

Changes in milk BHB of dairy cows with different number of lactation

We observed that milk BHB had a tendency of increasing with an increase of lactation number. Consequently, the highest level of BHB was estimated in the oldest cows of lactation ≥ 5 (18.519 % higher in comparison with cows of 1st lactation, $p<0.05$).

Evaluation of BHB in milk of dairy cows with different levels of lactation stage

The study showed that the average BHB in multiparous cows was 11.111% higher in comparison with primiparous cows ($p<0.001$).

The highest BHB in milk of primiparous cows was obtained in an early stage of lactation on 20, 40 and 90 days in milk (DIM), there after steady decline to 110 DIM, gradually increased to 180 DIM and then gradually declined in the later stage of lactation to 280 DIM. However, in multiparous cows steady increasing of BHB was observed from 40 to 110 DIM and highest BHB was obtained in the later stage of lactation on 200 and 220 DIM (Fig. 1) (Table 2).

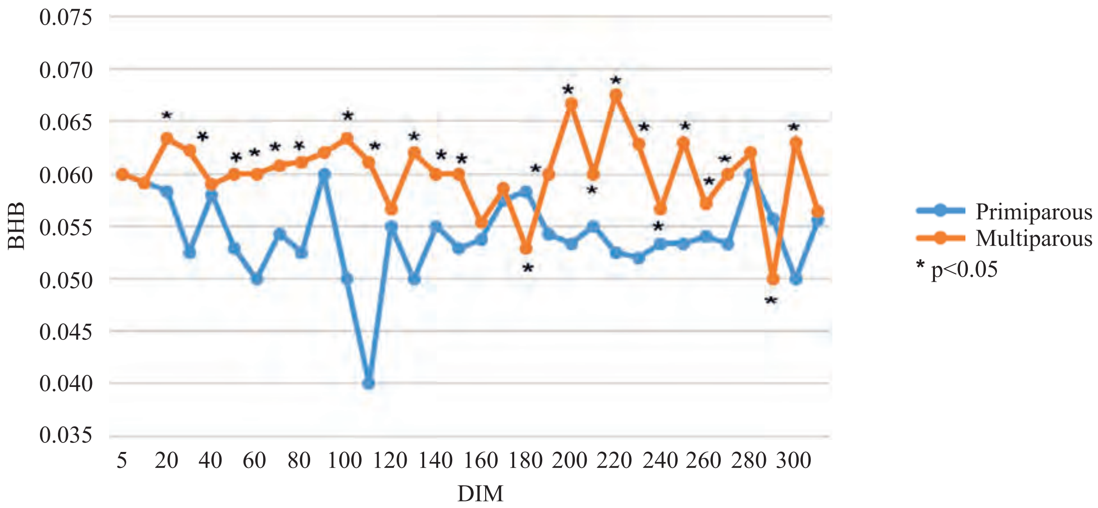

Fig. 1. Milk BHB in primiparous and multiparous cows based on lactation stage.

Table 2. Milk β-hydroxybutyrate (BHB) based on number of lactation.

Lactation number	BHB	95% CI
1 (n=147)	0.054±0.001[a]	0.053-0.056
2 (n=114)	0.059±0.001[b]	0.057-0.060
3 (n=49)	0.060±0.001[b]	0.058-0.062
4 (n=40)	0.061±.001[bc]	0.058-0.064
≥ 5 (n=28)	0.064±0.002[c]	0.061-0.067

[abc] Row means with different superscripts differ significantly at $p<0.05$

BHB in primiparous and multiparous cows by status of reproduction

We found higher ($p<0.001$) BHB concentration in the multiparous cows in all reproduction status groups (Fig. 2). The biggest difference in the BHB between groups was found in open cows (15.094%), lowest – in pregnant (7.272%) and fresh cows (8.923%).

BHB relationship with the milk yield of cows by status of reproduction

A strong positive statistically significant ($p<0.001$) relationship has been established between BHB and the average milk yield within all groups of primiparous cows although we found a statistically unreliable coefficient of correlation (from -0.202 to 0.057) between highest milk yield and BHB in primiparous and multiparous cows. Table 3 represents that the open, inseminated and pregnant primiparous cows have from 2.02 to 3.20 times higher correlation coefficients between BHB and average milk yield.

Discussion

This study investigated changes in the real-time measurements of milk β-hydroxybutyrate in relation to milk yield, lactation number and status of reproduction in dairy cows. According to Yu and Maeda (2017) an inline 'herd navigator' system that works automatically and provides real-time physiological information about lactating dairy cow for making farm management decisions is not only a novel tool for scientific research but also improves productivity, food safety, animal well-being, the environment and the public's perception of the dairy industry. Ježek et al. (2017) found a statistically significant positive correlation between the concentration of BHB in blood and milk.

According to our results BHB was higher (5.263%) in the fresh that of the pregnant ones. The highest BHB in milk of primiparous cows was obtained in an early stage of lactation on 20, 40 and 90 DIM, there after steady declined to 110 DIM gradually increased to 180 DIM and then gradually declined in the later stage of lactation to 280 DIM. However, in multiparous cows steady increasing of BHB was observed from 40 to 110 DIM and highest BHB was obtained in the later

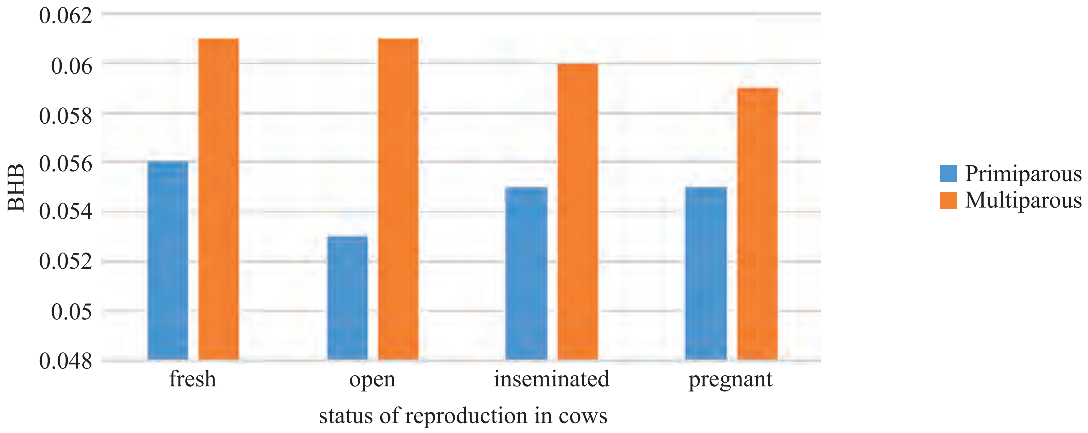

Fig. 2. Milk BHB in primiparous and multiparous cows based on reproduction status.

Table 3. Correlation of β-hydroxybutyrate (BHB) with milk yield of primiparous and multiparous cows

Milk yield	Fresh	Open	Inseminated	Pregnant
		Primiparous (n=147)		
Average milk yield	0.998***	0.729***	0.610***	0.615***
Highest milk yield	-0.202	-0.176	0.041	0.046
		Multiparous (n=231)		
Average milk yield	0.999***	0.227	0.302**	0.192
Highest milk yield	0.183	-0.130	0.057	0.044

* $p<0.05$, ** $p<0.01$, *** $p<0.001$

stage of lactation on 200 and 220 DIM. During the early postpartum period dairy cows mobilize fat and muscle to support lactation, this is associated with alterations in blood metabolite and hormone profiles which in turn influence milk yield and fertility (Wathes et al. 2007). According to Tatone et al. (2017) the peak ketosis prevalence occurred at 6 and 7 DIM decreased over 30 DIM. This finding is consistent with another works, indicating a higher prevalence in the first 2 weeks of lactation (Duffield et al. 1997, Rasmussen et al. 1999, McArt et al. 2012). McArt et al. (2012) reported an average hyperketonemia incidence of 43%, ranging from 26% to 56% with a peak incidence at 5 DIM when 22.3% of the cow had their first positive result. Peak prevalence was also at 5 DIM when 28.9% of cow were positive. Mean predicted blood BHB of cows with ketosis was higher in early lactation, but decreased rapidly with stage of lactation toward the l evel found in more healthy cows (Belay et al. 2017). Pryce et al. (2016) found a median ketosis frequency of 3.3% with a range from 0.24% in first lactation up to 17.2% in third lactation

We observed the highest level of BHB in the oldest cows. The biggest difference in the BHB between groups was estimated in open cows, lowest – in fresh. The open, inseminated and pregnant primiparous cows have from 2.02 to 3.20 times higher correlation coefficients between BHB and average milk yield. Multiparous animals with higher fat yield had lower odds of ketosis of the current lactation (Tatone at al. 2017). Increased dry days and longer calving intervals, for multiparous animals, and older age at first calving for primiparous animals increased the odds of ketosis. Piñeyrúa et al. (2018) found that multiparous cows showed a greater imbalance in metabolic and hormonal profiles than in primiparous cow, leading to abnormal ovarian activity. BHB was reportedly either higher (Vandehaar et al. 1999, Meikle et al. 2004) or lower (Santos et al. 2001) in primiparous than in multiparous cows. There are differences in the control of tissue mobilization between primiparous and multiparous cows which may promote nutrient partitioning into growth as well as milk during the first lactation (Wathes et al. 2007). According to Ruoff et al., (2007) primiparous cows had a lower prevalence and incidence of hypoglycemia than multiparous cows. According to Wathes et al. (2007) multiparous cows β-hydroxybutyrate was negatively correlated with yield. The analysis of reproductive performance revealed that ketosis, cystic ovaries and postpartum anoestrus highly influence the conception rates and length of breeding period, irrespective of length of the voluntary waiting period (Blom et al. 2015). According to Ruoff et al. (2017) hypoglycemia mainly occurred in multiparous cows, whereas primiparous cows were at a lower risk for hypoglycemia. Heringstad et al. (2005) reported mean frequency of ketosis ranging from 7.5% in first-lactation to 17.2% in the third-lactation cows.

We can conclude that the real-time measured β-hydroxybutyrate levels and changes in their dynamics correlate with different reproductive statuses, productivity and number of lactations.

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