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Original article

Beta-hydroxybutyrate in milk as screening test for subclinical ketosis in dairy cows

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Abstract

Ketosis is a very frequent metabolic disease in dairy cows, resulting in lower milk production, impaired fertility and increased frequency of other diseases. The course of the disease is often subclinical, so early detection is very important. The aim of the study was to investigate the relation between the concentration of beta-hydroxybutyrate in blood and milk and to determine the cut-off value in milk for detection of subclinical ketosis. The study included 94 cows, which were in the first third of lactation. Beta-hydroxybutyrate (BHB) concentrations were measured in blood and milk serum using a biochemical analyser. The average concentration of BHB in the blood serum samples was 1.14 mmol/L while in the milk it was about ten times lower at 0.117 mmol/L. A statistically significant positive correlation between the concentration of BHB in blood and milk ($r=0.705$, $p<0.001$) was found. In cows with BHB in blood below 2.0 mmol/L a stronger correlation between blood and milk BHB was established ($r=0.658$, $p<0.001$) than in cows with blood BHB above 2.0 mmol/L ($r=-0.292$, $p=0.206$). Therefore, BHB in milk is a very suitable indicator in the diagnosis of subclinical ketosis as there is a good correlation between BHB in the blood and milk of cows with subclinical ketosis. The cut-off concentration of BHB in milk set at ≥0.080 mmol/L (AUC=0.91±0.03; $p<0.001$) is a significant indicator for subclinical ketosis in dairy cows. The sensitivity of the test was 94% and specificity 74%. Beta-hydroxybutyrate in milk is a good indicator of subclinical ketosis in dairy cows and can be measured accurately with a biochemical analyser.

Key words: subclinical ketosis, biochemical analyser, BHB, milk, cows

Introduction

Ketosis is a common disease in dairy herds, associated with high milk production and a negative energy balance. Most cases occur in the first 6 weeks to 2 months after calving (Herdt 2000, Enjalbert et al. 2001, Fleming 2002). Ketosis results in lower milk yield, lower fertility and increased frequency of other diseases (abomasal displacement) (McArt et al. 2012, Suthar et al. 2013). Subclinical ketosis causes greater

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losses than clinical ketosis because it occurs more frequently (Geishauser et al. 2000) and often cannot be detected by the farmers. On average 40% of the cows have subclinical ketosis at least once during lactation (Dirksen et al. 1997, Berg and Vert 2014), while clinical ketosis affects on average 5% of cows (Kelton and et al. 1998, Oetzel 2004, Koeck et al. 2013). The prevalence of subclinical ketosis in ten European countries was on average 21.8% (from 11.2 to 36.6%), and clinical ketosis was 3.7% (0.4 to 11.1%). The average prevalence of subclinical ketosis in 24 herds in Slovenia was 24%, while in Serbia it was up to 19.5% in 42 herds (Suthar et al. 2013).

In view of the above mentioned facts, it is crucial to detect the disease as soon as possible, begin to treat it and to introduce preventive measures. The most reliable way to establish subclinical ketosis is measuring the concentration of beta-hydroxybutyrate (BHB) in the blood serum (Duffield 2000) or in the whole blood. The most accurate method is measuring BHB concentration in the laboratory with a biochemical analyser. In the literature different thresholds for the determination of subclinical ketosis are indicated: 1.40 mmol/L (Oetzel 2004), 1.20 mmol/L (Asl et al. 2011) and 1.00 mmol/L (Ospina et al. 2010, Whitaker 1997). Values of BHB above 2.99 mmol/L are most often associated with the clinical form of ketosis (Oetzel 2004).

Milk is a very convenient sample for the determination of ketosis because it is easily accessible also by farm personnel. In the case of sub-clinical ketosis the content of BHB in the milk is elevated but the concentrations are lower than in the blood. BHB concentration in milk can be measured in the field by using a semi quantitative colorimetric dipstick test. The cut-off value is 100 to 200 µmol/L, higher values indicate ketosis. Carrier (2004) found in his study that the test (KetoTest strip) detected 73% of the actual positive cows and 96% of the actually negative. When the BHB level in milk exceeds 100 µmol/L the risk of clinical ketosis increases (Francos et al. 1997).

The Fossomatic milk analyser is widely used for the testing of milk samples and has the possibility to test for BHB with Fourier transform infrared spectrometry (FTIR) as well. The results of Wilson and Goodel (2013) show that the BHB test methods agreed well for most non-ketotic cows, but the test did not agree well on classification of ketotic cows. They concluded that calibration improvements are necessary for improved testing of BHB in milk.

Biochemical analysers are routinely used and they are very accurate for measuring of BHB in blood serum. Regarding the producer instructions the RX Daytona biochemical analyser can be used also for biochemical analysis of milk serum. For this reason BHB was measured in blood and milk serum with the same analyser in this study. The authors were not able to find any data in the literature concerning the measurement of BHB in milk with a biochemical analyser.

The aim of this study was to assess the agreement between the concentration of BHB in blood and milk of cows measured by biochemical analyser. Additionally, the cut-off value of BHB in milk serum for identifying subclinical ketosis was calculated.

Materials and Methods

Ninety-four samples of blood and milk of cows were analysed. The cows were Holstein Friesian breed and in the first third of lactation. Average production of milk was 30.3±8.7 kg/day. Cows were milked twice a day and fed according to requirements. Blood was taken approximately four hours after the morning feed from the tail vein (*vena caudalis mediana*), and milk samples were collected at the same time. To obtain milk serum for biochemical analysis the milk samples were centrifuged at 4500 rotations for 15 minutes to obtain skimmed milk. Skimmed milk samples were centrifuged in Eppendorf tubes for 30 minutes at 13000 rotations to obtain a milk serum. BHB concentrations were measured in blood serum and milk serum using a RX Daytona biochemical analyser (RANDOX Laboratories Ltd., UK) and Rayto 1904cv (Rayto Electronics Inc. Shenzhen, China). A kinetic enzyme test with BHB dehydrogenase (reagent RANBUT, Art RB 1007) and software settings of the RANDOX manufacturer were used. Measurements in the milk serum were carried out in parallel and the mean value of both measurements was used.

Statistical analysis was performed using an SPSS (ver. 22) software package (IBM Analytics, USA) (Arbuckle 2013). Descriptive statistics for BHB in milk and blood were calculated and results were presented graphically as a frequency distribution with parameters of central tendency and variation. Correlation between BHB in blood and milk was determined using the Pearson correlation coefficient and linear regression. A difference between correlation coefficient in the groups of cows with blood BHB <2.0 mmol/L and ≥2.0 mmol/L was determined. A receiver operating characteristic (ROC) curve was used to illustrate performance of milk BHB for diagnostics of subclinical ketosis (analysis area under curve-AUC of ROC curve, sensitivity and specificity for optimal cut-off value of BHB in milk). For ROC analysis the cows were divided in accordance with BHB concentration in blood, to positive and negative to subclinical ketosis based on the cut-off at ≥1.00 mmol/L.

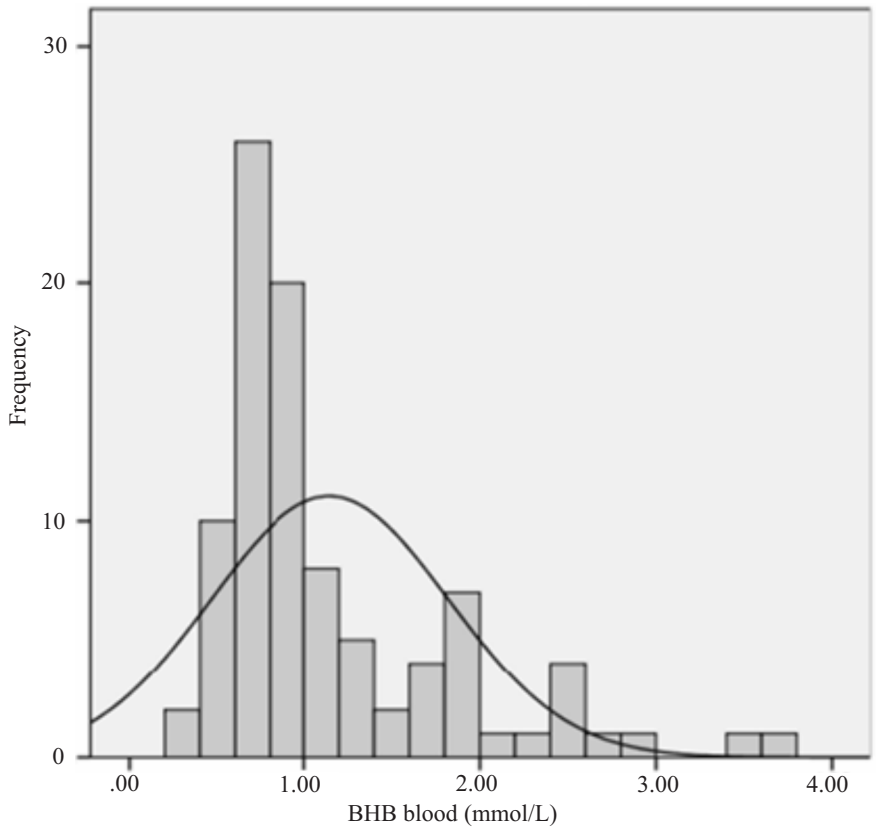

Fig. 1. Frequency distribution of beta-hydroxybutyrate (BHB) in blood.

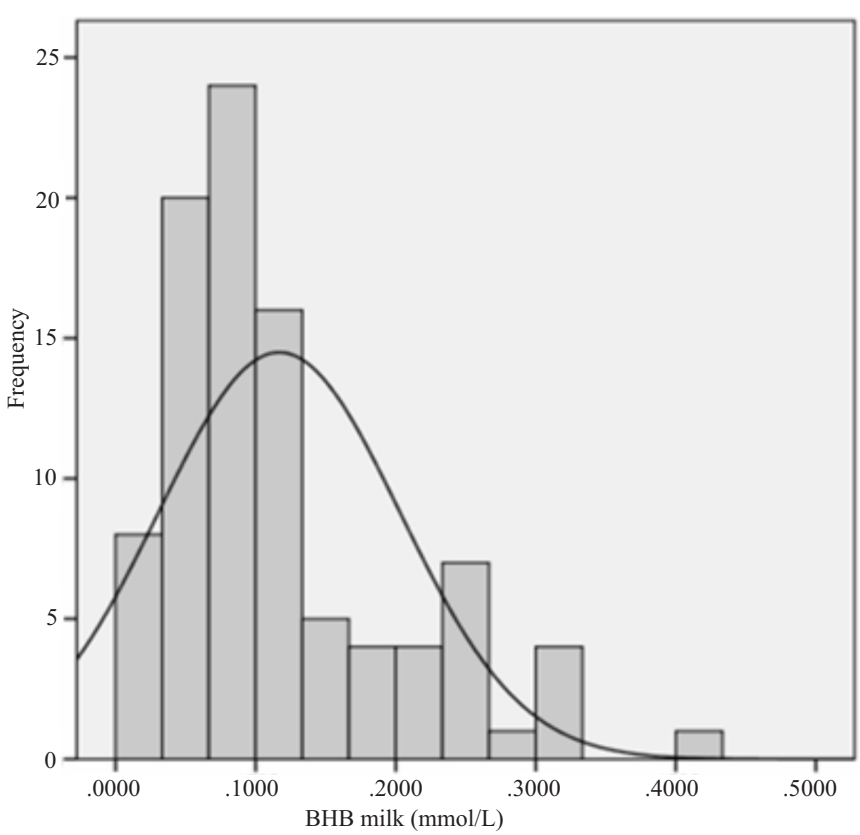

Fig. 2. Frequency distribution of BHB in milk.

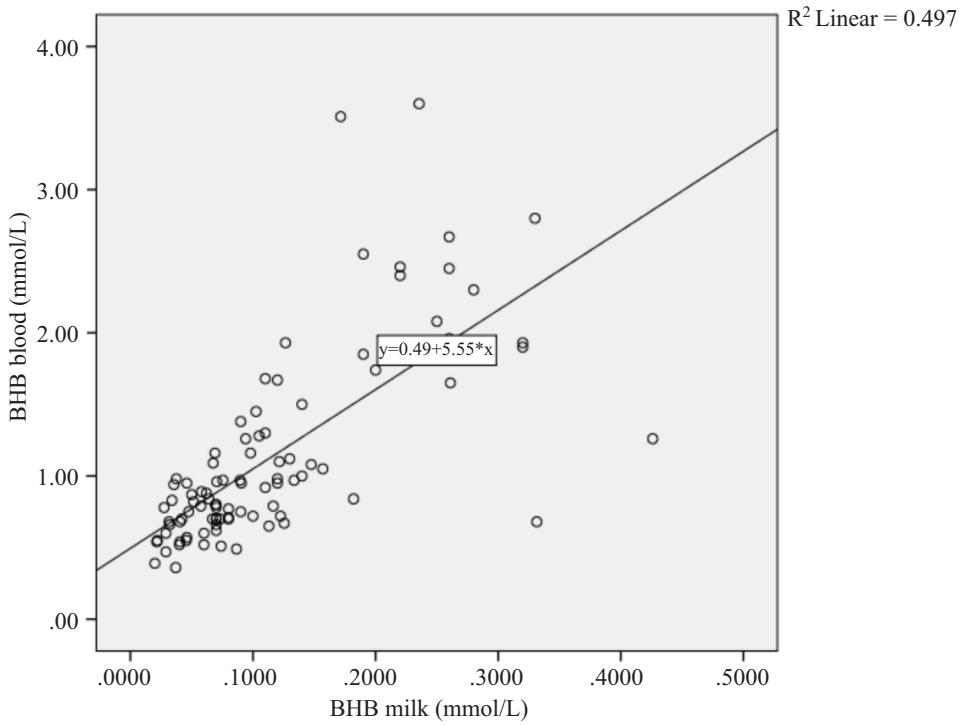

Fig. 3. Relation between concentration of BHB in blood and milk of cows.

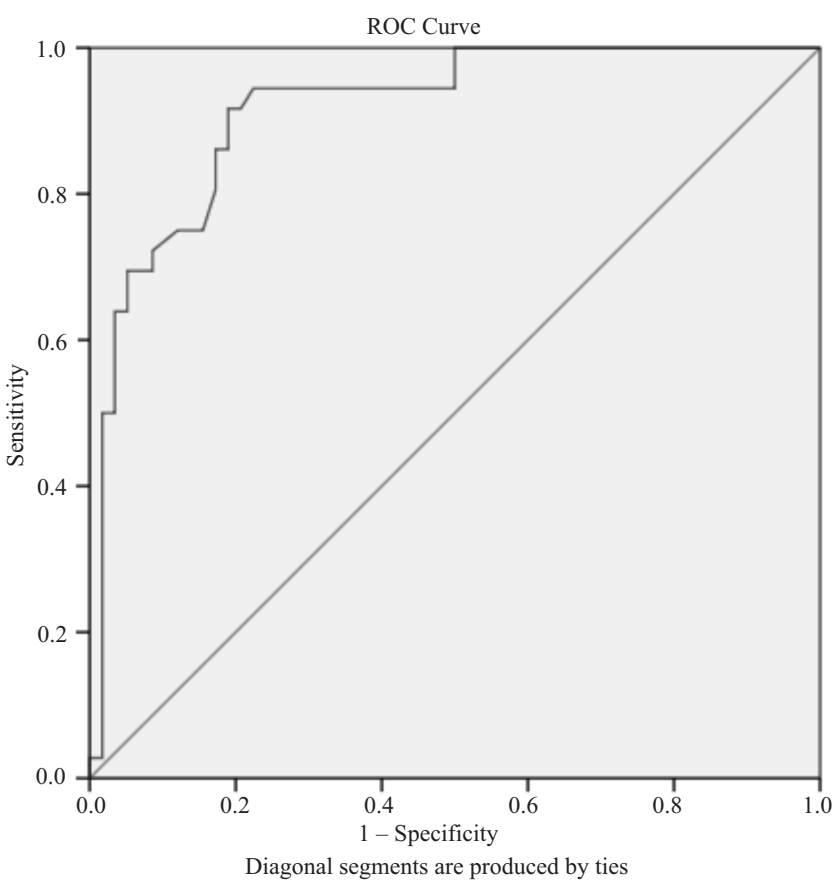

Fig. 4. Response operator characteristic (ROC) curve for BHB measurements in milk.

Results

The concentration of the BHB in milk was approximately ten times lower than in the blood (Figs. 1, 2). The average concentration of BHB in the blood serum samples was 1.14±0.68 mmol/L (median: 0.90 mmol/L) while in the milk it was 0.116±0.086 mmol/L (median: 0.089 mmol/L).

Comparing the concentration of BHB in blood and milk, a statistically significant positive correlation ($r=0.705$, $p<0.001$) was found (Fig. 3). Regression analysis showed that an increase of blood BHB concentration of 1 mmol/L means an increase of milk BHB concentration of 0.11 mmol/L. A much stronger correlation between the value of BHB of blood and milk was found when the value of BHB in blood ($n=84$) was below 2.0 mmol/L ($r=0.658$, $p<0.001$) in comparison to samples ($n=10$) with BHB levels above 2.0 mmol/L ($r=-0.292$, $p=0.206$). For this reason, there is a much greater dispersion of results with increasing values of BHB (Fig. 3).

The cut-off value of BHB in milk for subclinical ketosis was determined. The results of ROC analysis showed the best sensitivity and specificity at the borderline value 0.080-0.100 mmol/L (Fig. 4). Concentration of BHB in milk at ≥0.080 mmol/L (AUC=0.91±0.03; $p<0.001$) was a significant indicator of subclinical ketosis in dairy cows with 94% sensitivity and 74% specificity. At the cut-off value of 0.100 mmol/L, the sensitivity was 86% and specificity 82%.

Discussion

The concentration of BHB in milk was approximately ten times lower than in the blood. Because BHB can be used by the mammary gland for fatty acid synthesis, lower concentrations in milk than in blood could be expected (Enjalbert et al. 2001).

A statistically significant positive correlation was established between BHB concentration in blood and milk. Some studies have found a similar correlation between the content of BHB in the blood and milk of cows, namely $r=0.66$ ($p<0.001$) (Enjalbert et al. 2001), 0.48 and 0.85 (Hohler 2015). The differences in the results between studies can be explained by the fact that the ratio between different ketones in the blood varies. In healthy cows BHB constitutes 80% of the total quantity of ketones; however, in ketosis this ratio changes in favour of acetone and acetoacetate (Dirksen, et al. 1994). The metabolism of energy also changes in heat stress, when the supply of glucose to the mammary gland is decreased and uptake of BHB and non-esterified fatty acids in the mammary gland is increased (Belić et al. 2011).

In this study a much stronger correlation between the value of BHB of blood and milk was found when the value of BHB in blood was below 2.0 mmol/L in comparison to samples with BHB levels above 2.0 mmol/L. Therefore, the BHB in milk is a very suitable indicator in the diagnosis of subclinical ketosis because there is an even better correlation between BHB in blood and milk of cows with subclinical ketosis.

A cut-off value of BHB in blood at ≥1.00 mmol/L was used for ROC analysis to discriminate between positive and negative cows. This cut-off value is used in the laboratory where analyses were performed and is indicative of subclinical ketosis according to the authors' experience and in some literature (Whitaker 1997, Ospina et al. 2010). Zadnik (2003) compared blood BHB concentration between clinically healthy cows and cows with abomasal displacement. In clinically healthy cows BHB concentration was below 1.00 mmol/L and was significantly lower (0.71±0.22 mmol/L) than in cows with abomasal displacement (1.77±1.37 mmol/L). A cut-off value of BHB in milk for subclinical ketosis was determined. The best sensitivity and specificity was at the cut-off value 0.080-0.100 mmol/L. For practical use it is more appropriate to set a cut-off value at 0.080 mmol/L, which means the test is more sensitive and allows detection of the most cows with subclinical ketosis. Enjalbert et al. 2001, used a blood BHB cut-off value of 1.20 mmol/L, and they calculated a threshold for BHB in milk at 0.07 mmol/L, with 91.7% sensitivity and 64.4% specificity. Jorritsma et al. (1998) obtained better results in terms of sensitivity and specificity of the test at a threshold value of 0.1 mmol/L BHB in milk than at the value of 0.2 mmol/L. In their opinion, with the screening test for detection of cows with subclinical ketosis, higher sensitivity is more important than higher specificity. The differences between studies could be due to different analytical methods used for quantitative determination of BHB in blood and milk. In the German study, a large proportion of cows that had a concentration of BHB in milk between 0.05-0.09 mmol/L, an increased concentration of acetoacetate and acetone in the urine was found, indicating subclinical ketosis (Dirksen et al. 1994). The milk cut-off values proposed in these studies are very close to the cut-off value proposed in the present study.

Conclusions

A statistically significant correlation between the concentration of BHB in blood and milk serum of cows was confirmed. The best sensitivity and specificity for measurements of BHB in milk were observed

at the cut off value of 0.080 mmol/L (80 µmol/L). This cut off is suitable for practical use because it allows detection of the most cows with subclinical ketosis. Beta-hydroxybutyrate in milk serum is a good indicator of subclinical ketosis in dairy cows.

Acknowledgement

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