β-HYDROXYBUTYRATE, GLUCOSE, CALCIUM, PHOSPHORUS, AND VITAMIN C CONCENTRATIONS IN BLOOD OF DAIRY COWS WITH SUBCLINICAL KETOSIS DURING THE EARLY LACTATION

ZHIGANG ZHANG, GUOWEN LIU, XIAOBING LI, ZHE WANG, TAO KONG, NAISHENG ZHANG, AND CHANGMING GUO

Department of Clinical Veterinary Medicine, College of Animal Science and Veterinary Medicine, Jilin University, Changchun 130062, Jilin, China

wangzhe500518@sohu.com

Received for publication August 13, 2008

Abstract

The objective of this study was to examine the association between β-hydroxybutyrate (BHBA) and glucose, calcium (Ca), phosphorus (P), and vitamin C concentrations in dairy healthy and with subclinical ketosis cows during an early lactation period. The blood from 99 healthy cows and 26 sub-clinically ketotic cows within the first two months of lactation was sampled. Serum concentration of BHBA was measured by enzymatic-rate method, glucose by oxidase method, and P by colorimetric method. These tests were performed in a biochemical auto-analyser. Serum Ca concentration was measured by atomic absorption spectrometer followed by acid digestion. Vitamin C was measured by HPLC. The results showed that in ketotic cows serum BHBA was significantly increased (P<0.01) and glucose and Ca concentrations were significantly decreased (P<0.01) as compared to healthy cows, while vitamin C and P concentrations were similar in the both groups of cows (P>0.05). The serum BHBA concentration was negatively correlated with the serum glucose and Ca concentrations (P<0.01). Vitamin C and P status of cows with subclinical ketosis was not affected by BHBA. Serum Ca concentration can be used as a reference index for the diagnosis of subclinical ketosis.

Key words: dairy cows, subclinical ketosis, β-hydroxybutyrate, calcium, glucose, vitamin C.

Ketosis is a common metabolic disorder frequently observed in dairy cows during the early lactation period. It is a metabolic condition characterised by increased concentrations of ketone bodies in blood, urine, and milk. Subclinical ketosis (SCK) is defined as the elevated concentration of circulating ketone bodies in the absence of clinical signs of ketosis (2). In general, diseases, which are subclinical in their nature, are becoming the ones of the greatest economic importance in the dairy industry (4). SCK can result in a significant decrease in total milk production. Besides, researches showed that SCK plays a role in many diseases, such as infertility (18), metritis, cystic ovaries (8), and mastitis. The gold standard diagnostic test for SCK is the measurement of β-hydroxybutyrate (BHBA) in serum or plasma because of its stability (5, 9, 13).

Calcium (Ca) is an essential mineral for life in higher animals, involved in the normal function of a wide variety of tissues and physiologic processes. For these functions to be carried out properly, blood Ca concentration must be not only monitored but also regulated according to strictly defined limits. Low blood Ca concentration at calving predispose the cow to metabolic problems during the transition period such as parturient paresis (14), milk fever, subclinical hypocalcaemia, displaced abomasum (17), ketosis, metritis, and retained placenta (6). Serum Ca concentration is considered to be the primary source for the evaluation of Ca abnormalities in dairy cows.

In the past few years, researchers paid more and more attention to the relationship between nutritional metabolic diseases and immune function of dairy cows (11, 20). Vitamin C, widely distributed in the animal body, is a known antioxidant that protects the structural integrity of the cells of the immune system (3). The majority of vitamin C exists in animal body as ascorbic acid, which is then is reversibly oxidised to dehydroascorbic acid (DHAA). Vitamin C is not regarded as an essential dietary nutrient for healthy dairy cows; however, some studies have suggested that stressed cows possibly suffered from vitamin C deficiency (16, 19).

If a programme to prevent, monitor, and even treat SCK is to be considered, it is essential to have a
thorough understanding the associations between the serum concentrations of BHBA and glucose, Ca, phosphorus (P), and vitamin C (8). However, for cows with SCK, only limited correlations between the serum BHBA concentration and total Ca, P, glucose, and vitamin C concentrations have been reported. Therefore, the objective of this study was to compare the serum levels of these compounds in healthy and sub-clinically ketotic cows during the early lactation period, and to define the correlation between serum BHBA and other metabolites (Ca, glucose) concentrations to provide basis for the prevention, diagnosis, and treatment of SCK in dairy cows.

Material and Methods

Animals. The study was performed at a commercial dairy farm located in Changchun city, Jilin province. In total, 99 healthy and 26 sub-clinically ketotic cows within the first two months of lactation were used in the study. The cows were housed in a tethered stall (research barn), and fed according to the nutrition requirements of dairy cows at the early postpartum stage. These cows were multiparous ones in their second to fifth lactation. All the cows had no visible clinical signs of disease. All the animals were treated according to International Guiding Principles for Biomedical Research Involving Animals. Body condition score (BCS) and feed intake were not recorded.

Sample preparation and testing. Firstly, milk BHBA concentrations were determined, using a milk ketone body test strip, and when the serum BHBA concentrations were >1.200 µmol/L, the cows were considered to be affected with SCK (1, 9, 13, 21). Blood samples were taken only once from each animal, in the morning before feeding. The blood was obtained by jugular venipuncture using evacuated tubes; decoagulant was not used. The blood was centrifuged immediately after the collection at 4,000 g for 10 min. Serum was harvested and packaged in centrifuge tubes. One part of the tubes was frozen and stored at -20°C. Just before analysis, the samples had to be equilibrated to room temperature. Samples with visual haemolysis were excluded from the analysis. Other serum samples were acidified and reduced (using 1.12% m-phosphoric acid and 0.05% dithiothreitol) within 2 h after obtaining the sample. This process converted DHAA into ascorbic acid and prevented its oxidation (20). Vitamin C was detected by HPLC (19).

Serum samples without dithiothreitol treatment were used for the detection of glucose, BHBA, Ca, and P. BHBA was measured by serum BHBA kit (enzymatic-rate method), glucose by serum glucose kit (oxidase method), and P by P kit (colorimetric method). These tests were performed in a biochemical auto-analyser. Serum total Ca level was measured by atomic absorption spectrometer followed by acid digestion.

Statistical analysis. The SPSS13.0 software was used for the statistical analysis of the results. The data was expressed as means ± standard deviation. Student-t test was used for comparison of means. Spearman rank test (non-parametric tests) was performed to analyse the relationship between the concentration of serum BHBA and glucose and Ca. For all statistical tests a P-value of <0.05 was taken to be statistically significant.

Results

The results showed that serum glucose concentration in sub-clinically ketotic cows was significantly lower (P<0.01) and the BHBA concentration was significantly higher (P<0.01) than in healthy cows (Table 1). There were no significant differences in the average vitamin C and P concentrations between two groups of the cows (P>0.05). Serum total Ca concentration in sub-clinically ketotic cows decreased significantly (P<0.01) comparing to healthy ones. The serum BHBA concentration was negatively correlated (P<0.01) with glucose and Ca concentrations (Table 2).

Discussion

The early postpartum period is considered to be the major risk period for SCK occurrence because the negative energy balance (NEB), hypocalcaemia, metritis, and nutritional and management factors play a central role in its pathogenesis. Serum glucose...
concentration was lower in the sub-clinically ketotic cows than in the healthy ones and there was a negative relationship between them. These results suggest the insufficient glucose supply in the sub-clinically ketotic cows. Dairy cows experience a NEB because the drain of energy for milk production exceeds the energy uptake from the ingested feedstuffs. This imbalance leads to mobilisation of body fat reserves in the form of fatty acids, this results in an increase in ketone body production in the liver. Hypoglycaemia is correlated to SCK in the dairy cows as reported by Sakha et al. (15). SCK may start at serum BHBA concentrations above 1,000 µmol/L and clinical ketosis at about 2,600 µmol/L. However, in our studies serum BHBA concentrations in some cows was beyond 3,000 µmol/L, although they did not present clinical ketosis symptoms. This suggests that the level of BHBA at which an individual cow will express clinical signs of the disease is extremely variable. Studies using blood BHBA concentrations for assessing SCK reported a range of values from 1,000 to 1,400 µmol/L for defining a SCK threshold; however, the distribution of blood BHBA concentrations in our study seemed to suggest a cutoff of 1,200 µmol/L between cows with and without SCK. At present, there is no unified standard of the milk strip for SCK diagnosing. We used 200 µmol/L as the threshold according to the recommendation of the producer; despite, 100 µmol/L was used as cutoff point in a few studies, too (5). Further study should be carried out on the optimal cut-off point of the milk strip. It is an effective method for detecting the milk BHBA using milk strip before collecting blood samples for primary screening of sub-clinically ketotic cows.

Dairy cows are not dependent upon a feed source of vitamin C, because they can synthesise a sufficient amount of ascorbic acid from glucose in the liver (12, 19). In this study, glucose synthesis was not sufficient in cows with SCK; however, serum vitamin C concentration did not change. This suggested that the ketotic cows had the ability to produce ascorbic acid to meet their requirements in the early lactation period though glucose supply was not sufficient. Only few information on the control of ascorbic acid synthesis in dairy cows could be offered (19, 20). It might be considered that ascorbic acid synthesis was given a high metabolic-priority over glucose in the liver of ketotic cows. In a word, the concentration of BHBA and glucose in sub-clinically ketotic cows does not influence the level of vitamin C during the early period of lactation, meanwhile, it may limit its use as test index for the SCK of dairy cows, too.

The normal concentrations range of blood Ca and P reported in the dairy cow are of 2.22–2.70 mmol/L and 1.05–2.83 mmol/L, respectively. Serum total Ca 1.80, 1.90, or 2.00 mmol/L was used as the threshold of subclinical hypocalcaemia, mean serum Ca concentration was no lower than 1.80 mmol/L in our study, and serum P concentration was in normal range, too. The average blood Ca concentrations was significantly lower (P<0.01) in cows with SCK comparing to that of the healthy ones in the presented study, and a negative relationship existed between the serum total Ca and the BHBA concentrations in the cows. It suggests that the insufficient Ca supply in the ketotic cows and serum total Ca concentration can be used as a reference index for the diagnosis of SCK. The average blood P concentrations were not different in sub-clinically ketotic cows as compared to that of the healthy cows. It indicates that the metabolism of P is not affected by the BHBA concentration in cows with SCK.

The aetiology of SCK is complex. Insufficient forage ration of Ca can result in hypocalcaemia, which brings about a high risk of clinical ketosis (7). Ca absorption and metabolism are regulated by many hormones. The detailed mechanism of the action of these hormones in sub-clinically ketotic cows needs to be explained in a further study. On the farm, where our experiment was conducted, there was a sufficient supply of minerals at the feed ration for the cows. Otherwise, if the reason of Ca concentration decrease was a foraging reduction, the P concentration would decrease too. However, it did not change significantly, so that possibility could be excluded in our study. Thus, we are prone to think that the high concentration of BHBA impairs the absorption and utilisation of Ca in dairy cows with SCK during the early lactation period.

The greatest advances in dairy health in the last 25 years have been disease prevention rather than treatment, as well as the shifts from focusing on individual animals to groups and herds (11). From this paper, it is visible that detecting serum BHBA concentration as a routine monitoring programme could be beneficial for the husbandry of dairy cows and the cows should be examined regularly during the first two months after calving. Serum total Ca concentration can be used as a reference index for the diagnosis of SCK.

Acknowledgments: This study was funded by two grants financed by the China National Science Foundation Committee (30600441, 30571365).

References