Relationship between the Sodium Nitroprusside Test for Ketone Bodies in Urine and Serum β-Hydroxybutyrate Concentrations in Dairy Cows

Theera Rukkwamsuk1,*, Jenjira Suksiri1, Nonthisit Chutiyanawat1, Nathakorn Kaewsakhorn1 and Sunthorn Rungruang2

ABSTRACT

The relationship between the sodium nitroprusside (SNP) test for ketone bodies in urine and concentrations of serum β-hydroxybutyrate (β-HB) was studied in 50 Holstein Friesian cows on a commercial dairy farm, including 10 cows in the dry period, and 22, 8 and 10 cows and in week 2, 4 and 8 of lactation, respectively. Urine and blood samples were collected from all cows and were transported to the laboratory on the sampling date. Urine samples were analyzed for ketone bodies using the SNP test within 24 hours after collection. Serum samples were analyzed for the concentration of β-HB. Results of the SNP test for ketone bodies were classified as either N (negative), +1 or +2 based on the color reaction of SNP with a known amount of acetone in aqueous solution at 0 mmol (N), 1 to 5 mmol (+1) and 6 to 10 mmol (+2) respectively. Results of the SNP test for ketone bodies in urine were highly correlated with the concentrations of β-HB in the serum (r = 0.82). Concentrations of serum β-HB were 0.46 ± 0.03, 0.98 ± 0.10 and 2.63 ± 0.34 mmol/L for cows with the SNP test results of N (n = 31), +1 (n = 10) and +2 (n = 9), respectively. Concentrations of serum β-HB were 0.42 ± 0.04, 0.97 ± 0.21, 1.48 ± 0.44 and 0.89 ± 0.12 mmol/L for cows in the dry period and in week 2, 4 and 8 of lactation, respectively. The method was practical, simple and inexpensive and therefore, it would be very beneficial for the diagnosis of subclinical ketosis or the early detection of ketosis in dairy cows, particularly on small-holder farms.

Key words: β-hydroxybutyrate, dairy cow, ketosis, sodium nitroprusside

INTRODUCTION

Ketosis is a common metabolic disorder in dairy cows, which is frequently observed in the first few weeks of lactation (Andrews 1998; Grummer, 1993). It is known that dairy cows approaching parturition have a marked decrease in their feed intake due to hormonal and physiological changes (Goff and Horst, 1997). Therefore, the amount of energy obtained from the feed source cannot adequately supply the energy required for maintenance and milk production. As a result, cows during the periparturient period must mobilize their body energy reserves, mainly fat from adipose tissues, to compensate for the energy deficits. Increased lipolysis results in increased non-esterified fatty acid concentration in the blood, which is
metabolized further in the liver to generate ATP or to form ketone bodies (Bruss, 1993). In practice, ketotic cows are prone to develop poor health, low milk production and infertility (Dohoo and Martin, 1984; Fourichon et al., 1999; Fourichon et al., 2000).

In principle, the diagnosis of ketosis in dairy cows depends on the determination of ketone bodies [acetoacetate, acetone and β-hydroxybutyrate(ß-HB)] in the blood, urine and milk (Geishauser et al., 1998). Increased concentrations of these ketone bodies in the body fluid indicate ketosis. Blood ß-HB concentration has frequently been used for detection of ketosis and a cut-off point of 1.2 mmol/L is widely accepted by several investigators (Duffield et al., 1998; Geishauser et al., 1998; Jorritsma et al., 1998) to distinguish between ketotic and healthy dairy cows. However, it is not always practical to collect blood samples for determination of ß-HB concentration. Moreover, this determination requires special equipment such as spectrophotometer and the procedure is time-consuming. Alternative cow-side tests, such as the Ketostix urine strip test (Bayer Corporation, Elkhart, Indiana, USA), the KetoCheck powder test on milk (Great States Animal Health, St. Joselp, Missouri, USA) and the KetoTest milk strip test (Sanwa Kagaku Kenkyusho Co., Ltd., Nagoya, Japan), have been applied to detect ketosis in dairy cows (Geishauser et al., 1998; Carrier et al., 2004). These tests are more practical for use by farmers and are reliable to detect ketosis in dairy cows. However, it is still costly for small-scale dairy farms in Thailand and these commercial tests are not easily accessible to most Thai farmers.

The objective of this study was therefore to efficiently use the sodium nitroprusside (SNP) test, which is a traditional qualitative method, to determine ketone levels in urine by evaluating the SNP test results with serum ß-HB concentrations.

**MATERIALS AND METHODS**

**Cows and sampling**

In total, 50 Holstein Friesian dairy cows were randomly selected from a commercial herd: 10 cows in the dry period and 10, 22 and 8 cows in week 2, 4 and 8 of lactation, respectively. Blood and urine samples were collected from all cows once on the same day. Five milliliters of blood were collected from the coccygeal vein or artery into a vacuum tube without added preservatives. The blood samples were allowed to clot at room temperature for 30 minutes and were transported in an ice box to the laboratory and thereafter were centrifuged at 1300 × g for 10 min. Serum samples were harvested and stored at -20°C until determination of ß-HB concentrations. Cows were manually stimulated to urinate and the first stream of the urine was discarded. Thereafter, approximately 50 milliliters were collected into a dark brown glass bottle with a screw cap and the samples were transported to the laboratory in an ice box. All urine samples were stored at 4°C until analysis for ketones within 24 h after collection.

**Determination of serum β-hydroxybutyrate**

Serum ß-HB concentrations (Ranbut-RB1007, Randox Laboratories Ltd., UK) were determined by an enzymatic method using spectrophotometry and a commercially available test kit.

**Determination of ketone bodies in urine**

Urine samples were qualitatively determined for ketone bodies using a modified Rothera test (Free et al., 1958). In principle, sodium nitroprusside (SNP) {Na₄[Fe(CN)₆NO]·2H₂O} is dissociated in an alkaline solution to sodium ferrocyanide [Na₄(CN)₃], sodium nitrite (NaNO₂) and ferric hydroxide [Fe(OH)₃]. These molecules are strong oxidizing agents which react with ketone bodies in the sample to form a reddish purple ring.
Exactly 5 ml of urine sample were place in a test tube containing 1 g of mixture powder (1 g of SNP and 99 g of ammonium sulphate). After these ingredients had completely dissolved, 1 ml of concentrated ammonium hydroxide was gently added to the test tube. A purple ring developed immediately at the interface between the bottom of the urine sample and the top of the concentrated ammonium hydroxide. The intensity of the ring color was classified into N (negative), +1 and +2 based on the color reaction of the SNP test with a known amount of acetone in aqueous solution at 0 mmol (N), 1 to 5 mmol (+1) and 6 to 10 mmol (+2), respectively.

**Statistical analyses**

Data on serum β-HB concentrations were tested for a normal distribution using the Shapiro-Wilk W test (Patrie and Watson, 1999), and the homogeneity of variances was verified using Levene’s test (Patrie and Watson, 1999). Comparison of data between different groups of cows was conducted using ANOVA. The relationship between the results of the SNP test for ketone bodies in urine and serum β-HB concentrations was evaluated using Pearson’s correlation (Patrie and Watson, 1999).

**RESULTS AND DISCUSSION**

**Serum β-hydroxybutyrate**

Serum β-HB concentrations in the 50 cows are shown in Figure 1. Average serum concentrations of β-HB were 0.42 (± 0.04), 0.97 (± 0.21), 1.48 (± 0.44) and 0.89 (± 0.12) mmol/L for cows in the dry period and in week 2, 4 and 8 of lactation, respectively. Cows in the dry period had the lowest serum β-HB concentrations and the concentrations were within the normal range for Thailand (Rukkwamsuk et al., 2005). As indicated by the higher serum β-HB concentrations, cows in their lactating period had an increase in fat mobilization from adipose tissue. This result indicated that early-lactating cows usually go into some degree of negative energy balance which is a common phenomenon during this period (Rukkwamsuk et al., 2006). Based on a cut-off concentration of 1.2 mmol/L of serum β-HB, 0% (0/10), 23% (5/22), 38% (3/8) and 20% (2/10) of dry cows and cows in week 2, 4 and 8 of lactation, 0% (0/10), 23% (5/22), 38% (3/8) and 20% (2/10) of data represent mean and SEM. Different letters indicate mean concentrations differed between each sampling period at P < 0.05.

**Figure 1** Comparison of serum β-hydroxybutyrate concentrations (mmol/L) between cows in the dry period (n = 10) and cows in week 2 (n = 10), week 4 (n = 22) and week 8 (n = 8) of lactation. Data represent mean and SEM. Different letters indicate mean concentrations differed between each sampling period at P < 0.05.
respectively were classified as having subclinical ketosis. Overall prevalence was 20% (10/50). The highest prevalence was observed in cows in week 4 of lactation, which was also reported by Dohoo and Martin (1984). In an experimental trial of fatty liver development, cows with a negative energy balance during their postparturient period had an increase in blood concentration of non-esterified fatty acids, which mainly originate from lipolysis (Rukkwamsuk et al., 1998). The peak concentrations were observed in the first 2 weeks after calving (Rukkwamsuk et al., 1998). These non-esterified fatty acids were further metabolized in the liver to cause an increase in the production of ketone bodies. This is one of the reasons why higher concentrations of serum β-HB were observed at week 4 than at week 2 of lactation. After the peak concentrations at week 4, cows could adapt to a negative energy balance. Therefore the concentrations of serum β-HB concentrations gradually declined to a normal lactation level and reached the dry period level by week 12 of lactation (Van den Top et al., 1996).

**Sodium nitroprusside test**

When data were pooled and arbitrarily divided into 3 groups according to the SNP test results, cows with negative (n = 31), +1 (n = 10) and +2 (n = 9) SNP test results had average serum β-HB concentrations of 0.46 (± 0.03), 0.98 (± 0.10) and 2.63 (± 0.34) mmol/L, respectively. Cows with a +2 SNP test result had the highest serum β-HB concentrations (Figure 2). Sodium nitroprusside test results and serum β-HB concentrations are shown in Table 1. For this study, SNP test results of +1 and +2 and serum β-HB concentrations could adapt to a negative energy balance.

![Comparison of serum β-hydroxybutyrate concentrations (mmol/L) between cows with sodium nitroprusside test result as negative (n = 31), +1 (n = 10) and +2 (n = 9). Data represent mean and SEM. Different letters indicate means concentrations differed between each sampling periods at P < 0.05.](image)

**Figure 2**

**Table 1** Sodium nitroprusside (SNP) test results and serum β-HB concentrations in 50 dairy cows.

<table>
<thead>
<tr>
<th>Number of cows</th>
<th>Serum β-HB ≥ 1.20 mmol/L</th>
<th>Serum β-HB &lt; 1.20 mmol/L</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ SNP test</td>
<td>10</td>
<td>9</td>
<td>19</td>
</tr>
<tr>
<td>- SNP test</td>
<td>0</td>
<td>31</td>
<td>31</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>40</td>
<td>50</td>
</tr>
</tbody>
</table>
greater than 1.2 mmol/L were classified as indicating subclinical ketosis. The results of the SNP test were positively correlated (r = 0.82) with serum β-HB concentrations in the diagnosis of subclinical ketosis. The sensitivity and specificity of the SNP test in this study were calculated to be 100% (10/10) and 77.5% (31/40). In the study by Nielen et al. (1994), two cow-side tests for subclinical ketosis in dairy cows were evaluated and the results showed that the SNP test on urine samples for diagnosis of subclinical ketosis had a sensitivity of 100% and a specificity of 67%. However, the authors used a cut-off concentration for serum β-HB of 1.4 mmol/L instead of 1.2 mmol/L as in this study.

CONCLUSION

To alleviate any adverse effects of ketosis on the health, milk production and reproduction in lactating dairy cows, early diagnosis of this metabolic disorder is crucially important. In practice, dairy farmers prefer a cowside test for ketosis, which should be simple and inexpensive. This study demonstrated that the SNP test for ketone bodies using a urine sample could be an appropriate tool for dairy farmers in early detection of subclinical ketosis in their dairy cows. This would lead to early treatment or prevention of this disorder at the farm level.

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LITERATURE CITED


