Calcium and β-hydroxybutyrate serum concentrations in early postpartum of Jersey cows

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ABSTRACT

The experiment was conducted to determine serum [Ca\(^{2+}\)]. β-hydroxybutyrate in blood, milk and urine during peripartum and urinary pH. Ninety Jersey cows were selected during their last month of pregnancy. Five days before calving, at calving and 12, 24, 48 and 72 h after calving blood samples were taken. Ca\(^{2+}\), and β-hydroxybutyrate (β-HB) in blood serum, milk and urine were determined. Correlations and one-way ANOVA were used for the analyses. Calcium and glucose (mmolL\(^{-1}\)) decreased throughout the peripartum: Ca\(^{2+}\) [2.65 to 2.31±0.11 (p<0.05)]; glucose [3.74 to 3.11±0.26 (p<0.05)]. NEFA, blood β-HB, urine β-HB and milk β-HB (mmolL\(^{-1}\)) increased over time: NEFA [0.36 to 0.45±0.05 (p<0.05)]; blood β-HB [0.69 to 1.29±0.07 (p<0.05)]; urine β-HB [0.10 to 0.37±0.09 (p<0.05)]; milk β-HB [0.09 to 0.18±0.04 (p<0.05)]. Urine pH did not change: 7.31 to 6.95±0.34 (p>0.05). No correlation was found between β-HB in blood and β-HB in milk. β-HB in blood and milk did not have a functional relationship in Jersey cows that was useful in diagnosing metabolic disorders.

Key words: Calcium, Hypocalcemia, β-hydroxybutyrate, Ketosis.

INTRODUCTION

In the last 30 years individual milk production of dairy cattle has increased by ~430% as the result of breeding, feeding and integrated management. However, susceptibility to diseases has increased and reproductive efficiency has decreased (Melendez et al. 2004; Martinez et al. 2012; Yildiz and Erisir 2016). Today’s dairy cattle is the result of genetic selection for higher milk production, which depends on the feed they consume and their body reserves at the beginning of lactation. Metabolic processes in the period before and after calving undergo a series of abrupt changes, which can affect cow health and reproductive performance (Espino et al. 2005; Bauman et al. 2006; Martinez et al. 2012; Yildiz and Erisir 2016). A low serum Ca\(^{2+}\) concentration at calving predisposes the cows to metabolic disorders during the transition period (Melendez et al. 2002; Suthar et al. 2013; Xiao et al. 2017), such as milk fever, subclinical hypocalcemia, ketosis and displacement of the abomasum (Walsh et al. 2007; Hesam et al. 2011). A cow with subclinical hypocalcemia shows signs of depression, reduces voluntary intake, and has decreased milk production (Chapinal et al. 2011; Xiao et al. 2017). Homeostatic mechanisms maintain Ca\(^{2+}\) blood concentrations within a range of 2.24 - 2.74 mmolL\(^{-1}\), but during peripartum, these mechanisms fail; Ca\(^{2+}\) content decreases to ≤1.74 mmolL\(^{-1}\) and hypocalcemia occurs (Goff, 2000; Goff et al. 2002). A decrease in dry matter intake (DMI) lowers blood Ca\(^{2+}\) concentration, and the cows enter into a negative energy balance, increasing the risk of subclinical ketosis. This disease, related to hypocalcemia (Walsh et al. 2007), is characterized by a blood concentration of β-hydroxybutyrate above 1.4 mmol L\(^{-1}\), but with no clinical symptoms (Ospina et al. 2010). Ketosis has a higher incidence in cows that have had two or more calvings and at the beginning of lactation when milk production is higher (Espino et al. 2005; Enjalbert et al. 2001; Gilliund et al. 2001; Ingvartsen et al. 2003; Overton et al. 2004). The dairy cow induced ketosis when the concentration of NEFA product more than the demands of body (Chuang et al. 2016) Some breeds of dairy cattle, such as Jersey and Guernsey have a high propensity to contract metabolic disorders because they produce colostrum with a higher concentration of Ca\(^{2+}\) than Holstein cows and because the 1.25 (OH)\(_2\)D\(_3\) receptors are fewer in Jersey cows (Ingvartsen et al. 2003; Andresen , 2001). In general, the diseases that occur at a subclinical stage are of great importance in the dairy industry because of economic and health consequences (Suthar et al. 2013). For detection of hypocalcemia or subclinical ketosis in cows, it is important to have test results that can be related, for timely treatment of both disorders (Hesam et al. 2011). For this reason, the objective of this study was to determine the serum concentrations of Ca\(^{2+}\) and β-hydroxybutyrate in milk during pre- and post-partum, and urinary pH 5 days prepartum in Jersey cows, as well as to determine functional relationships among these variables.

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MATERIALS AND METHODS

Study area: The study was conducted on the San Carlos farm, Villa de Reyes, San Luis Potosí, Mexico (21° 51’ N, 100° 53’ W, altitude 1810 m).

Experimental design: Ninety Jersey cows, 460 ± 37 (SD) kg liveweight, were selected at random during the last month of pregnancy. Their body condition was 2.75 to 3.25 (scale 1–5). Average milk production in the previous lactation period was 7808.5±366.4 L (30 second lactation, 30 third and 30 fourth or more lactations). The cows were fed a prepartum diet of 70% forage (alfalfa, oats, and silaged maize) and 30% concentrate with anionic salts (NH₄Cl) ad libitum once daily. After calving, they were given a diet of alfalfa, silaged maize and concentrate with 20% crude protein (Table 1).

Sampling: Five days before (-120 h) the likely calving date, at calving (0 h), 12, 24, 48 and 72 h after calving, blood samples were taken from the coccygeal vein with BD Vacutainers™. Blood samples were centrifuged at 3,000 rpm at 4 ºC (Thermo Scientific™ IEC) to obtain serum, which was frozen (-0.4ºC) and stored for later analysis. In the blood sample, Ca²⁺ concentration was determined with o-cresolphthalein complexone (Becton Dickinson de México, S. A. de C. V.) at a wavelength of 550 nm in a UV/VIS spectrophotometer. Blood, urine and milk serum concentrations of β-hydroxybutyrate were determined with the UV NAD-dependent enzyme method (International Headquarters Randox Laboratories LTD, UK) at a wavelength of 340 nm. We considered that cows had subclinical ketosis when β-hydroxybutyrate concentration was ≥ 1200 µmol L⁻¹ (Geishauser et al. 2000). On the same dates as blood sampling, urine pH was measured with a potentiometer (HANNA Instruments® HI 8014) that was calibrated before each blood sampling with a buffer solution that had pH 4.0, 7.0 and 10.0. To obtain urine, the lower part of the vulva was massaged repeatedly (Goff, 2000).

Statistical analysis: Data were analyzed with simple linear correlation and a one-way ANOVA in the GLM module of the STATISTICA v10 software, and Sigma Plot v11 was used to construct the Figs.

RESULTS AND DISCUSSION

Values of serum, urine and milk concentrations of the metabolites studied are presented in Fig 1. Eight cows (8.88%) were found to have slight clinical hypocalcemia and ten cows (11.11%) had slight clinical ketosis. Fig 1 shows the significant changes in metabolite concentrations throughout the period analyzed (*p< 0.05). Another aspect analyzed was correlation of [Ca²⁺] over the different times of the peripartum period; results are shown in Table 2.

The results of our study contrast with those of others report that a period of major fluctuations in serum Ca²⁺ occurs between day 7 prepartum (168 hours before calving) and day 7 postpartum (168 hours after calving) and that, as the number of calvings or age increases, cow serum concentrations of Ca²⁺ decrease, and incidence of clinical hypocalcemia increases (Samad et al. 2002; Meléndez et al. 2004) and can cause other associated metabolic disorders (Chapinal et al. 2011; Salgado et al. 2009; Chuang et al. 2016).

No correlation was found between β-HB concentrations in blood and β-HB concentrations in milk, as has been found in other studies (Enjalbert et al. 2001), possibly because β-hydroxybutyrate circulating in the blood

Table 1: Composition of diets fed to cows during the experimental period.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Prepartum</th>
<th>Postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize silage</td>
<td>35</td>
<td>20</td>
</tr>
<tr>
<td>Oats</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>Alfalfa</td>
<td>15</td>
<td>24</td>
</tr>
<tr>
<td>Ground maize</td>
<td>10</td>
<td>31</td>
</tr>
<tr>
<td>Soybean paste</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Coconut paste</td>
<td>-</td>
<td>1.7</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td>Premixture of vitamins and minerals</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Anionic salts</td>
<td>7.9</td>
<td>3.9</td>
</tr>
<tr>
<td>Calculated nutritional content</td>
<td>1.63</td>
<td>1.66</td>
</tr>
</tbody>
</table>

Table 2: Coefficient of partial correlation and probability > R of the [Ca²⁺] serum in prepartum and postpartum.

<table>
<thead>
<tr>
<th>Sampling period</th>
<th>Coefficients of correlation</th>
<th>Probability &gt; R</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 d prepartum – at calving</td>
<td>0.5166</td>
<td>0.0002</td>
</tr>
<tr>
<td>5 d prepartum – 12 h postpartum</td>
<td>0.7120</td>
<td>0.0001</td>
</tr>
<tr>
<td>5 d prepartum – 24 h postpartum</td>
<td>0.6629</td>
<td>0.0001</td>
</tr>
<tr>
<td>5 d prepartum – 48 h postpartum</td>
<td>0.6500</td>
<td>0.0001</td>
</tr>
<tr>
<td>5 d prepartum – 72 h postpartum</td>
<td>0.6100</td>
<td>0.0001</td>
</tr>
<tr>
<td>Calving – 12 h postpartum</td>
<td>0.5532</td>
<td>0.0001</td>
</tr>
<tr>
<td>Calving – 24 h postpartum</td>
<td>0.3981</td>
<td>0.0051</td>
</tr>
<tr>
<td>Calving – 48 h postpartum</td>
<td>0.4211</td>
<td>0.0016</td>
</tr>
<tr>
<td>Calving – 72 h postpartum</td>
<td>0.5621</td>
<td>0.0001</td>
</tr>
<tr>
<td>12 h postpartum – 24 h postpartum</td>
<td>0.7316</td>
<td>0.0001</td>
</tr>
<tr>
<td>12 h postpartum – 48 h postpartum</td>
<td>0.7218</td>
<td>0.0001</td>
</tr>
<tr>
<td>12 h postpartum – 72 h postpartum</td>
<td>0.7404</td>
<td>0.0001</td>
</tr>
<tr>
<td>24 h postpartum – 48 h postpartum</td>
<td>0.7512</td>
<td>0.0001</td>
</tr>
<tr>
<td>24 h postpartum – 72 h postpartum</td>
<td>0.7639</td>
<td>0.0001</td>
</tr>
<tr>
<td>48 h postpartum – 72 h postpartum</td>
<td>0.7599</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

**p< 0.01 highly significant**
Fig 1: Serum concentrations of metabolites in blood, urine and milk samples taken during prepartum (-120, 0, 24, 48 and 72 hours). A. Serum concentrations of Ca$^{2+}$. B. Serum concentrations of glucose. C. Serum concentration of non-esterified fatty acids (NEFA). D. Serum concentration of β-hydroxybutyrate in blood (β-HB). E. Serum concentration of β-hydroxybutyrate in milk. F. Serum concentration of β-hydroxybutyrate in blood. (*p< 0.05). Dotted blue line represent the 120 h prepartum.

is used by the mammary gland to synthesize fatty acids, and acetate is then converted into butyrate (Clark et al. 2005; Ospina et al. 2010). For this reason, low β-HB concentrations can be expected in milk. However, there are studies that point out that Ca$^{2+}$ concentration correlates negatively with increased β-hydroxybutyrate (Sakha et al. 2006; Zhigang et al. 2009). Cows that have subclinical ketosis have lower concentrations of Ca$^{2+}$ (≤2.03 mmol/L), which cause hypocalcemia, decreased DM intake and a negative energy balance, suggesting that insufficient serum Ca$^{2+}$ can be used as a diagnostic index of subclinical ketosis (Suthar et al. 2013; Xiao et al. 2017).

Has been reported two quite different types of metabolic disorders in which ketosis can occur, type I ketosis and type II ketosis, respectively (Holtenius and Holtenius, 1996). Type I, generally occurs 3-6 weeks after calving in cows whose milk secretion is so extensive that the demand for glucose exceeds the capacity for glucose production, was characteristic with a decrease in appetite and milk production, and weight loss. In addition, there was a decrease on glucose, but the concentrations of beta-hydroxybutyric acid (BHBA) and non-esterified fatty acid (NEFA) were increased (Berge and Vertenten, 2014). However, type II, generally occurs earlier in lactation, leads to the concentrations of BHBA, glucose and NEFA were increased (Oikawa and Oetzel, 2006).

In our experiment, blood β-HB concentrations remained stable, coinciding with other studies that reported that β-HB concentrations in blood were constant 15 days before calving and up to 10 days postpartum (Le Blanc et al. 2005). β-HB concentrations in milk correlated positively with the diet with anionic salts before calving, prior to postpartum milk days and milk production in previous lactation. However, it has been reported that high milk production in the previous lactation does not increase the risk of ketosis in the following lactation (Oetzel, 2000); however, an increase in milk production increases risk of ketosis (Piepenbrink et al. 2000). Moreover, it has been observed that a prolonged interval between calvings increases the risk of ketosis in the following lactation (Beaudeau et al. 2000; Yildiz and Erisir, 2016).

This suggests that the homeostatic mechanisms that regulate Ca$^{2+}$ metabolism are different in each cow, so that a low concentration of serum Ca$^{2+}$ does not necessarily indicate clinical hypocalcemia (Melendez et al. 2002; Melendez et al. 2004). Some authors have indicated that the cause of clinical hypocalcemia is not attributed only to Ca$^{2+}$ serum concentrations, but to diverse, sometimes unknown, causes...
(Schultz, 1998). The low incidence of hypocalcemia can be related to urinary pH that was found to be within the range recommended for measuring the effectiveness of anionic salts (Overton et al. 2004; Goff et al. 2003).

As calving approaches, and up to 48 h postpartum, the serum concentrations of Ca$^{2+}$ gradually decrease. Serum concentrations of Ca$^{2+}$ in prepartum correlated with concentrations in postpartum, suggesting the importance of adequate feeding and management of the cow during the dry period (Walsh et al. 2007; Hesam et al. 2011; Xiao et al. 2017). According to the reference values, all the cows had subclinical hypocalcemia in a period of 48 h postpartum. The incidence of clinical hypocalcemia was 21.4%; of this percentage, 33% had serum concentrations of Ca$^{2+}$ above 1.63 mmol L$^{-1}$. In contrast, 43% of the healthy cows had lower Ca$^{2+}$ serum concentrations. No relationship was found between urine pH and Ca$^{2+}$ concentrations, indicating that urine pH was not a good indicator of Ca$^{2+}$ serum concentrations. However, other studies report that administration of anionic salts induce metabolic acidosis, which increases parathormone production as well as fine tuning of receptors, production of 1-25(OH)$_2$D$_3$, production of bone resorption and intestinal absorption of Ca$^{2+}$ (Duffield, 2000; Goff et al. 2003; Salgado et al. 2009). β-hydroxybutyrate concentrations in blood did not correlate with β-HB milk concentrations.

There is a relationship between low serum Ca$^{2+}$ concentrations and increases in β-hydroxybutyrate because when Ca$^{2+}$ decreases in blood, intake decreases because of hypocalcemia, and the cows have a negative energy balance, indicating that subclinical ketosis is correlated with hypocalcemia (Sakha et al. 2006; Zhigang et al. 2009; Martinez et al. 2012).

CONCLUSION

In this study, we concluded that a low serum concentration of Ca$^{2+}$ was not indicative of clinical hypocalcemia. However, it does tend to correlate with urine pH, which can be used as an indicator of some problem of hypocalcemia. The other variables such as β-hydroxybutyrate in blood and milk in our experiment with Jersey cows did not have a functional relationship that was useful in diagnosing metabolic disorders. Early diagnosis of β-hydroxybutyrate in blood can help to prevent ketosis.

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REFERENCES


