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Accuracy of a cow-side test for the diagnosis of hyperketonemia and hypoglycemia in lactating dairy cows

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ABSTRACT

The objective of this study was to evaluate the use of a cow-side device (FreeStyle Precision Neo™) to diagnose ketosis and hypoglycemia based on measures of blood β-hydroxybutyrate (BHBA) and glucose. Eleven commercial dairy farms were visited and blood samples were taken from Holstein cows between 2 and 14 days in milk, yielding 441 samples for BHBA analysis and 308 samples for glucose analysis. Concentrations of BHBA and glucose were measured in two ways, 1) using the cow-side device with whole blood immediately after sampling and 2) serum samples analyzed with a standard laboratory assay (Animal Health Laboratory, University of Guelph, Canada). The accuracy of the device was determined by comparing the results to the laboratory method as well as the ability to diagnose ketosis (BHBA ≥ 1.2 mmol/L) and hypoglycemia (glucose < 2.5 mmol/L). The concordance correlation coefficient (CCC), Bland-Altman plot and Kappa coefficient were calculated to evaluate agreement between the 2 methods using SAS (version 9.3). The CCC was 0.92 for BHBA and 0.56 for glucose measurements. The 95% confidence intervals of the Bland-Altman plot encompassed 97% and 95% of the mean difference between methods for BHBA and glucose measurements, respectively. The Kappa coefficients were 0.78 for BHBA and 0.23 for glucose measurements. These results indicate that the cow-side device is accurate for rapid measurement of blood BHBA and diagnosis of ketosis on farms but is not accurate for measurement of blood glucose concentrations and diagnosis of hypoglycemia.

1. Introduction

During early lactation cows are in a state of negative energy balance. Their body fat stores are mobilized to increase blood concentrations of non-esterified fatty acids (NEFA) to be used by the liver for gluconeogenesis in order to reduce the energy deficit (Herdt, 2000). However, excess NEFA will result in incomplete oxidation in the liver and the production of ketone bodies, such as β-hydroxybutyrate (BHBA), which can accumulate in the body and lead to ketosis (Herdt, 2000). Subclinical ketosis is defined as the excess of circulating ketone bodies without clinical signs (Andersson, 1988) with threshold plasma BHBA concentrations of > 1.2 mmol/L (Duffield et al., 2009), whereas clinical ketosis has a threshold of ≥ 3.0 mmol/L (Oetzel, 2004) and includes clinical symptoms such as reduced appetite, loss of body weight and decreased milk yield (Duffield, 2000). Even at sub-clinical levels, excess BHBA increases the risk of other transition diseases, such as displaced abomasum and metritis (Duffield et al., 2009), decreases milk yield, reduces fertility and increases the risk of culling from the herd (McArt et al., 2012). The incidence of subclinical ketosis is variable; McArt et al. (2011) measured sub-clinical ketosis on 4 farms in the US and found a range of incidence between 26 and 56%, with an average of 43%. Clinical ketosis has a much lower incidence of 2 to 15% (Duffield, 2000).

As ketosis has a significant incidence on farm and represents a health and economic concern, it is important to monitor transition cows. The early detection of ketosis allows for timely treatment, preventing any loss in production or negative effects on cow health. Currently, the gold standard for diagnosing ketosis is the measurement of plasma or serum BHBA (Duffield, 2000) conducted in laboratories, which is expensive and takes too long to implement an intervention plan. In the last decade ketone sensors used for humans have been adopted and evaluated for use as cow-side diagnostic tools that are timely and less expensive (Jeppesen et al., 2006; Voyvoda and Erdogan, 2010; Süss et al., 2016). A newer device, the FreeStyle Precision Neo™ (Abbot Laboratories), has had minimal studies conducted to evaluate its use as a cow-side BHBA meter (Süss et al., 2016) and, to our knowledge,

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no studies evaluating its use as a cow-side glucometer. Although BHBA concentrations in the blood are the primary method of determining ketosis, blood glucose concentrations can be a valuable supplemental for differentiating between Type I and Type II ketosis (Herdt, 2000). Specifically, Type I ketosis is concurrent with low blood glucose levels, known as hypoglycemia (glucose < 2.5 mmol/L; Li et al., 2012) and occurs closer to peak lactation when glucose demands are not being met despite maximally functioning gluconeogenic pathways, requiring treatment intervention to directly supply glucose (Herdt, 2000). Even at subclinical BHBA values, cows can exhibit clinical signs of ketosis when also experiencing hypoglycemia (Li et al., 2012). Type II ketosis occurs soon after parturition when large amounts of NEFA are delivered to the liver, yet gluconeogenic pathways are not maximally functioning (Herdt, 2000). In this case blood glucose levels are normal and the cow benefits more from a propylene glycol treatment to stimulate gluconeogenesis as opposed to treatment with glucose (Gordon et al., 2013). Blood glucose measurements may give insight into treatment decisions regarding ketosis and having one device that measures both BHBA and glucose is advantageous. Therefore, the objective of this study was to evaluate the accuracy of the Precision Neo as a cow-side device to measure both blood BHBA and glucose concentrations in comparison to standard laboratory methods.

2. Materials and methods

All procedures were conducted in accordance with the guidelines of the Canadian Council on Animals Care (CCAC, 2009), and all procedures were approved by Alberta Agriculture and Forestry.

2.1. Animal, housing and feeding

During May to October 2015, 11 commercial Dairy farms in central and northern Alberta were visited up to 10 times and one blood sample was taken from individual Holstein cows on each farm that were currently between 2 and 14 days in milk at the time of the visit, for a total of 139 primiparous and 302 multiparous cows. Average parity of cows sampled was 2.7 ± 0.07 (mean ± SEM) ranging from 1st to 8th lactation. Number of cows sampled per farm varied, based on farm size, with a range of 6 to 129 cows per farm. The criteria for farm selection required a free stall barn, a herd > 100 milking cows, and for producers to either use Dairy Comp 305 (Valley Agricultural Software, Tulare, CA, USA) or DHI services (CanWest DHI, Guelph, ON, Canada). All farms fed a totally mixed ration, once daily, to meet or exceed the dietary requirements for a lactating cow weighing approximately 680 kg and producing 45 kg of 3.5% fat-corrected milk (NRC, 2001).

2.2. Sample collection and assays

A total of 441 blood samples were collected and analyzed for BHBA, whereas 308 blood samples were collected and analyzed for glucose. Blood was taken from the coccygeal vessels into vacuum tubes containing no preservative (Vacutainer; Becton Dickinson and Co., Franklin Lakes, NJ, USA). The concentration of BHBA and glucose in whole blood was measured by the electronic cow-side device (FreeStyle Precision Neo™; Abbot Diabetes Care Inc., Mississauga, ON, Canada) immediately following collection. Sample tubes were left at room temperature for 3 to 4 h before centrifugation (3000 × *g* for 20 min), at which point serum was collected, frozen, and stored at −20 °C until submission to the Animal Health Laboratory (University of Guelph, Canada) for determination of BHBA and glucose concentrations. Both assays were conducted within 1 week of sampling using commercial reagent kits and an automated analyzer (Hitachi 911 Analyzer, Laval, QC) as previously described by Duffield et al. (2009) and Wittrock et al. (2013). The intra-assay CV was 1.3% and 1.7% for BHBA and glucose analyses, respectively. The inter-assay CV was 2.9% for low BHBA samples and 2.4% for high BHBA samples, whereas the inter-assay CV was 1.7% for low glucose samples and 1.8% for high glucose samples.

2.3. Statistical analyses

Based on the laboratory standard method for BHBA, cows were categorized as normal (< 1.2 mmol/L), sub-clinical ketosis (1.2 ≤ and > 3.0 mmol/L), or clinical ketosis (≥ 3.0 mmol/L). Similarly for glucose, cows were categorized as hypoglycemic (< 2.5 mmol/L), normal (2.5 ≤ and > 3.6), or high (≥ 3.6 mmol/L). Statistical analyses were performed using SAS (version 9.3; SAS Institute Inc., Cary, NC). Pearson's correlation coefficients were used to test the strength of the relationship between the cow-side device and the laboratory methods; however, correlation coefficients do not indicate the agreement between methods. Bland-Altman plots depict the difference between methods, in which good agreement is shown by values that lie close to the 0 mean difference line and between the 95% confidence interval limits of agreement (Bland and Altman, 2007). Lin's concordance correlation coefficients (CCC) were also included to determine agreement amongst results; the coefficient ranges from −1 to 1 with −1, 0 and 1 indicating perfect disagreement, independence and perfect agreement, respectively (Crawford et al., 2007). Contingency tables based on the BHBA and glucose concentration categories were created to calculate a Cohen's Kappa (weighted) coefficient to indicate the agreement of ketosis and hypoglycemia diagnosis beyond chance. The Kappa coefficient ranges from 0 to 1 with a higher value indicating better agreement between methods (Dohoo et al., 2009). The true positive rate, sensitivity (Se), was calculated as the proportion of cows that has sub-clinical ketosis, clinical ketosis, and/or hypoglycemia which were correctly identified by the device. The false positive rate, specificity (Sp), was calculated as the proportion of cows not ketotic and/or hypoglycemic, which were correctly identified by the device.

3. Results

Based on the 11 commercial farms used in this study the prevalence of sub-clinical ketosis was 12.5% (BHBA concentration ≥ 1.2 mmol/L) and the prevalence of clinical ketosis was 1.6% (BHBA concentration ≥ 3.0 mmol/L). The Pearson's correlation coefficient indicated a strong relationship between the cow-side meter and the standard lab method for measuring BHBA concentrations (Table 1) that was highly significant. The CCC between the 2 methods of measuring for BHBA concentrations shows a strong agreement (Table 1). Despite this strong agreement, Fig. 1 shows that the cow-side device was generally overestimating BHBA concentrations and that the overestimation increased as concentrations of BHBA in the blood increased. The Bland-Altman plot also indicated agreement between the two methods with 97% of differences falling between the 95% confidence intervals (Fig. 2). This analysis also specifies that the mean difference between

Table 1
Strength of association and agreement between the laboratory method and cow-side device (FreeStyle Precision Neo™) for measuring blood BHBA concentration and diagnosing ketosis.

	Value	Lower 95% CI[a]	Upper 95% CI[a]	*P*-value
Pearson's correlation[b]	0.98	0.97	0.98	< 0.0001
Concordance correlation[c]	0.92	0.91	0.93	–
Kappa coefficient[d]	0.78	0.70	0.86	< 0.001

* Four hundred and forty one blood samples were collected and analyzed from individual cows between 2 and 14 days in milk at 11 different farms.

[a] The lower and upper 95% confidence intervals represent limits that are ± 1.96 SD from the mean.

[b] Pearson's measures the strength of association between two continuous variables.

[c] The Concordance correlation is a measure of agreement between two methods that accounts for the accuracy and precision of BHBA measurement, a continuous variable.

[d] The Kappa coefficient measures agreement between two methods, specifically the ability to diagnose ketosis (BHBA ≥ 1.2 mmol/L), a dichotomized variable.