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Accuracy of milk ketone bodies from flow-injection analysis for the diagnosis of hyperketonemia in dairy cows

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ABSTRACT

The objectives of this study were (1) to determine the correlations between blood β-hydroxybutyrate (BHBA) and milk components [BHBA, acetone, fat, protein, and fat:protein (F:P) ratio], and (2) to establish optimal thresholds for milk components to predict hyperketonemia in dairy cows. Data on 163 cows from 37 herds were used in this cross-sectional study. Herds were visited once during the study period, and cows between 2 and 90 d in milk were blood sampled within 4 h of milk sampling for the Dairy Herd Improvement test. Blood BHBA concentrations were measured using a cow-side electronic meter, Precision Xtra, which was considered the gold standard test in this study. Milk BHBA and acetone concentrations were measured in Dairy Herd Improvement milk samples by flow-injection analysis; whereas, milk fat and protein were tested using Fourier transform infrared spectroscopy. Hyperketonemia was defined by a blood BHBA concentration ≥1.4 mmol/L. The prevalence of hyperketonemia (based on blood BHBA values) in this study population was 21.0%. Pearson correlation coefficients between blood BHBA and milk BHBA, acetone, fat, protein, and F:P ratio were 0.89, 0.73, 0.21, 0.04, and 0.17, respectively. Receiver operating characteristic curves were generated and thresholds for each individual milk component were determined based on the maximal sum of sensitivity and specificity. Optimal threshold values for hyperketonemia were milk BHBA ≥0.20 mmol/L, acetone ≥0.08 mmol/L, fat ≥4.2%, and F:P ratio ≥1.3. Based on these thresholds, milk BHBA and acetone had greater sensitivity (84 and 87%, respectively) and greater specificity (96 and 95%, respectively) than the other milk components (fat, protein, and F:P). Series and parallel testing slightly improved the accuracy of milk BHBA and acetone values to predict hyperketonemia. A multivariable model that accounted for milk BHBA and milk acetone values simultaneously had the highest accuracy of all tested models for predicting hyperketonemia. These results support that milk BHBA and milk acetone values from flow-injection analysis are accurate diagnostic tools for hyperketonemia in dairy cows and could potentially be used for herd-level hyperketonemia surveillance programs.
Key words: hyperketonemia, milk, flow-injection analysis, β-hydroxybutyrate

INTRODUCTION

Hyperketonemia is defined as an abnormally high concentration of circulating ketone bodies (Andersson and Emanuelsson, 1985) and is an indicator of excessive negative energy balance in transition dairy cows (Duffield et al., 2009). The gold standard for diagnosing hyperketonemia is the measurement of BHBA in serum or plasma (Duffield et al., 1998). A serum BHBA concentration of 1.4 mmol/L or greater during the first weeks postpartum is often used to define hyperketonemia and was shown to be associated with impaired subsequent health, production, and reproduction in dairy cows (LeBlanc et al., 2005; Duffield et al., 2009; Ospina et al., 2010).

Accurate and user-friendly tools that facilitate cow-side measurement of BHBA concentrations are needed. Precision Xtra (Abbott, Mississauga, Ontario, Canada) is an electronic BHBA hand-held meter that has excellent accuracy (Iwersen et al., 2009). However, one of the main drawbacks of the Precision Xtra technology is the additional farm labor resources required to systematically test all animals at risk for hyperketonemia. Implementing a hyperketonemia surveillance program using DHI monthly milk test data could be a more practical and less labor-intensive approach. Flow-injection analysis (Gustafsson and Emanuelsson, 1996) and Fourier transform infrared (**FTIR**) spectroscopy (Heuer et al., 2001; van Knegsel et al., 2010; van der Drift et al., 2012) are laboratory techniques that have been developed for use with DHI milk testing to measure concentrations of BHBA, acetone, fat, and protein in milk. Interestingly, flow-injection analysis is generally used to calibrate FTIR spectroscopy for BHBA and ac-

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etone measurement (de Roos et al., 2007). However, the ability of milk ketone bodies values from flow-injection analysis to accurately diagnose hyperketonemia, as compared with whole blood or serum BHBA, has not been evaluated.

Therefore, the first objective of this study was to determine the correlations between blood BHBA and milk components measured by flow-injection analysis (BHBA and acetone) and FTIR (fat and protein). The second objective was to establish threshold values for these milk components to predict hyperketonemia in dairy cows.

MATERIALS AND METHODS

Experimental Design

This cross-sectional study was conducted from June to September 2010. Holstein dairy cows (n = 200) from client herds of the Ruminant Field Service at the Université de Montréal (Saint-Hyacinthe, Québec, Canada) were enrolled in the study. A convenience sample of 37 herds, located within a 25-km radius around the veterinary college, were selected for participation in this study. Sample-size calculations were performed (PASS 11, NCSS Statistical Software, Kaysville, UT) based on the following assumptions: correlation coefficients between 0 and 0.3 or between 0.70 and 1, a rho value of 0.05 to adjust for herd clustering, and power of 80%. All procedures were approved by the Animal Care Committee (10-Rech-1556) of the Université de Montréal.

Participating herds were visited once by a research technician within 4 h of milk sampling on the monthly DHI herd test day (Valacta, Sainte-Anne-de-Bellevue, Québec, Canada). During these farm visits, the 5 cows that had most recently calved and that were greater than 2 and less than 90 DIM were enrolled on the study and blood sampled. A 1-mL whole blood sample was drawn from the coccygeal vessels of each enrolled cow and immediately analyzed for BHBA concentration using the Precison Xtra meter. On the herd test day, DHI technicians were responsible for collecting individual composite milk samples from each enrolled cow using the approved metering device (International Committee on Animal Recording). Milk samples were preserved using bronopol tablets (Brotab, Systems Plus, Baden, ON, Canada), transported overnight on ice, and then analyzed at the DHI laboratory (Valacta). All DHI milk samples were analyzed for ketone bodies (BHBA and acetone) with a continuous flow analyzer (San^{++}, Skalar, Breda, the Netherlands) using the procedure described by de Roos et al. (2007). Milk BHBA and acetone values were calculated based on a standard subtraction method for the continuous flow analyzer, which may lead to small negative numbers. These negative numbers suggest very low concentrations of BHBA and acetone. Milk samples were also analyzed for fat and CP using FTIR (MilkoScan FT+, FOSS, Hillerod, Denmark).

Statistical Analyses

Data were entered into a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA). All statistical analyses were performed using SAS (version 9.3, SAS Institute Inc., Cary, NC). A variable titled fat-to-protein (**F:P**) ratio was created by dividing the fat value by the CP value for each sample. The F:P ratio was considered a milk component. The MEANS procedure was used to generate descriptive statistics for the blood BHBA and milk component data. The FREQ procedure was used to calculate the true prevalence (based on blood BHBA) and apparent prevalence (based on milk components) of hyperketonemia.

Correlation Between Blood BHBA and Milk Components. The CORR procedure was used to calculate Pearson correlation coefficients for blood BHBA and individual milk components.

Optimal Thresholds for Milk Components to Predict Hyperketonemia. The MIXED procedure was used to construct generalized linear mixed models to examine the univariable relationship between blood BHBA concentration (continuous variable) and each individual milk component (continuous variables), while controlling for the random effect of herd. Any variable with a P-value <0.05 in the univariable models was used to build receiver operating characteristic curves to illustrate graphically the ability of each milk component to correctly classify cows as hyperketonemia positive (defined as blood BHBA $\geq$1.4 mmol/L) or negative (defined as blood BHBA $<$1.4 mmol/L).

The LOGISTIC procedure was used to calculate the area under the curve (**AUC**) for each milk component. The AUC represents the probability of a randomly selected cow with hyperketonemia having a positive milk test result for BHBA, acetone, fat, protein, or F:P compared with a randomly selected cow without hyperketonemia (Dohoo et al., 2003). The Wald chi-squared contrast test was used to compare differences in AUC. An optimal threshold for predicting hyperketonemia was established for each milk component as the value that yielded the maximum sum of sensitivity (**Se**) and specificity (**Sp**). These optimal thresholds were used to determine the positive predictive value (**PPV)** and negative predictive value (**NPV**) for each milk component compared with blood BHBA values. Cohen's kappa (Dohoo et al., 2003) was used to evaluate the