Detection of hyperketonemia in dairy cows: "in-lab" milk components performance

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Introduction

Over the past years, hyperketonemia has been shown to be associated with health and production of postpartum dairy cows. Many tests are available to detect hyperketonemia in blood, urine, and milk. A procedure that quantifies ketone bodies in milk is now available through the dairy herd improvement association (DHIA) sampling process; however, conflicting data are reported on the accuracy of this procedure. The main objective of this study was to validate the relationship between blood β-hydroxybutyrate acid (BHBA) concentration and the concentrations of various milk components such as ketone bodies (BHBA and acetone), fat, protein, lactose, urea, and somatic cell count (SCC). A second objective was to identify thresholds for these indicators to diagnose hyperketonemia in dairy cows for surveillance purposes.

Materials and Methods

From July to September 2010, blood samples were collected from postpartum Holstein cows within four hours of the DHIA milk sample collection on 40 commercial dairy herds. Study herds represented a convenience sample of herds in the region of St-Hyacinthe (QC, Canada). Blood samples were collected from coccygeal vessels and analyzed on-farm with an electronic handheld BHBA meter (Precision Xtra; Abbott, Mississauga, ON, Canada). Individual composite milk samples were collected from all cows during on-farm DHIA sampling. Milk samples were preserved with bronopol and refrigerated until analyzed at a DHIA laboratory (Valacta, Ste-Anne-de-Bellevue, QC, Canada). For the DHIA analysis, ketone body (BHBA and acetone) concentrations in milk were quantified with a continuous flow analyzer (San ++, Skalar, Breda, Netherlands); milk fat, protein, lactose, and urea concentrations were determined using Fourier transform infrared spectrometry (MilkoScan, FOSS, Hillerod, Denmark), and SCC was determined by flow cytometry (Fossomatic FC, FOSS, Hillerod, Denmark). Correlation coefficients between concentrations of blood BHBA and milk components (BHBA, acetone, fat, protein, lactose, urea, SCC) were determined with SAS 9.3. (PROC CORR). A logarithmic transformation was applied to milk acetone and SCC data to normalize their distributions prior to determination of their respective correlation coefficients. Milk fat-to-protein ratio was calculated and its correlation coefficient was also determined. Hyperketonemia was defined as a blood BHBA concentration > 1.4 mmol/L. Receiver operating characteristic (ROC) curves were produced (PROC LOGISTIC) to determine the threshold (dichotomized data) for each milk component that maximized the sum of sensitivity (Se) and specificity (Sp) for detection of hyperketonemia. Milk components were then tested in series and in parallel to increase the Se and Sp sum.

Results

A total of 176 Holstein cows of all parities from two to 89 days-in-milk were sampled once for this study. Mean ± SD blood BHBA concentration was 1.14 ± 0.99 mmol/L (range, 0.2 to 6.3 mmol/L); and true prevalence of hyperketonemia in the population was 21.6%. Mean ± SD concentrations for milk BHBA, acetone, and urea, and percentage fat, protein, lactose, and urea were 0.184 ± 0.167 mmol/L, 0.100 ± 0.287 mmol/L, 9.35 ± 4.37 mg/dL, 4.10 ± 1.36 %, 3.08 ± 0.43 %, and 4.53 ± 0.27 %, respectively. Correlation coefficients were 0.937 ($P < 0.001$), 0.744 ($P < 0.001$), 0.208 ($P = 0.008$), 0.043 ($P = 0.587$), -0.25 ($P = 0.001$), -0.170 ($P = 0.031$), -0.028 ($P = 0.722$), and 0.175 ($P = 0.026$), for BHBA, log-transformed acetone, fat, protein, lactose, urea, log-transformed SCC, and fat-to-protein ratio, respectively. When hyperketonemia was defined as a blood BHBA concentration ≥1.4 mmol/L, the threshold for maximizing the sum of Se and Sp were > 0.20 mmol/L for milk BHBA (Se = 83%; Sp = 96%), > 0.08 mmol/L for milk acetone (Se = 87%; Sp = 95%), > 4.2% for milk fat (Se = 59%; Sp = 73%), < 4.6% for milk lactose (Se = 78%; Sp = 49%), < 9.75 mg/dL for milk urea (Se = 69%; Sp = 51%), and > 1.3 for fat-to-protein ratio (Se = 69%; Sp=66%). The use of milk BHBA and acetone threshold tests in series and in parallel, resulted in a sensitivity of 83% and 90%, respectively, and a specificity of 99% and 91%, respectively.

Significance

Similar to previous studies, we found significant correlations between blood BHBA and milk ketone bodies (BHBA and acetone) concentrations. Smaller, but statistically significant correlations were obtained for fat percentage, lactose percentage, urea concentration, and fat-to-protein ratio. The sensitivity and specificity for detecting hyperketonemia can be improved by testing milk BHBA and acetone concentrations in series or in parallel. In conclusion, measurement of ketone bodies with a continuous flow analyzer at a DHIA laboratory is an method to diagnose hyperketonemia, and may be useful for herd surveillance.