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Technical note: Validation of the BHBCheck blood β-hydroxybutyrate meter as a diagnostic tool for hyperketonemia in dairy cows

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ABSTRACT

Accurate cow-side blood β-hydroxybutyrate (BHB) detection meters are valuable tools for rapid diagnosis of hyperketonemia. The main objective of this study was to compare the blood BHB measured in whole blood by the BHBCheck meter (PortaCheck, Moorestown, NJ) to a previously validated meter, Precision Xtra meter (Abbott Laboratories, Abbott Park, IL) and a colorimetric laboratory assay. Samples (n = 426) were collected from postpartum primiparous and multiparous Holstein cows (n = 79 cows) enrolled in 1 of 2 experiments (Exp) with different sampling schedules (Exp 1: n = 39 cows, 58 samples; Exp 2: n = 40 cows, 368 samples). In both Exp, whole-blood samples were collected from the coccygeal vessels after morning milking, before morning feeding. Blood samples were used immediately for BHB quantification via the BHBCheck meter and the Precision Xtra meter. Blood was also collected into evacuated tubes containing no additive (Exp 1) or potassium oxalate/sodium fluoride (Exp 2), which were centrifuged for serum or plasma separation and stored at −20°C for subsequent analysis. Laboratory quantification of BHB concentration was done by the BHB LiquiColor Assay (EKF Diagnostics-Stanbio, Boerne, TX; certified for serum and plasma). Data were analyzed by UNIVARIATE, CORR, FREQ, REG, and LOGISTIC procedures of SAS 9.4 (SAS Institute Inc., Cary, NC). Within this sample set, average parity was 3.3 lactations and DIM was 14 d. The proportion of samples classified as hyperketonemia (BHB ≥1.2 mmol/L) was 25, 28, and 31% as determined by the colorimetric assay, BHBCheck meter, and Precision Xtra meter, respectively. The correlation for BHBCheck meter BHB concentration compared with the colorimetric assay concentrations was r = 0.96, with a sensitivity of 91% and specificity of 93%. Correlation, sensitivity, and specificity of the Precision Xtra meter concentrations were 0.97, 98%, and 92%, respectively. Bland-Altman plots demonstrated minimal bias for both meters. Area under the receiver operator characteristic curve suggests adequate diagnostic accuracy of both meters. Overall, accuracy, sensitivity, and specificity of the BHBCheck meter was similar to the Precision Xtra meter and laboratory assay, indicating the BHBCheck meter is appropriate for use as a cow-side diagnostic test for hyperketonemia in dairy cows.

Key words: ketosis, cow-side diagnostic tool, transition cow

Technical Note

During the postpartum period, dairy cows are at increased risk of developing metabolic disorders, largely due to negative energy balance and subsequent mobilization of triglyceride (**TG**) from adipose tissue (Grummer, 1993; Drackley, 1999; Duffield, 2000). Fates of mobilized TG within the liver include complete oxidation to energy, incomplete oxidation to ketone bodies, or storage as TG (Grummer, 1993). Although some peripheral tissues can use ketone bodies as an energy source, production of ketone bodies beyond tissue usage results in hyperketonemia (**HYK**). When untreated, HYK is associated with negative effects on animal health, production, and profitability (Herdt, 2000; McArt et al., 2015). Hyperketonemia is defined as blood BHB ≥1.2 mmol/L (Iwersen et al., 2009; McArt et al., 2012b; Gordon et al., 2013, 2017), and the average postpartum prevalence of HYK worldwide ranges from 15 to 22%, although it is highly variable by farm (Suthar et al., 2013; Chandler et al., 2015; Santschi et al., 2016). The average cost per case is $289 (McArt et al., 2015); however, early treatment can reduce the costs and negative outcomes (McArt et al., 2012a), making detection protocols that are composed of labor- and cost-effective diagnostics essential to managing HYK.

Previous validation of a handheld blood ketone meter, the Precision Xtra meter (Abbott Laboratories, Abbott Park, IL), provided a valuable quantitative tool for diagnosing HYK using a minimally invasive blood sample

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with greater sensitivity and specificity than previously used milk and urine tests (Oetzel, 2004; Iwersen et al., 2009). Recently, several additional meters have been developed and some have been evaluated for potential use in bovine HYK diagnostics (Bach et al., 2016). Validation of these diagnostic tests in a controlled setting, before field application, is essential. A relatively new meter that has not yet been validated for use in bovine HYK diagnostics is the BHBCheck meter (PortaCheck, Moorestown, NJ). This meter quantifies BHB via a 0.7-μL whole-blood sample, which is less blood than other meters (which require between 0.8 to 1.5 μL of blood), and quantification is completed in under 5 s compared with other meters that average 10 s. We hypothesized that, similar to other handheld BHB diagnostic meters, the BHBCheck meter would provide adequate accuracy for HYK diagnostics in dairy cattle. The objectives of our study were (1) to determine the efficacy of the BHBCheck meter as a cow-side BHB-monitoring device by validating it against a colorimetric laboratory assay and (2) to compare the BHBCheck meter to an industry standard cow-side meter (Precision Xtra).

We conducted 2 animal experiments that were approved by the University of Wisconsin-Madison College of Agricultural and Life Sciences Animal Care and Use Committee (protocol numbers A05467 and A01569). For both experiments (**Exp**), blood was collected from Holstein cows immediately following morning milking and before feeding. The samples from Exp 1 were collected from 39 primiparous and multiparous cows between 4 and 18 DIM during twice-weekly sampling on a privately owned dairy in south-central Wisconsin from May to June 2016. Experiment 1 was intended to provide a balanced data set of an equal number of samples that were above and below the 1.2 mmol/L HYK threshold. This was accomplished by collecting whole blood from cows between 4 and 18 DIM into evacuated collection tubes without additives and immediately analyzing blood using the Precision Xtra test. When a cow with HYK was identified, she was included in the study along with the next cow that was negative for HYK. Whole-blood samples were also analyzed using the BHBCheck meter. Samples were collected over several farm visits and some cows contributed more than 1 sample to the data set. Although the balanced design in Exp 1 resulted in a nonrepresentative sample set relative to the population prevalence, it avoided having a sample set with a minimal proportion of samples above the 1.2 mmol/L threshold. Whole-blood tubes were then centrifuged (2,500 × *g*, 15°C, 15 min) and serum was aliquoted and stored in 1.5-mL microtubes at −20°C until subsequent analysis. Experiment 2 was conducted at the University of Wisconsin-Madison Dairy Cattle Center from May to October 2016. Blood samples were collected from 40 multiparous cows between 1 and 45 DIM into an evacuated tube without additives as well as into an evacuated tube containing potassium oxalate and sodium fluoride. Whole blood from the tube without additives was immediately analyzed on the BHBCheck and Precision Xtra meter. The tube containing potassium oxalate and sodium fluoride was centrifuged (2,000 × *g*, 4°C, 15 min) and plasma was aliquoted into 1.5-mL microtubes and stored at −20°C for subsequent analysis.

Serum samples from Exp 1 and plasma samples from Exp 2 were analyzed using the LiquiColor colorimetric assay (EKF Diagnostics-Stanbio, Boerne, TX; certified for serum and plasma collected with EDTA, heparin, or sodium fluoride) per the manufacturer's protocol. Samples were quantified in duplicate and samples with intra-assay coefficient of variation greater than 10% were reanalyzed. The interassay coefficient of variation of the laboratory assay was 6.7%. Sample BHB concentration determined by the laboratory assay served as the gold standard BHB concentration for statistical analysis.

Data analysis was completed in SAS 9.4 (SAS Institute Inc., Cary, NC). Descriptive statistics were analyzed by PROC UNIVARIATE. Residuals were produced by PROC REG with colorimetric laboratory assay BHB as the dependent variable and BHBCheck or Precision Xtra meter-quantified BHB as the independent variable. Pearson correlations between the BHB concentrations determined by both meters and by the colorimetric assay were analyzed by PROC CORR. To account for correlation among residual errors due to the repeated measures on the experimental unit (cow) across time, different error structures (i.e., compound symmetry, first-order autoregressive, and spatial power) were tested using PROC MIXED. A log-likelihood ratio test was performed between the repeated measure models and the null model and indicated accounting for repeated structure of the data did not significantly alter model fit; therefore, analysis of the data continued without accounting for repeated measures. Sensitivity and specificity were calculated by PROC FREQ. Receiver operating characteristic (**ROC**) curves and associated area under the curve were determined by PROC LOGISTIC. Results for Exp 1 and 2 are provided both separately and combined to provide the diagnostic accuracy of the meters in 2 sample sets with different proportion of samples ≥1.2 mmol/L.

Demographics of the samples from Exp 1, 2, and overall are provided in Table 1. Samples were collected from 79 Holstein cows with an average parity of 3.3 ± 0.1 and average DIM of 14.3 ± 0.6 across the 2 experiments (Table 1). The mean BHB was 1.3 ± 0.12, 1.0 ± 0.03, and 1.0 ± 0.03 mmol/L for samples from Exp 1,