J. Dairy Sci. 101:1–16
https://doi.org/10.3168/jds.2017-13209

Predicting hyperketonemia by logistic and linear regression using test-day milk and performance variables in early-lactation Holstein and Jersey cows

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ABSTRACT

Although cowside testing strategies for diagnosing hyperketonemia (HYK) are available, many are labor intensive and costly, and some lack sufficient accuracy. Predicting milk ketone bodies by Fourier transform infrared spectrometry during routine milk sampling may offer a more practical monitoring strategy. The objectives of this study were to (1) develop linear and logistic regression models using all available test-day milk and performance variables for predicting HYK and (2) compare prediction methods (Fourier transform infrared milk ketone bodies, linear regression models, and logistic regression models) to determine which is the most predictive of HYK. Given the data available, a secondary objective was to evaluate differences in test-day milk and performance variables (continuous measurements) between Holsteins and Jerseys and between cows with or without HYK within breed. Blood samples were collected on the same day as milk sampling from 658 Holstein and 468 Jersey cows between 5 and 20 d in milk (DIM). Diagnosis of HYK was at a serum β-hydroxybutyrate (BHB) concentration ≥1.2 mmol/L. Concentrations of milk BHB and acetone were predicted by Fourier transform infrared spectrometry (Foss Analytical, Hillerød, Denmark). Thresholds of milk BHB and acetone were tested for diagnostic accuracy, and logistic models were built from continuous variables to predict HYK in primiparous and multiparous cows within breed. Linear models were constructed from continuous variables for primiparous and multiparous cows within breed that were 5 to 11 DIM or 12 to 20 DIM. Milk ketone body thresholds diagnosed HYK with 64.0 to 92.9% accuracy in Holsteins and 59.1 to 86.6% accuracy in Jerseys. Logistic models predicted HYK with 82.6 to 97.3% accuracy. Internally cross-validated multiple linear regression models diagnosed HYK of Holstein cows with 97.8% accuracy for primiparous and 83.3% accuracy for multiparous cows. Accuracy of Jersey models was 81.3% in primiparous and 83.4% in multiparous cows. These results suggest that predicting serum BHB from continuous test-day milk and performance variables could serve as a valuable diagnostic tool for monitoring HYK in Holstein and Jersey herds.

Key words: ketosis, Fourier transform infrared spectrometry, acetone, β-hydroxybutyrate

INTRODUCTION

Hyperketonemia (**HYK**), a metabolic disorder characterized by elevated blood ketone bodies (Herdt, 2000), affects between 40 and 60% of dairy cows and results in decreased production, impaired reproduction, and increased comorbidities (Duffield et al., 2009; McArt et al., 2012). Ketone bodies can be detected in blood, urine, and milk for diagnosis of HYK (Andersson, 1988), and early treatment can ameliorate production losses (McArt et al., 2011). Enzymatically quantifying blood BHB is considered the gold standard of HYK diagnosis. Although enzymatic tests are available as hand-held meters for cowside use (Iwersen et al., 2009), their associated cost and labor may limit routine testing. Strong correlations between blood and milk ketone bodies (Andersson, 1984) present quantifying BHB and acetone in milk as a potential strategy for diagnosing HYK (Marstorp et al., 1983; Enjalbert et al., 2001). Milk ketone bodies can be accurately measured by flow-injection analysis, GLC, or enzymatic assays, but these techniques are time consuming, expensive, difficult to automate, and not applicable on farm. Crude diagnostics that rapidly estimate ketone bodies in milk cowside are practical but inaccurate diagnostic tools (Geishauser et al., 2000; Carrier et al., 2004). Alternatively, use

Received May 22, 2017.
Accepted November 12, 2017.
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of Fourier transform infrared (**FTIR**) spectrometry in routine milk analysis might allow for prediction of milk ketone bodies for accurate and practical HYK monitoring in dairy herds.

Several groups attempted to validate infrared prediction of milk ketone bodies to couple routine testing with HYK monitoring (Hansen, 1999; Heuer et al., 2001; de Roos et al., 2007). Milk ketone bodies previously predicted by FTIR spectrometry had correlation coefficients of 0.79 and 0.85 to chemically determined milk BHB (**mBHB**) and acetone and their respective FTIR predictions (de Roos et al., 2007). After calibration, prediction equations and recommended diagnostic thresholds for HYK had less than desirable sensitivities (69–70%) but suggested that FTIR predictions of mBHB and acetone could be part of useful screening tools (de Roos et al., 2007). When compared with HYK diagnosis by blood, FTIR-predicted milk ketone bodies determined by the equations developed by de Roos et al. (2007) demonstrated only moderate diagnostic sensitivity and specificity (van der Drift et al., 2012). To reconcile low specificity, categorical and continuous test-day variables were included along with FTIR-predicted mBHB and acetone in logistic models to diagnose HYK (van der Drift et al., 2012). This strategy increased specificity but considerably decreased sensitivity, and the resulting models were deemed inadequate to accurately diagnose individual animals (van der Drift et al., 2012). Milk ketone bodies predicted by FTIR have only been recommended to monitor herd prevalence of HYK.

Predicting blood BHB and applying a conventional diagnostic threshold may offer an alternative strategy for HYK diagnosis. Accuracy may be improved by including performance variables known to alter HYK risk, such as production, parity, and dry period length (McArt et al., 2012, 2013). The objectives of the current study were to (1) develop linear and logistic regression models using all available test-day milk and performance variables for predicting HYK and (2) compare prediction methods (FTIR milk ketone bodies, linear regression models, and logistic regression models) to determine which is the most predictive of HYK. Given the data available, a secondary objective was to evaluate differences in test-day milk and performance variables (continuous measurements) between Holsteins and Jerseys and between cows with or without HYK within breed. We hypothesized that predicting serum BHB concentrations from continuous test-day milk and performance variables using multiple linear regression would improve HYK detection compared with individual tests of FTIR milk ketone bodies or multiple logistic models.

MATERIALS AND METHODS

Study Population

Between February 2014 and October 2015, commercial Holstein (n = 10) and Jersey (n = 6) herds in the midwestern United States (described in Supplemental Table S1; https://doi.org/10.3168/jds.2017-13209) were enrolled in a cross-sectional study, and 658 Holstein and 468 Jersey cows were sampled. Target animal numbers were based on power calculations using preliminary data from another study within the laboratory to achieve 80% power with an α of 5% between HYK and non-HYK groups for Holstein and Jersey cattle using the POWER procedure in SAS 9.4 (SAS Institute Inc., Cary, NC). Blood BHB means for non-HYK and HYK groups were 0.6 and 1.4 mmol/L with a standard deviation of 0.7, assuming an HYK prevalence of 20%, resulting in a minimum of 82 total cows within each breed to detect a difference in blood BHB between HYK and non-HYK if prevalence was 20%. Based on model statistic output, a post hoc decision was made to further separate Holstein and Jersey groups into primiparous and multiparous rather than including parity as a model variable; therefore, power calculations were not done a priori for those subgroups. Retrospective power calculations of these subgroups (using means and standard deviations in Supplementary Tables S2 and S3; https://doi.org/10.3168/jds.2017-13209) indicate power of >0.99 to detect differences in blood BHB for all subgroups for both Holstein and Jersey cows. A priori sample size calculations for models could not be performed as we did not identify variables to be included a priori; rather, statistical criteria were used to determine variable inclusion as detailed below. Herds were selected based on the following criteria: enrollment in at least monthly DHI milk testing, use of a farm management software program, availability of headlocks or management rails for blood sampling, and willingness to participate in the proposed data collection. During the study, herds were visited at least once, or up to 5 times, on a scheduled DHI test day for milk sampling, and all cows between 5 and 20 DIM were enrolled at each visit. All animal use and handling protocols were approved by the University of Wisconsin–Madison College of Agricultural and Life Sciences (protocol no. A01569) and University of Missouri Animal Care and Use (protocol no. 8447) committees.

Sample Collection and Analysis

Sampling of individual cows consisted of a single paired milk and blood sample collected on the same day,