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

T. L. Chandler,* R. S. Pralle,* J. R. R. Dórea,* S. E. Poock,† G. R. Oetzel,‡ R. H. Fourdraine,§ and H. M. White*[1]
*Department of Dairy Science, University of Wisconsin, Madison 53706
†Veterinary Medical Extension and Continuing Education, University of Missouri, Columbia 65211
‡School of Veterinary Medicine, University of Wisconsin, Madison 53706
§International Center for Biotechnology, Cooperative Resources International, Verona, WI 53593

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.
[1]Corresponding author: heather.white@wisc.edu

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,

and individual cows enrolled in the study were sampled only once. Milk samples were collected at the morning (n = 14 herds) or noon (n = 2 herds) milking. Test-day milk samples were acquired by trained sampling technicians as part of the herd's routine monthly DHI testing. Animal identification numbers were recorded by automatic radio-frequency identification collection and verified by visual identification of animal identification tags to prevent inaccurate identification recording. All milk samples were collected by an International Committee on Animal Recordings–approved sampling system using a proportional sampler that was previously calibrated within 12 mo of milk sampling. Milk samples were immediately preserved with 2-bromo-2-nitropropane-1,3-diol (Advanced Instruments Inc., Norwood, MA) before transport and analysis of milk composition according to standard test-day procedures in the laboratory of AgSource Cooperative Services (Menomonie, WI). All milk samples were gently preheated to 40°C and mixed before analysis of milk fat and milk protein by FTIR using the Foss MilkoScan FT+ (Foss Analytical, Hillerød, Denmark) in accordance with the instrument manufacturer's instructions and ISO 9622/IDF 141 (ISO, 2013) and AOAC International (2016) method 972.16 as done previously (Barros et al., 2017; Dórea et al., 2017; Rathbun et al., 2017). Analysis of SCC was performed using Fossomatic FC (Foss Analytical). Milk BHB and milk acetone (**mACE**) concentrations were predicted by FTIR using Foss Ketolab (Foss Analytical). Additionally, Foss MilkoScan FT+ analysis of milk samples collected in 2015 (Jersey cows only) included predictions of the content of SFA, USFA, *trans* fatty acids (**FA**), and short-, medium-, and long-chain FA in milk based on Foss FTIR FA prediction models (Foss Analytical, 2011). Prediction equation version numbers used for samples tested during Holstein and Jersey sampling were Foss 1.6.1 and 1.6.2r, respectively. The quality control standards and equipment calibrations were maintained by the DHI. Per DHI standard operating procedures, milk samples were analyzed on equipment that is calibrated weekly with 12 standards, and standards are rechecked daily and hourly with a subset of 6 of the 12 standards. Intra-assay coefficients of variation (**CV**) for all variables were maintained at <7%. Interassay CV are not available for all variables; however, interassay CV for fat and protein are maintained at <2 and <1.5%, respectively.

On the same day of milk sampling, a blood sample was collected from cows either directly before or after milking but before the postmilking feeding for HYK diagnosis. Blood samples were collected from a coccygeal vessel into a nonanticoagulant serum collection tube containing clot activator (BD Diagnostics, Franklin Lakes, NJ). Tubes were centrifuged at 3,000 × *g* for 15 min for serum separation, and serum was stored at −20°C until subsequent analysis. Serum BHB concentration was quantified by colorimetric assay (Stanbio Laboratory Inc., Boerne, TX) based on the method of Williamson et al. (1962). Intra- and interassay CV of BHB assays were 3.9 ± 0.07% and 6.1 ± 1.5%, respectively. A threshold of ≥1.2 mmol/L of serum BHB concentration was used as the HYK diagnostic reference. Animals with circulating serum BHB concentrations <1.2 mmol/L were considered non-HYK.

Data Collection

Milk sample results, along with test-day milk production, were recorded by and obtained from AgSource Cooperative Services (Menomonie, WI). Cow records that included previous lactation length, dry period length, gestation length, previous mature-equivalent 305-d milk production, and age at calving were exported from DairyComp305 (Valley Agricultural Software, Tulare, CA) or PCDART (DRMS, Ames, IA) on the day of sampling with permission from herd owners. All milk and performance variables were recorded as continuous values and were not categorized.

Analysis of Breed, Parity, and DIM Group Data

Breed differences in serum BHB concentration, test-day milk, and performance variables were analyzed using the MIXED procedure of SAS 9.4 in a model that accounted for the fixed effect of breed and random effect of cow within herd. Apparent breed differences observed in the current data set (Table 1) and the difference in available test-day variables warranted separation of breeds before further analysis. Animals within each breed were stratified by parity to primiparous and multiparous because of differences in available performance variables and increased incidence of HYK previously reported in cows in second and greater lactation (van der Drift et al., 2012; McArt et al., 2013). The prevalence of HYK was calculated for each parity group within breed, and differences were analyzed by the GLIMMIX procedure of SAS 9.4. Differences in test-day milk and performance variables between non-HYK and HYK animals were analyzed using the MIXED procedure of SAS 9.4 in a model that accounted for the fixed effect of parity, diagnosis, and parity × diagnosis interaction and the random effect of cow within herd. Before predicting serum BHB by multiple linear regression, parity groups within breed were further stratified into 5 to 11 DIM and 12 to 20 DIM groups because of elevated HYK incidence reported during the first 10 d of lactation (McArt et al., 2012). Within breed, differences in serum BHB and test-day milk and perfor-

Table 1. Summary (±SE) of breed differences between test-day variables collected from Holstein (n = 10 farms) and Jersey (n = 6 farms) cows between 5 and 20 DIM

Variable	Holstein	Jersey	*P*-value
Cows, no.	606	399	
Hyperketonemia[1] prevalence, %	14	19	
Serum BHB, mmol/L	0.80 ± 0.03	0.92 ± 0.03	<0.01
Milk variables			
BHB, mmol/L	0.073 ± 0.004	0.106 ± 0.004	<0.01
Acetone, mmol/L	0.101 ± 0.01	0.159 ± 0.01	<0.01
Fat, %	4.45 ± 0.04	4.40 ± 0.05	0.46
Protein, %	3.33 ± 0.02	3.64 ± 0.02	<0.01
Fat:protein	1.34 ± 0.01	1.22 ± 0.02	<0.01
SCC (×1,000)	192 ± 43	494 ± 52	<0.01
Performance variables			
Production on test day, kg	29.8 ± 0.4	19.3 ± 0.5	<0.01
DIM	12.1 ± 0.18	11.8 ± 0.22	0.34
Lactation	1.9 ± 0.04	2.2 ± 0.05	<0.01
Previous 305-d milk production, kg	14,023 ± 117	9,060 ± 146	<0.01
Previous lactation length, d	336 ± 2.9	318 ± 3.6	<0.01
Dry period length, d	58.7 ± 0.6	60.1 ± 0.7	0.18
Gestation length, d	277 ± 0.36	280 ± 0.44	<0.01

[1]Defined as a serum BHB ≥1.2 mmol/L.

mance variables between parity and DIM groups were analyzed using the MIXED procedure of SAS 9.4 in a model that accounted for the fixed effects of parity, DIM group, and parity × DIM group interaction and the random effect of cow within herd. Means were considered different when $P \leq 0.05$ and tended to differ at $0.5 < P \leq 0.10$. For mixed models, Tukey–Kramer studentized adjustments were used to separate means when the interaction of main effects was considered significant or a trend toward significance.

Milk Threshold Determination

Correlation coefficients between serum BHB, mBHB, and mACE were calculated by the multivariate function of the statistical software package JMP (JMP Pro 11.0; SAS Institute Inc.). The diagnostic value of FTIR-predicted mBHB and mACE as single tests for the detection of HYK was first investigated by using threshold values that were previously recommended as ≥0.10 mmol/L of mBHB and ≥0.15 mmol/L of mACE (de Roos et al., 2007). To determine whether optimal threshold values differed in the current data set compared with those previously recommended, the nominal LOGISTIC method of JMP was used to produce receiver operating characteristic (**ROC**) curves. To assess the potential diagnostic ability of milk ketone bodies as single tests, area under the curve (**AUC**) of the ROC curves was calculated. The optimal mBHB or mACE threshold was determined based on concentrations that maximized sensitivity and specificity for predicting HYK. Finally, because the assumed cost of a false negative is greater than the cost of further testing and treatment associated with a false positive, thresholds were identified and investigated when sensitivity was ≥90%, as previously suggested (Enjalbert et al., 2001). Sensitivity, specificity, and 95% confidence intervals were calculated for classification of animals as non-HYK or HYK according to the thresholds described above using the In Vitro Diagnostic Performance Add-In package of JMP. Positive predictive values (**PPV**) and negative predictive values (**NPV**) were also calculated.

Logistic Regression

Logistic regression models that predicted HYK diagnosis from variables were constructed for parity groups within each breed using the nominal LOGISTIC method of JMP. Test-day milk and performance variables eligible for logistic models were milk fat percentage, milk protein percentage, fat:protein ratio, SCC, mBHB, mACE, FA groups (Jersey models only; total SFA, MUFA, PUFA, total unsaturated FA, *trans* FA, and short-chain, medium-chain, and long-chain FA), production on sampling day, DIM, lactation number, previous mature-equivalent 305-d milk production, previous lactation length, dry period length, gestation length, and age at calving. Potential explanatory variables for multiple logistic regression models were first screened by simple logistic regression analysis to calculate a respective *P*-value and an AUC of the ROC curve. For each variable, both a chi-squared test of $P \leq 0.15$ and an AUC of ≥0.60 were used as selection criteria. Subsequently, these variables were evaluated for collinearity using linear regression of the binomial dependent variable and calculating the variance infla-

tion factor (**VIF**) for each predictive variable retained in the least squares model function of JMP. When the VIF was ≥10, collinearity was suspected, the variable with the greatest VIF was removed, and models were rebuilt until the VIF for all variables was <10. Final logistic models were constructed from the previously screened variables using forward stepwise selection by including variables with a *P*-value ≤0.15 and using the corrected Akaike information criterion as a selection criterion. A herd effect (random intercept per herd) was evaluated for each model and determined to be nonsignificant. The resulting logistic models were validated using a repeated 10-fold cross-validation method (repeated 50 times) as described previously (Shetty et al., 2017; Weigel et al., 2017) in the CARET Package of R (http://cran.r-project.org/web/packages/caret/). For each repetition, animals were diagnosed as non-HYK or HYK based on the logistic probability that maximized sensitivity and specificity. Sensitivity, specificity, accuracy, their respective confidence intervals, and predictive values were calculated from the binary cross-validation results when compared with observed serum BHB diagnosis. Final logistic models were evaluated for herd-level testing performance by comparing true HYK prevalence based on observed serum BHB and predicted herd prevalence estimated using logistic models for each parity group.

Linear Regression

Multiple linear regression models that predicted serum BHB concentration from test-day milk and performance variables were built for parity and DIM groups within each breed. The same variables eligible for logistic regression were eligible for linear regression models. Prediction model variables were selected in the REG procedure of SAS 9.4 using forward stepwise selection, retaining variables with a *P*-value ≤0.15, and using selection criteria of sequential sums of squares, error sums of squares, and Akaike information criterion. Collinearity was evaluated in each model by calculating VIF for each retained variable in the REG procedure of SAS 9.4. When VIF was ≥10, collinearity was suspected, and the variable was removed and models were rebuilt. This process continued until the VIF for all variables was <10. Normality of model predictions was checked by examining residual plots of the studentized residuals. A herd effect (random intercept per herd) was evaluated and determined to be nonsignificant. Statistical parameters including coefficient of determination and root mean squared error (**RMSE**) were calculated to evaluate model fit.

Each model was validated using a 10-fold cross-validation method in the DAAG Package of R (http://cran.r-project.org/web/packages/daag/). Serum BHB predicted by the cross-validation procedure was tested against the observed value to calculate coefficient of determination, concordance coefficient correlation, and root mean squared error of prediction for each model. Individuals were then classified as non-HYK or HYK based on their predicted serum BHB from the cross-validation procedure using a threshold of ≥1.2 mmol/L. This diagnosis was used to calculate sensitivity, specificity, accuracy, and their respective confidence intervals in the FREQ procedure of SAS. The PPV and NPV were also calculated. Apparent herd HYK prevalence was estimated from classification of individual animals based on prediction models for the stratified DIM and parity groups combined as a complete diagnostic tool. Herd-level testing performance was evaluated by comparing apparent HYK prevalence with true HYK prevalence and calculating the number of herds that were correctly identified at a 10% prevalence alarm level. Alarm levels are subjective and reflect the diagnostic cut-off used and the outcomes examined. For HYK, alarm levels of 10 to 15% have been suggested based on negative effects on milk production or reproduction (Oetzel, 2004; Ospina et al., 2010; van der Drift et al., 2012; Dubuc and Denis-Robichaud, 2017).

RESULTS

Cow records were excluded from analysis in cases of incorrect stage of lactation at sampling (n = 6), unknown calving date (n = 5), missing sample results (n = 76), or analytical errors in FTIR milk results (n = 2). To avoid confounding factors associated with HYK treatment on animal physiology, animals that had a recorded HYK diagnosis within 1 wk of sampling or that were undergoing current treatment for HYK were also excluded (n = 32).

As a result of exclusions, the final statistical analysis was performed on 606 Holstein and 399 Jersey cow records. Of the 10 Holstein herds sampled, any one herd did not contribute more than 18% of cow records to the data set, and no more than 25% was contributed by any of the 6 Jersey herds. Breed differences in test-day milk and performance variables observed in this study are summarized in Table 1. Overall prevalence of HYK was 14% for Holstein cows and 19% for Jersey cows. Hyperketonemia prevalence of sampled herds ranged from 0 to 28.6% in Holstein herds and 11.4 to 25.0% in Jersey herds. Prevalence of HYK was greater ($P < 0.01$) in multiparous Holsteins compared with primipa-

rous Holsteins (19.9 vs. 4.0%) but not different ($P = 0.61$) between multiparous and primiparous Jerseys (19.8 vs. 17.8%).

When stratified by parity and diagnosis (non-HYK vs. HYK), differences ($P < 0.05$) were detected in test-day milk and performance variables for both Holstein and Jersey cows (Supplemental Tables S2 and S3). Almost all milk variables were different ($P < 0.05$) by diagnosis for both breeds. Of the reported performance variables, lactation number, previous mature-equivalent 305-d milk production, dry period length, gestation length, and age at calving were different ($P < 0.05$) between HYK and non-HYK Holstein cows (Supplemental Table S2), whereas only dry period length was different ($P < 0.01$) by diagnosis in Jersey cows (Supplemental Table S3). Differences were observed in test-day milk and performance variables for both Holstein and Jersey cows when stratified by parity and DIM groups (Supplemental Tables S4 and S5; https://doi.org/10.3168/jds.2017-13209). The DIM groups differed in mACE, milk fat, milk protein, and SCC concentrations as well as milk production on test day in both Holstein and Jersey cows (Supplemental Table S4 and S5). Milk FA groups also differed between DIM groups in Jersey cows (Supplemental Table S4).

mBHB and mACE Thresholds

Correlation coefficients between serum BHB and mBHB and between serum BHB and mACE, respectively, were as follows: 0.40 and 0.79 in primiparous Holsteins; 0.62 and 0.58 in multiparous Holsteins; 0.47 and 0.50 in primiparous Jerseys; 0.69 and 0.74 in multiparous Jerseys. Three diagnostic thresholds of FTIR-predicted milk ketone bodies that diagnosed HYK were evaluated in this study: previously recommended thresholds (de Roos et al., 2007), thresholds that maximized sensitivity and specificity in the current data set, and thresholds that diagnosed HYK with at least 90% sensitivity in the current data set. Table 2 presents the AUC as well as sensitivity, specificity, accuracy, PPV, and NPV of these 3 diagnostic thresholds for parity groups within breed. The AUC of mBHB and mACE diagnostic tests ranged from 0.75 to 0.96, with the lowest AUC observed for mACE in multiparous Holsteins and the highest observed for mACE in primiparous Holsteins. Diagnostic tests of mACE produced an AUC that was either similar to or greater than mBHB tests in the same animal group. For almost all diagnostic thresholds tested in this study, sensitivity of mBHB and mACE tests was higher in primiparous cows compared with multiparous cows. A similar pattern was observed for specificity and accuracy. When previously recommended diagnostic thresholds of mBHB and mACE (de Roos et al., 2007) were applied to Holstein and Jersey data sets, sensitivity ranged from 39.5 to 92.6% and specificity ranged from 60.8 to 91.5% (Table 2). Accuracy of thresholds recommended by de Roos et al. (2007) was greater for diagnostic tests of mACE than mBHB within the same parity group (Table 2).

Optimal diagnostic threshold values for predicting HYK based on maximized sensitivity and specificity in the current data set differed from those previously recommended (de Roos et al., 2007) and ranged from 0.07 to 0.11 mmol/L of mBHB or 0.08 to 0.19 mmol/L of mACE. Except for diagnostic tests of mACE in Jersey cows, threshold values of mBHB and mACE that optimized sensitivity and specificity differed in each parity group and were lower in multiparous cows compared with primiparous cows. When combined sensitivity and specificity were maximized, sensitivity remained the same or was improved compared with previously recommended thresholds (de Roos et al., 2007) for all diagnostic tests except mACE in multiparous Jerseys. In Holstein cows, diagnostic thresholds of mBHB and mACE were decreased to increase sensitivity to 90%. When sensitivity exceeded 90%, diagnostic tests of mACE resulted in greater accuracy than tests of mBHB in primiparous cows; the opposite pattern was observed in multiparous cows.

Logistic Regression

Final logistic models that predicted HYK in primiparous and multiparous Holstein and Jersey cows are presented in Table 3. Table 4 summarizes the AUC, sensitivity, specificity, accuracy, and predictive values of the multiple logistic regression models cross-validated for parity groups within breed. The AUC exceeded 0.80, specificity exceeded 95%, and accuracy exceeded 80% for the final logistic models (Table 4). Sensitivity ranged from 31.6 to 55.6%, PPV from 62.3 to 70.3%, and NPV from 84.9 to 98.2%. The primiparous Holstein model demonstrated the highest AUC, sensitivity, specificity, accuracy, and NPV.

Linear Regression

Stratified breed, parity, and DIM groups were used to build 8 separate multiple linear regression models that predicted serum BHB from test-day milk and performance variables. A summary of the variables retained in the 4 Holstein and 4 Jersey models is presented in Tables 5 and 6, respectively, along with coefficient of determination and RMSE. Variables that were retained in the predictive models for Holstein cows included mBHB, mACE, milk protein percentage, fat:protein

Table 2. Sensitivity, specificity, accuracy, and predictive values of proposed thresholds of Fourier transform infrared spectrometry–predicted milk BHB and acetone for predicting hyperketonemia[1] in Holstein (n = 10 farms) and Jersey (n = 6 farms) cows between 5 and 20 DIM[2]

	Holstein				Jersey			
	Primiparous (n = 224)		Multiparous (n = 382)		Primiparous (n = 152)		Multiparous (n = 247)	
Variable	Milk BHB	Milk acetone	Milk BHB	Milk acetone	Milk BHB	Milk acetone	Milk BHB	Milk acetone
Area under the curve	0.91	0.96	0.76	0.75	0.79	0.85	0.79	0.86
Recommended threshold[3]	0.10	0.15	0.10	0.15	0.10	0.15	0.10	0.15
Sensitivity, % (95% CI)	88.9 (56.5, 98.0)	88.9 (56.5, 98.0)	56.6 (45.4, 67.1)	39.5 (29.2, 50.7)	81.5 (63.3, 91.9)	92.6 (76.6, 97.9)	77.6 (64.1, 87.0)	69.4 (55.5, 80.5)
Specificity, % (95% CI)	83.3 (77.7, 87.7)	86.0 (80.8, 90.0)	79.8 (75.0, 83.9)	91.5 (87.9, 94.2)	60.8 (52.0, 68.9)	62.4 (53.7, 70.4)	65.7 (58.8, 71.9)	86.9 (81.5, 90.9)
Accuracy,[4] % (95% CI)	83.5 (78.0, 88.1)	86.2 (80.9, 90.4)	75.2 (70.6, 79.4)	81.2 (76.9, 85.0)	64.5 (56.9, 72.1)	67.8 (60.3, 75.2)	68.0 (62.2, 73.8)	83.4 (78.8, 88.0)
Positive predictive value, %	18.2	21.1	41.0	53.6	31.0	34.7	35.8	56.7
Negative predictive value, %	99.4	99.5	88.1	85.9	93.8	97.5	92.2	92.0
Optimized threshold[5]	0.11	0.19	0.08	0.08	0.09	0.17	0.07	0.17
Sensitivity, % (95% CI)	88.9 (56.5, 98.0)	88.9 (56.5, 98.0)	77.6 (67.1, 85.5)	77.6 (67.1, 85.5)	92.6 (76.6, 97.9)	92.6 (76.6, 97.9)	93.8 (83.5, 97.9)	67.3 (53.4, 78.8)
Specificity, % (95% CI)	87.9 (82.9, 91.6)	93.0 (88.8, 95.7)	61.6 (56.0, 66.8)	60.6 (55.0, 65.9)	57.6 (48.8, 65.9)	68.6 (60.2, 76.3)	50.5 (43.6, 57.4)	91.4 (86.7, 94.6)
Accuracy, % (95% CI)	87.9 (83.0, 91.9)	92.9 (88.7, 95.9)	64.8 (59.7, 69.5)	64.0 (58.9, 68.8)	63.8 (56.2, 71.5)	73.0 (66.0, 80.1)	59.1 (53.0, 65.2)	86.6 (82.4, 90.9)
Positive predictive value, %	23.5	34.8	33.3	32.8	32.1	39.1	31.9	66.0
Negative predictive value, %	99.5	99.5	91.7	91.6	97.3	97.7	97.1	91.9
90% sensitivity threshold[6]	0.07	0.13	0.05	0.03	0.09	0.17	0.07	0.09
Sensitivity, % (95% CI)	100 (70.1, 100)	100 (70.1, 100)	94.7 (87.2, 97.9)	90.8 (82.2, 95.5)	92.6 (76.6, 97.9)	92.6 (76.6, 97.9)	93.8 (83.5, 97.9)	91.8 (84.2, 99.5)
Specificity, % (95% CI)	57.7 (51.0, 64.1)	81.4 (75.7, 86.0)	29.3 (24.5, 34.6)	25.4 (20.9, 30.6)	57.6 (48.8, 65.9)	68.6 (60.2, 76.3)	50.5 (43.6, 57.4)	50.5 (43.3, 57.7)
Accuracy, % (95% CI)	59.4 (52.6, 65.9)	82.1 (76.5, 86.9)	42.3 (37.3, 47.4)	38.4 (33.5, 43.5)	63.8 (56.2, 71.5)	73.0 (65.2, 79.9)	59.1 (53.0, 65.2)	58.7 (52.6, 64.8)
Positive predictive value, %	9.0	18.4	24.9	23.2	32.1	39.1	31.9	31.5
Negative predictive value, %	100	100	95.7	91.8	97.3	97.7	97.1	96.2

[1]Defined as a serum BHB ≥1.2 mmol/L.

[2]Observed prevalence of hyperketonemia: 4% in primiparous Holsteins and 19.9% in multiparous Holsteins; 17.8% in primiparous Jerseys and 19.8% in multiparous Jerseys.

[3]Threshold values (mmol/L) of milk BHB and milk acetone proposed by de Roos et al. (2007).

[4]Calculated as the proportion of correctly identified subjects among all subjects tested.

[5]Threshold values (mmol/L) of milk BHB and milk acetone determined when sensitivity and specificity were maximized based on receiver operating characteristic curves.

[6]Threshold values (mmol/L) of milk BHB and milk acetone determined when sensitivity was ≥90%.

Table 3. Final logistic models based on continuous test-day milk and performance variables for diagnosing hyperketonemia[1] in Holstein (n = 10 farms) and Jersey (n = 6 farms) cows between 5 and 20 DIM

	Primiparous			Multiparous		
Variable	Regression coefficient	SE	*P*-value	Regression coefficient	SE	*P*-value
Holstein						
Intercept	−54.41	19.82	0.006	−7.62	1.07	<0.001
Milk BHB, mmol/L	—	—	—	14.78	4.63	0.001
Milk acetone, mmol/L	9.34	3.20	0.004	3.53	2.28	0.121
Fat:protein	3.68	1.78	0.038	1.63	0.59	0.006
Lactation	—	—	—	0.40	0.14	0.004
Dry period length, d	—	—	—	0.02	0.01	0.052
Gestation length, d	0.16	0.067	0.019	—	—	—
Jersey						
Intercept	−3.41	1.14	0.003	−0.65	3.17	0.838
Milk BHB, mmol/L	—	—	—	8.92	5.61	0.112
Milk acetone, mmol/L	2.81	1.31	0.031	7.35	3.91	0.060
Milk protein, %	—	—	—	−1.23	0.68	0.073
Fat:protein	—	—	—	−3.99	1.96	0.042
MUFA, % of milk	2.50	0.98	0.010	2.36	1.17	0.044
trans fatty acids, % of milk	—	—	—	22.56	7.72	0.004
Short-chain fatty acids, % of milk	−6.35	2.64	0.016	—	—	—
Dry period length, d	—	—	—	0.038	0.02	0.097

[1]Defined as a serum BHB ≥1.2 mmol/L.

ratio, production on test day, DIM, lactation number, length of dry period, previous lactation, and gestation. In Jersey models, mBHB, mACE, milk protein percentage, fat:protein ratio, SCC, PUFA, total UFA, *trans* FA, short-chain FA, medium-chain FA, DIM, and gestation length were retained as variables to predict serum BHB.

The coefficient of determination, concordance coefficient correlation, and root mean squared error of prediction (mmol/L) between observed and predicted serum BHB based on cross-validation are presented in Table 7. The values of predicted serum BHB for each DIM group calculated by the cross-validation procedure were combined by parity group within breed and used to calculate sensitivity, specificity, accuracy, PPV, and NPV and are presented in Table 8. Primiparous Holstein models more accurately diagnosed HYK than multiparous models, likely a result of low HYK prevalence; the opposite was true in Jerseys. Accuracy exceeded 80% for all models and was highest for primiparous Holstein cows. The NPV exceeded 85% for all models.

Herd-Level Prevalence Identification

Logistic models correctly identified 8 of 10 Holstein herds (80% accuracy) at a 10% prevalence alarm level, and the remaining 2 herds identified as 1 false positive and 1 false negative (87.5% sensitive and 50% specific; Table 9). Jersey herds were identified with 100% accuracy (6 of 6 Jersey herds correctly identified) by the logistic models at a 10% prevalence alarm level. When individual multiple linear regression models were combined to predict herd prevalence of HYK as a diagnostic tool, Holstein models correctly identified 5 of 10 herds (50% accuracy; 50% sensitivity, 50% specificity; 5

Table 4. Area under the curve, sensitivity, specificity, accuracy, and predictive values of the cross-validation for logistic regression models predicting hyperketonemia[1] in Holstein (n = 10 farms) and Jersey (n = 6 farms) cows between 5 and 20 DIM

	Holstein		Jersey	
Variable	Primiparous	Multiparous	Primiparous	Multiparous
Cows, no.	224	382	152	247
Area under the curve	0.96	0.82	0.83	0.90
Sensitivity, % (95% CI)	55.6 (50.9, 60.1)	31.6 (30.1, 33.0)	39.8 (37.9, 41.8)	42.4 (40.5, 44.4)
Specificity, % (95% CI)	99.0 (98.8, 99.2)	95.3 (94.9, 95.6)	95.8 (95.4, 96.2)	95.1 (94.7, 95.5)
Accuracy,[2] % (95% CI)	97.3 (96.9, 97.5)	82.6 (82.1, 83.2)	87.7 (84.1, 85.4)	84.7 (84.0, 85.3)
Positive predictive value, %	70.0	62.3	70.3	68.4
Negative predictive value, %	98.2	84.9	86.6	87.0

[1]Defined as a serum BHB ≥1.2 mmol/L.

[2]Calculated as the proportion of correctly identified subjects among all subjects tested.

Table 5. Final multiple linear regression models based on continuous test-day milk and performance variables for the prediction of serum BHB in primiparous and multiparous Holstein (n = 10 farms) cows

	Primiparous					Multiparous				
Variable	R^2	RMSE[1]	Regression coefficient	SE	*P*-value	R^2	RMSE	Regression coefficient	SE	*P*-value
5 to 11 DIM	0.77	0.28				0.56	0.30			
Intercept			−2.38	1.13	0.037			−0.22	0.15	0.132
Milk acetone, mmol/L			1.22	0.08	<0.001			1.09	0.10	<0.001
Milk protein, %			−0.10	0.07	0.143			—	—	—
Fat:protein			0.24	0.11	0.032			0.49	0.09	<0.001
Production on test day, kg			−0.003	0.002	0.076			—	—	—
Lactation			—	—	—			0.04	0.02	0.082
Dry period length, d			—	—	—			0.004	0.002	0.020
Gestation length, d			0.01	0.004	0.004			—	—	—
12 to 20 DIM	0.68	0.26				0.67	0.44			
Intercept			−1.11	0.70	0.116			−1.26	0.59	0.035
Milk BHB, mmol/L			—	—	—			2.45	0.96	0.011
Milk acetone, mmol/L			1.23	0.07	<0.001			4.46	0.53	<0.001
Milk protein, %			−0.10	0.05	0.038			0.21	0.12	0.090
Fat:protein			0.26	0.07	<0.001			0.35	0.12	0.005
DIM			−0.01	0.005	0.129			0.04	0.01	0.003
Lactation			—	—	—			0.05	0.03	0.083
Previous lactation length, d			—	—	—			−0.0007	0.0004	0.132
Gestation length, d			0.006	0.002	0.006			—	—	—

[1]Root mean squared error.

incorrectly identified herds identified as 2 false positives and 3 false negatives) and Jersey models detected 6 of 6 herds (100% accuracy) at a 10% prevalence alarm level (Table 9).

DISCUSSION

The objectives of our study were to (1) develop linear and logistic regression models utilizing all available

Table 6. Final multiple linear regression models based on continuous test-day milk and performance variables for the prediction of serum BHB in primiparous and multiparous Jersey (n = 6 farms) cows

	Primiparous					Multiparous				
Variable	R^2	RMSE[1]	Regression coefficient	SE	*P*-value	R^2	RMSE	Regression coefficient	SE	*P*-value
5–11 DIM	0.57	0.44				0.82	0.36			
Intercept			1.47	0.36	<0.001			−0.95	0.55	0.088
Milk acetone, mmol/L			0.85	0.26	0.002			2.69	0.22	<0.001
Milk protein, %			—	—	—			0.17	0.10	0.091
Fat:protein			—	—	—			−0.82	0.23	0.0004
SCC (×1,000)			—	—	—			−0.00003	0.00002	0.054
PUFA, % of milk			−3.33	0.96	0.001			—	—	—
Total UFA, % of milk			0.95	0.18	<0.001			1.04	0.17	<0.001
Short-chain fatty acids, % of milk			−1.08	0.57	0.061			—	—	—
Medium-chain fatty acids, % of milk			−0.34	0.15	0.029			—	—	—
DIM			—	—	—			0.05	0.02	0.011
12–20 DIM	0.75	0.52				0.62	0.43			
Intercept			−2.08	3.19	0.517			−3.87	2.05	0.061
Milk BHB, mmol/L			—	—	—			2.99	0.35	<0.001
Milk acetone, mmol/L			3.67	0.54	<0.001			1.92	0.61	0.002
Milk protein, %			−1.26	0.28	<0.001			—	—	—
SCC (×1,000)			0.0006	0.00007	<0.001			—	—	—
Total UFA, % of milk			0.38	0.25	0.142			—	—	—
trans fatty acids, % of milk			−7.70	1.48	<0.001			2.58	1.33	0.055
Short-chain fatty acids, % of milk			3.90	0.82	<0.001			—	—	—
Medium-chain fatty acids, % of milk			—	—	—			−0.14	0.05	0.010
DIM			0.07	0.03	0.037			—	—	—
Gestation length, d			0.01	0.01	0.179			0.016	0.007	0.033

[1]Root mean squared error.

Table 7. Parameter evaluation of serum BHB predictions by breed, parity, and DIM groups after internal cross-validation of linear models retaining test-day variables collected from Holstein (n = 10 farms) and Jersey (n = 6 farms) cows between 5 and 20 DIM

	Holstein				Jersey			
	Primiparous		Multiparous		Primiparous		Multiparous	
Variable	5–11 DIM	12–20 DIM	5–11 DIM	12–20 DIM	5–11 DIM	12–20 DIM	5–11 DIM	12–20 DIM
Cows, no.	113	111	167	215	73	79	122	125
R^2	0.71	0.63	0.49	0.62	0.47	0.20	0.51	0.59
CCC[1]	0.78	0.80	0.62	0.76	0.68	0.41	0.69	0.74
RMSEP[2]	0.32	0.29	0.32	0.47	0.48	0.92	0.58	0.44

[1]Concordance correlation coefficient.

[2]Root mean squared error of prediction.

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. Our hypothesis was that predicting serum BHB concentrations from test-day variables using multiple linear regression would improve HYK detection compared with individual tests of FTIR milk ketone bodies or multiple logistic models and could be used as a herd-level diagnostic tool to monitor HYK prevalence. The primary goal of this research was to use predictions of individual cow serum BHB to determine herd-level HYK prevalence, calculated after predicting individual cow diagnosis, and thus provide a management decision-making tool. Within this study, multiple linear regression models exhibited improved reliability for diagnosing HYK in both Holstein and Jersey cattle compared with multiple logistic models.

The overall prevalence of HYK in Holstein animals in the current study was similar to some Holstein reports (Geishauser et al., 2000; van der Drift et al., 2012; Suthar et al., 2013) yet lower than others (Duffield et al., 2009; Denis-Robichaud et al., 2014). The lack of HYK surveys in Jersey herds did not allow for a prevalence comparison with other Jersey populations, but greater HYK prevalence in Jersey cows was similar to reports of high prevalence in Holsteins (Duffield et al., 2009; Denis-Robichaud et al., 2014). Increased prevalence in multiparous Holstein cows was expected as previous studies reported increased HYK as parity increased (Gröhn et al., 1989; Rasmussen et al., 1999; McArt et al., 2013). The same pattern was not observed in Jersey cows as primiparous Jerseys experienced greater HYK prevalence than multiparous Jerseys. Differences between breeds justified stratifying animals by breed and parity before predicting HYK instead of including them as variables within the models. Given that this was a post hoc decision and that HYK prevalence was lower than anticipated within the primiparous Holstein cows, that subgroup had a low sample size, which should be considered when interpreting results.

Although reports of HYK prevalence or incidence from individual research studies are useful, these studies can introduce selection bias based on farm and cow within farm selection. Having the ability to determine HYK prevalence in many dairies across regions and countries using herd-level diagnostics would be beneficial to understanding disease patterns and effects. Although herd-level diagnostics can also contain population bias dependent on herd DHI participation, it is easier to

Table 8. Sensitivity, specificity, accuracy, and predictive values of cross-validated linear regression models that predicted serum BHB and diagnosed hyperketonemia[1] in Holstein (n = 10 farms) and Jersey (n = 6 farms) cows between 5 and 20 DIM

	Holstein		Jersey	
Variable	Primiparous	Multiparous	Primiparous	Multiparous
Cows, no.	224	382	152	247
Sensitivity, % (95% CI)	55.6 (23.1, 86.3)	53.3 (42.1, 64.4)	74.1 (57.5, 90.6)	63.3 (49.8, 76.8)
Specificity, % (95% CI)	99.5 (98.6, 100)	90.9 (87.7, 94.1)	82.9 (76.3, 89.6)	88.4 (83.9, 92.9)
Accuracy,[2] % (95% CI)	97.8 (95.8, 99.7)	83.3 (79.6, 87.1)	81.3 (75.1, 87.6)	83.4 (78.8, 88.0)
Positive predictive value, %	83.3	59.4	48.8	57.4
Negative predictive value, %	98.2	88.6	93.6	90.6

[1]Defined as a serum BHB ≥1.2 mmol/L.

[2]Calculated as the proportion of correctly identified subjects among all subjects tested.

Table 9. Observed, logistic regression–predicted, and linear regression–predicted hyperketonemia[1] (HYK) prevalence between 5 and 20 DIM for Holstein (n = 10 farms) and Jersey (n = 6 farms) herds

	HYK prevalence,[2] %		
Herd	Observed	Logistic regression	Linear regression
Holstein			
A	11.4	35.2	14.8
B	0.0	0.0	0.0
C	6.7	22.2	11.1
D	26.4	41.7	20.8
E	15.8	63.2	23.7
F	11.0	36.7	7.3
G	16.1	19.4	16.1
H	13.2	34.6	9.4
I	28.6	35.7	7.1
J	3.8	34.6	15.4
Jersey			
A	25.0	18.8	18.8
B	20.0	32.7	23.6
C	15.9	15.9	13.6
D	18.4	42.1	27.2
E	22.2	49.2	24.6
F	11.4	18.2	11.4

[1]Defined as a serum BHB ≥1.2 mmol/L.

[2]Observed prevalence of HYK was calculated as the proportion of animals that had a serum BHB ≥1.2 mmol/L. Logistic regression prevalence was calculated as the proportion of animals predicted to have HYK from logistic regression models based on a threshold that maximized sensitivity and specificity. Linear regression prevalence was calculated as the proportion of animals that had a predicted serum BHB ≥1.2 mmol/L based on multiple linear regression models.

procure data from populations of animals across a large number of dairies using these broader methods. For example, herd-level diagnosis based on mBHB concentration determined by FTIR data collected over 4 yr indicated HYK prevalence in first, second, and third and greater lactations of 18.7, 19.5, and 27.6%, high variability by herd, and seasonality effects (Santschi et al., 2016). Development and strengthening of herd-level diagnostic tools, such as the one just described (Santschi et al., 2016) and those in the current research, could provide valuable information to improve management of HYK at the herd level.

Using mBHB and mACE as a Strategy for Diagnosing HYK

Using Previously Recommended Milk Ketone Body Thresholds. The ability of FTIR milk ketone bodies to diagnose HYK in the current study, based on AUC, was slightly lower than in previous reports (van Knegsel et al., 2010; van der Drift et al., 2012) and demonstrated only fair to good diagnostic accuracy (Dohoo et al., 2003) with the exception of primiparous Holsteins. Thresholds of mBHB (0.10 mmol/L) and mACE (0.15 mmol/L) recommended by de Roos et al. (2007) detected HYK with similar sensitivity in Jerseys but considerably diminished sensitivity in multiparous Holsteins. Specificity of milk ketone body tests in Jerseys was also lower than in previous studies (van Knegsel et al., 2010; van der Drift et al., 2012). Previously recommended diagnostic thresholds (de Roos et al., 2007) detected HYK with greatest accuracy in primiparous Holstein cows. Although sensitivity and specificity exceeded 80% for both ketone body tests and NPV were >99%, PPV were lowest in this group compared with others, which is likely a result of the low HYK prevalence observed given that PPV increases as disease prevalence increases (Parikh et al., 2008). Apart from primiparous Holsteins, the recommended thresholds detected HYK with less than satisfactory accuracy and PPV, especially in Jersey cows.

Using Optimized Milk Ketone Body Thresholds. Diagnostic thresholds of FTIR milk ketone bodies that maximized sensitivity and specificity in the current population differed from those recommended previously (de Roos et al., 2007). Milk acetone thresholds likely depend on the milk production potential of the target population because mACE is correlated with milk production (Heuer et al., 2001). This may help explain the poor diagnostic value of previously recommended thresholds (de Roos et al., 2007) in the current study, especially Jerseys, as those thresholds were developed using data collected from Holstein animals. To prevent the economic losses associated with a false negative, threshold values that obtained at least 90% sensitivity were selected as formerly suggested (Enjalbert et al., 2001). Threshold values that achieved 90% sensitivity were either the same as or lower than those that optimized sensitivity and specificity and lower still than those thresholds previously recommended (de Roos et al., 2007). Although this strategy may improve the detection of HYK animals, it does so with limited efficiency as false positive rates are increased; however, if it was used as a screening tool and coupled with a quantitative cowside detection tool to promote accuracy of monitoring programs, cows with HYK could be identified and treated.

mACE Versus mBHB. Milk acetone appeared to be a more accurate predictor of HYK than did mBHB and demonstrated stronger correlations with serum BHB and greater AUC of most ROC curves. A greater AUC was also observed for an individual test of mACE compared with mBHB when diagnosing HYK (van der Drift et al., 2012). Others improved AUC of FTIR milk ketone bodies by series or parallel testing of mBHB and mACE to predict HYK (Denis-Robichaud et al., 2014). Including both FTIR-predicted mBHB and mACE in a logistic model did not increase AUC above individual mBHB and mACE for either breed in the current data set (data not shown). Milk BHB and mACE may have

been less accurate predictors of HYK here because of the weak to moderate correlations observed for serum BHB and FTIR-predicted mBHB and mACE. Others reported stronger correlations between blood ketone bodies and mACE rather than mBHB when milk ketone bodies were measured by analytical chemistry (Andersson, 1984; Enjalbert et al., 2001). This could be reflective of the timely nature of mACE peak, compared with mBHB, as discussed below.

Accuracy of FTIR milk ketone bodies between this and previous studies may have differed because of sampling period discrepancies. The first week of lactation is considered the highest risk period for HYK, with incidence peaking at 4 to 5 DIM (McArt et al., 2012) before decreasing as lactation progresses (van der Drift et al., 2012; Jorjong et al., 2015). Based on our objective of predicting HYK during the highest risk period, animals were sampled only between 5 and 20 DIM, a shorter time than the periods of 5 to 60 DIM previously sampled to validate FTIR-predicted milk ketone bodies (de Roos et al., 2007; van Knegsel et al., 2010; van der Drift et al., 2012). Longer sampling periods in previous studies may have limited the diagnostic accuracy of mBHB or mACE. Milk ketone bodies also appear to peak at different weeks of lactation (de Roos et al., 2007; van Knegsel et al., 2010). During the first week of lactation, milk samples with elevated mACE did not have elevated mBHB, which did not peak until 3 wk postpartum when samples no longer had elevated mACE (de Roos et al., 2007). The uncoordinated peaks of milk ketone bodies during early lactation suggest that other factors influence the concentration of milk ketone bodies. Furthermore, the mammary use of ketone bodies differs depending on blood BHB concentrations, resulting in different correlations between blood and mBHB concentrations reflective of HYK status (Schwalm et al., 1972; Oliveira et al., 2017). Together, these factors may explain the differences in optimal thresholds of milk ketone bodies between studies and groups, further detracting from their practical use to monitor HYK.

Using Multiple Logistic Regression Models as a Strategy for Diagnosing HYK

Performance of Logistic Models. Multiple logistic regression models were built in an effort to improve the detection of HYK over single tests of milk ketone bodies by including the available test-day data collected during the study. These models more accurately diagnosed HYK over single tests of FTIR milk ketone bodies, demonstrated by considerably improved specificity, accuracy, and PPV (Table 4). Logistic models also displayed comparable or greater AUC. Similar to FTIR milk ketone bodies, the model that predicted HYK in primiparous Holsteins displayed the highest AUC, sensitivity, specificity, and accuracy as well as NPV, again likely attributable to the lower prevalence of HYK in that group. A similar logistic model (van der Drift et al., 2012) predicted HYK with lower specificity (84%) but greater sensitivity (82%) than observed in the current study. Sensitivity was likely overestimated in the previous study because the final model was not validated by a cross- or external-validation procedure (van der Drift et al., 2012). A logistic model that included only mBHB and mACE predicted HYK with >90% sensitivity, specificity, PPV, and NPV (Denis-Robichaud et al., 2014). The diagnostic performance of the aforementioned model was most likely greater than the models described here because milk ketone bodies were determined by flow-injection analysis rather than FTIR, which is more strongly correlated with serum ketone body concentrations (Denis-Robichaud et al., 2014).

Milk Variables Retained in Logistic Models. Although FTIR-predicted milk ketone bodies were moderate predictors of HYK, they were retained in logistic models that demonstrated improved accuracy and offer more practical application than milk ketone bodies analyzed by flow injection. Although mACE was retained in all logistic models, mBHB was not, even though Holstein and Jersey cows diagnosed with HYK had increased mBHB (Supplemental Tables S2 and S3). Along with milk ketone bodies, fat:protein ratio has traditionally been used as a screening tool for HYK (Duffield et al., 1997; Krogh et al., 2011; van der Drift et al., 2012). Hyperketonemia was associated with increased fat and decreased protein content of milk in previous studies (Miettinen and Setala, 1993; Lean et al., 1994; Miettinen, 1994; Rasmussen et al., 1999). Milk fat percentage was not retained in the logistic models presented here, but milk protein was retained in the Jersey model predicting HYK in multiparous cows. In agreement with this, milk protein percentage was decreased in Jersey cows diagnosed with HYK, whereas milk fat percentage was only numerically higher (Supplemental Table S3). Milk fat:protein ratio was increased in both Holstein and Jersey cows diagnosed with HYK (Supplemental Tables S2 and S3) and was retained in 3 of the 4 logistic models described here. Previously, the ratio alone predicted HYK in individual cows with only 58 to 69% sensitivity and 66 to 71% specificity (Duffield et al., 1997; van Knegsel et al., 2010; Denis-Robichaud et al., 2014) and demonstrated an AUC of 0.70 (Denis-Robichaud et al., 2014) and 0.75 (van Knegsel et al., 2010). Although previous studies also observed increased fat:protein ratio in cows experiencing HYK (Miettinen, 1994; van der Drift et al.,

2012), the ratio alone is not considered an accurate tool for diagnosing HYK in individual cows.

Milk FA predictions are currently being incorporated into routine milk testing systems. Specific milk FA may serve as potential biomarkers for early detection of HYK because FA mobilized from body fat have high concentrations of long-chain FA C16:0, C18:0, and C18:1 (Van Haelst et al., 2008; Hostens et al., 2012), which are taken up by the mammary gland and secreted in milk fat. Gas-liquid chromatography identified individual FA, FA ratios, and FA groups that had some diagnostic power to detect HYK (Van Haelst et al., 2008; Jorjong et al., 2015; Mann et al., 2016), and the ratio of C18:1 to C15:0 revealed the most discriminating factor for diagnosis in the study described by Jorjong et al. (2015). Although the latter study (Mann et al., 2016) did not associate the ratio of C18:1 and C15:0 to HYK, independent associations were identified for C18:1, C15:0, and several short-chain FA. Of the FTIR-predicted FA groups that were retained in logistic models, Jersey cows diagnosed with HYK produced milk fat with higher concentrations of MUFA and *trans* FA and lower concentrations of short-chain FA compared with non-HYK cows (Supplemental Table S3).

Performance Variables Retained in Logistic Models. Predictive logistic models for multiparous cows also included performance variables as lactation number was retained in the Holstein model and dry period length was retained in both the Holstein and Jersey models. It followed that lactation number was greater in Holstein cows diagnosed with HYK, and HYK cows of both breeds experienced longer dry periods (Supplemental Tables S2 and S3). Multiple studies have demonstrated that incidence of HYK increases as parity increases (Gröhn et al., 1989; Rasmussen et al., 1999; McArt et al., 2013), and it was not unexpected that the variable was retained here. Limited data exist that associates dry period length and HYK risk; however, an observational study identified increased odds of elevated mBHB at first milk test as days dry increased (Tatone et al., 2017). Increased dry period length may increase body condition at calving, which is a known risk factor for HYK (Rajala and Gröhn, 1998; Rasmussen et al., 1999; Nielsen et al., 2005). Gestation length was retained in the logistic model that predicted HYK in primiparous Holstein cows. Primiparous cows diagnosed with HYK carried their calves 7 d longer than non-HYK animals, but older Holstein and Jersey cows diagnosed with HYK did not (Supplemental Tables S2 and S3). To our knowledge, this is the first study to identify a relationship between gestation length and HYK. Dry period length and gestation length represent 2 variables that may be useful for identifying at-risk animals eligible for preventative management of HYK. However, because animals were sampled only once and may have developed the disease later, performance variables such as dry period and gestation length should continue to be investigated for any associated risk of HYK.

Predicting Serum BHB by Linear Regression as a Strategy for Diagnosing HYK

Before building linear regression models, parity groups within breed were further separated into 2 DIM periods: 5 to 11 DIM, considered the highest risk period, and a lower risk period of 12 to 20 DIM. This was done because the incidence of HYK decreases sharply each day after 5 to 11 DIM (McArt et al., 2012). When stratified by DIM group, mACE concentration was decreased in the 12 to 20 DIM group compared with the 5 to 11 DIM group, and mBHB was unchanged for both Holstein and Jersey cows (Supplemental Tables S4 and S5). Other variables that were retained in any of the logistic or linear models also differed between the 2 DIM groups. Stratifying parity groups into DIM groups was not considered for logistic modeling because the strategy would have limited the number of HYK animals for an appropriate cross-validation of the final logistic models.

Predicting serum BHB by linear regression was explored in the current study to apply a conventional diagnostic threshold used to diagnose HYK across populations. This strategy has more practical application as the arbitrary thresholds of single milk ketone body tests and logistic models that optimize sensitivity and specificity will vary across populations and have limited physiological relevance. Predicting a continuous variable with linear regression rather than the probability of disease in logistic regression may prove more useful, especially across groups that are stratified based on disease etiology. The sensitivity and specificity of the current linear models, combined with the infrequency of DHI milk testing, do not result in an individual cow diagnostic tool that is as precise as blood diagnostics; however, improving individual animal predictions does provide a benefit through improving accuracy of the herd-level prevalence that is subsequently calculated from the individual animal predictions.

Milk Variables Retained in Linear Models. Similar to logistic models, mBHB was excluded from all linear models with the exception of models that predicted serum BHB in multiparous cows 12 to 20 DIM. This was not unexpected considering the temporal difference of mBHB and mACE peaks previously described (de Roos et al., 2007; van Knegsel et al., 2010). The fact that mACE was retained in all logistic and linear models further suggests that it more accurately predicted

HYK. Linear regression models built for Jersey cows also included FTIR predictions of milk FA, with diagnosis affecting the percentage of total UFA, *trans* FA, and short-chain FA in milk (Supplemental Table S3). When specific FA and FA ratios were investigated previously, studies reported weak to moderate correlations between FA and HYK (Van Haelst et al., 2008; Jorjong et al., 2015; Mann et al., 2016), but diagnostic ability may have been limited because of uncoordinated blood and milk sampling. Predictive ability of FA may have differed here because previous investigations collected data from Holstein cows. Unfortunately, milk FA composition variables were not available for Holsteins at the time of sampling and comparisons between breeds could not be made. The inclusion of FA in predictive models would have likely differed between breeds, as a previous trial revealed that Holstein cows produced milk with increased C16:1 and C18:1 and decreased short-chain FA (White et al., 2001), and warrants further investigation.

Performance Variables Retained in Linear Models. The performance variables retained in logistic models (dry period length, gestation length, and lactation number) were also retained in linear models, reiterating a connection to HYK. Other performance variables that predicted serum BHB have been connected to HYK risk, including DIM (McArt et al., 2012), milk production (Ingvartsen et al., 2003; Nielsen et al., 2005), and mastitis or elevated SCC (Gröhn et al., 1989; Raboisson et al., 2014). Although dry period and previous lactation length were studied here rather than calving interval, a prolonged calving interval increased the risk of HYK (Dohoo and Martin, 1984) and odds of elevated mBHB at first milk test (Tatone et al., 2017). Cows diagnosed with HYK did have numerically longer previous lactations (Supplemental Tables S2 and S3). The current study was not designed to identify causation between performance variables and HYK, but continued investigation of the predictive variables identified here may improve detection and ultimately lead to prevention strategies.

Comparing Logistic and Linear Regression Models as Strategies for Diagnosing HYK

Cross-validated linear regression models that predicted serum BHB diagnosed HYK with improved accuracy in Holstein cows but diminished accuracy in Jersey cows when compared with logistic models. Improved sensitivity and specificity contributed to increased accuracy in Holsteins. Although sensitivity was considerably improved in Jerseys, specificity was reduced enough to limit accuracy. Decreased specificity but increased sensitivity suggests that Jersey models had fewer false negative tests but a higher rate of false positives than Holstein models. Although the predictive value of a negative test exceeded 88% for all models, predictive values of a positive test were considerably lower and more variable than NPV but were improved compared with single tests of mBHB or mACE within parity group. Compared with logistic models, PPV was marginally improved in primiparous Holstein cows but decreased in linear models that predicted serum BHB in multiparous Holsteins and all Jersey cows. Nonetheless, accuracy exceeded 80% for all models and suggests that serum BHB predicted by linear regression is a viable tool for monitoring HYK at the herd level in Holstein and Jersey dairy cattle.

Improved accuracy in Holstein models and sensitivity in Jersey models suggests that linear models diagnosed HYK more reliably than individual tests of milk ketone bodies or logistic models previously described. Although diagnostic ability was improved for some models, linear regression did not achieve the performance of a quantitative cowside blood BHB test that can detect HYK with 88% sensitivity and 96% specificity (Iwersen et al., 2009). In addition, the high proportion of false-positive test results in some parity groups caused the diagnostic tool to overestimate HYK prevalence in some herds. Use of such models in a clinical setting would be primarily at a herd level to indicate HYK prevalence during DHI milk analysis, which is typically every 4 to 5 wk. This identification of herd-level prevalence would provide valuable information to guide management and nutritional decisions. On a larger scale, as mentioned previously, prevalence data over time, season, geography, and management or farm systems would provide useful information regarding HYK. Although logistic models appeared to more accurately classify Holstein herds at a 10% alarm level than linear models, applying logistic models considerably overestimated HYK and predicted the majority of herds to have >30% prevalence (Table 9). Herd prevalence estimated by linear models was more similar to observed prevalence for both Holstein and Jersey herds, which would provide optimal value in determining the herd-level prevalence at a farm. All Jersey herds in this study had a true prevalence >10%, which may have prevented full analysis of the ability of Jersey models to correctly identify herds above and below the threshold.

Treating individual animals based on these models is limited by both the model accuracy and the frequency of DHI milk testing. Given the current model sensitivity and specificity, false positives would be mistreated and false negatives would remain untreated. Furthermore, the frequency of DHI milk testing would result in half of cows going untested during the HYK high-risk period and tested cows being tested at only a single time point

during the high-risk period. It would be feasible on some farms to increase the frequency of milk testing of postpartum cows, which could provide the opportunity to use models such as these to identify suspected HYK cows, which could then be tested with more precise diagnostics such as blood BHB quantification.

CONCLUSIONS

Individual test-day milk and performance variables are poor predictors of HYK in postpartum cows, but combining these variables using logistic and multiple linear regression models improved HYK predictive ability. The relatively high proportion of false positives is reconciled from previously described strategies but remains a limitation for application of linear models to detect HYK in individual cows. However, suspected animals could be identified for quantification of blood ketone bodies and subsequent treatment. Models that predicted serum BHB from test-day milk and performance variables available on test day could be incorporated into routine milk testing programs to accurately estimate herd HYK prevalence. Differences in the diagnostic accuracy of milk ketone bodies across breed and parity and distinct variables in models stratified by breed, parity, and DIM emphasize possible differences in HYK etiology that need to be accounted for in strategies of predictive, model-based diagnosis.

ACKNOWLEDGMENTS

The authors extend their gratitude to the farms that participated in the study. The authors also acknowledge Peter Crump for his assistance with statistical analysis. Partial financial and milk sampling support for this research was provided by CRI AgSource (Verona, WI) and the American Jersey Cattle Association (Reynoldsburg, OH). T. L. Chandler was partially funded by the John Brandt Memorial Scholarship awarded by the Land O'Lakes Foundation (St. Paul, MN).

REFERENCES

Andersson, L. 1984. Concentrations of blood and milk ketone bodies, blood isopropanol and plasma glucose in dairy cows in relation to the degree of hyperketonemia and clinical signs. Zentralbl. Veterinarmed. A 31:683–693.

Andersson, L. 1988. Subclinical ketosis in dairy cows. Vet. Clin. North Am. Food Anim. Pract. 4:233–251.

AOAC International. 2016. Official Methods of Analysis. 20th ed. AOAC International, Gaithersburg, MD.

Barros, T., M. A. Quaassdorff, M. J. Aguerre, J. J. Olmos Colmenero, S. J. Bertics, P. M. Crump, and M. A. Wattiaux. 2017. Effects of dietary crude protein concentration on late-lactation dairy cow performance and indicators of nitrogen utilization. J. Dairy Sci. 100:5434–5448. https://doi.org/10.3168/jds.2016-11917.

Carrier, J., S. Stewart, S. Godden, J. Fetrow, and P. Rapnicki. 2004. Evaluation and use of three cowside tests for detection of subclinical ketosis in early postpartum cows. J. Dairy Sci. 87:3725–3735. https://doi.org/10.3168/jds.S0022-0302(04)73511-0.

de Roos, A. P. W., H. J. C. M. van den Bijgaart, J. Hørlyk, and G. de Jong. 2007. Screening for subclinical ketosis in dairy cattle by Fourier transform infrared spectrometry. J. Dairy Sci. 90:1761–1766. https://doi.org/10.3168/jds.2006-203.

Denis-Robichaud, J., J. Dubuc, D. Lefebvre, and L. DesCôteaux. 2014. Accuracy of milk ketone bodies from flow-injection analysis for the diagnosis of hyperketonemia in dairy cows. J. Dairy Sci. 97:3364–3370. https://doi.org/10.3168/jds.2013-6744.

Dohoo, I. R., and S. W. Martin. 1984. Disease, production and culling Holstein-Friesian cows II. Age, season and sire effects. Prev. Vet. Med. 2:655–670.

Dohoo, I. R., S. W. Martin, and H. Stryhn. 2003. Veterinary Epidemiologic Research. 1st ed. Atlantic Veterinary College, Charlottetown, PE, Canada.

Dórea, J. R. R., E. A. French, and L. E. Armentano. 2017. Use of milk fatty acids to estimate plasma nonesterified fatty acid concentrations as an indicator of animal energy balance. J. Dairy Sci. 100:6164–6176. https://doi.org/10.3168/jds.2016-12466.

Dubuc, J., and J. Denis-Robichaud. 2017. A dairy herd-level study of postpartum diseases and their association with reproductive performance and culling. J. Dairy Sci. 100:3068–3078. https://doi.org/10.3168/jds.2016-12144.

Duffield, T. F., D. F. Kelton, K. E. Leslie, K. D. Lissemore, and J. H. Lumsden. 1997. Use of test day milk fat and protein to detect subclinical ketosis in dairy cattle in Ontario. Can. Vet. J. 38:713–718.

Duffield, T. F., K. D. Lissemore, B. W. McBride, and K. E. Leslie. 2009. Impact of hyperketonemia in early lactation dairy cows on health and production. J. Dairy Sci. 92:571–580. https://doi.org/10.3168/jds.2008-1507.

Enjalbert, F., M. C. Nicot, C. Bayourthe, and R. Moncoulon. 2001. Ketone bodies in milk and blood of dairy cows: Relationship between concentrations and utilization for detection of subclinical ketosis. J. Dairy Sci. 84:583–589. https://doi.org/10.3168/jds.S0022-0302(01)74511-0.

Foss Analytical. 2011. Application note 64. P/N no. 1026556. Foss Analytical, Hillerød, Denmark.

Geishauser, T., K. Leslie, J. Tenhag, and A. Bashiri. 2000. Evaluation of eight cow-side ketone tests in milk for detection of subclinical ketosis in dairy cows. J. Dairy Sci. 83:296–299. https://doi.org/10.3168/jds.S0022-0302(00)74877-6.

Gröhn, Y. T., H. N. Erb, C. E. McCulloch, and H. S. Saloniemi. 1989. Epidemiology of metabolic disorders in dairy cattle: Association among host characteristics, disease, and production. J. Dairy Sci. 72:1876–1885. https://doi.org/10.3168/jds.S0022-0302(89)79306-1.

Hansen, P. W. 1999. Screening of dairy cows for ketosis by use of infrared spectroscopy and multivariate calibration. J. Dairy Sci. 82:2005–2010. https://doi.org/10.3168/jds.S0022-0302(99)75437-8.

Herdt, T. H. 2000. Ruminant adaptation to negative energy balance. Influences on the etiology of ketosis and fatty liver. Vet. Clin. North Am. Food Anim. Pract. 16:215–230. https://doi.org/10.1016/S0749-0720(15)30102-X.

Heuer, C., H. J. Luinge, E. T. G. Lutz, Y. H. Schukken, J. H. van der Maas, H. Wilmink, and J. P. T. M. Noordhuizen. 2001. Determination of acetone in cow milk by Fourier transform infrared spectroscopy for the detection of subclinical ketosis. J. Dairy Sci. 84:575–582. https://doi.org/10.3168/jds.S0022-0302(01)74510-9.

Hostens, M., V. Fievez, J. L. M. R. Leroy, J. Van Ranst, B. Vlaeminck, and G. Opsomer. 2012. The fatty acid profile of subcutaneous and abdominal fat in dairy cows with left displacement of the abomasum. J. Dairy Sci. 95:3756–3765. https://doi.org/10.3168/jds.2011-5092.

Ingvartsen, K. L., R. J. Dewhurst, and N. C. Friggens. 2003. On the relationship between lactational performance and health: Is it yield or metabolic imbalance that cause production diseases in dairy

cattle? A position paper. Livest. Prod. Sci. 83:277–308. https://doi.org/10.1016/S0301-6226(03)00110-6.
ISO. 2013. ISO 9622/IDF 141. Milk and liquid milk products–Guidelines for the application of mid-infrared spectrometry. 2:14. International Organization for Standardization, Geneva, Switzerland.
Iwersen, M., U. Falkenberg, R. Voigtsberger, D. Forderung, and W. Heuwieser. 2009. Evaluation of an electronic cowside test to detect subclinical ketosis in dairy cows. J. Dairy Sci. 92:2618–2624. https://doi.org/10.3168/jds.2008-1795.
Jorjong, S., A. T. M. van Knegsel, J. Verwaeren, R. M. Bruckmaier, B. De Baets, B. Kemp, and V. Fievez. 2015. Milk fatty acids as possible biomarkers to diagnose hyperketonemia in early lactation. J. Dairy Sci. 98:5211–5221. https://doi.org/10.3168/jds.2014-8728.
Krogh, M. A., N. Toft, and C. Enevoldsen. 2011. Latent class evaluation of a milk test, a urine test, and the fat-to-protein percentage ratio in milk to diagnose ketosis in dairy cows. J. Dairy Sci. 94:2360–2367. https://doi.org/10.3168/jds.2010-3816.
Lean, I. J., M. L. Bruss, H. F. Troutt, and J. C. Galland. 1994. Bovine ketosis and somatotrophin: Risk factors for ketosis and effects of ketosis on health and production. Res. Vet. Sci. 57:200–209.
Mann, S., D. V. Nydam, A. L. Lock, T. R. Overton, and J. A. A. McArt. 2016. Short communication: Associations of milk fatty acids with early lactation hyperketonemia and elevated concentration of nonesterified fatty acids. J. Dairy Sci. 99:5851–5857. https://doi.org/10.3168/jds.2016-10920.
Marstorp, P., T. Anfalt, and L. Andersson. 1983. Determination of oxidized ketone bodies in milk by flow injection analysis. Anal. Chim. Acta 149:281–289.
McArt, J. A. A., D. V. Nydam, and G. R. Oetzel. 2012. Epidemiology of subclinical ketosis in early lactation dairy cattle. J. Dairy Sci. 95:5056–5066. https://doi.org/10.3168/jds.2012-5443.
McArt, J. A. A., D. V. Nydam, and G. R. Oetzel. 2013. Dry period and parturient predictors of early lactation hyperketonemia in dairy cattle. J. Dairy Sci. 96:198–209. https://doi.org/10.3168/jds.2012-5681.
McArt, J. A. A., D. V. Nydam, P. A. Ospina, and G. R. Oetzel. 2011. A field trial on the effect of propylene glycol on milk yield and resolution of ketosis in fresh cows diagnosed with subclinical ketosis. J. Dairy Sci. 94:6011–6020. https://doi.org/103168/jds.2011–4463.
Miettinen, P. V. A. 1994. Relationship between milk acetone and milk yield in individual cows. Zentralbl. Veterinarmed. A 41:102–109.
Miettinen, P. V. A., and J. J. Setala. 1993. Relationships between subclinical ketosis, milk production and fertility in Finnish dairy cattle. Prev. Vet. Med. 17:1–8.
Nielsen, N. I., N. C. Friggens, M. G. G. Chagunda, and K. L. Ingvartsen. 2005. Predicting risk of ketosis in dairy cows using in-line measurements of β-hydroxybutyrate: A biological model. J. Dairy Sci. 88:2441–2453. https://doi.org/10.3168/jds.S0022-0302(05)72922-2.
Oetzel, G. R. 2004. Monitoring and testing dairy herds for metabolic disease. Vet. Clin. North Am. Food Anim. Pract. 20:651–674. https://doi.org/10.1016/j.cvfa.2004.06.006.
Oliveira, R. C., S. J. Erb, R. S. Pralle, T. L. Chandler, K. J. Sailer, T. N. Mack, K. A. Weld, and H. M. White. 2017. Mammary utilization and secretion of β-hydroxybutyrate differs in dairy cows with hyperketonemia. J. Dairy Sci. 100(E-Suppl. 2):E163. (Abstr.)
Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010. Associations between the proportion of sampled transition cows with increased nonesterified fatty acids and beta-hydroxybutyrate and disease incidence, pregnancy rate, and milk production at the herd level. J. Dairy Sci. 93:3595–3601. https://doi.org/10.3168/jds.2010-3074.
Parikh, R., A. Mathai, S. Parika, G. C. Sekhar, and R. Thomas. 2008. Understanding and using sensitivity, specificity and predictive values. Indian J. Opthalmol. 56:45–50.
Raboisson, D., M. Mounié, and E. Maigné. 2014. Diseases, reproductive performance, and changes in milk production associated with subclinical ketosis in dairy cows: A meta-analysis and review. J. Dairy Sci. 97:7547–7563. https://doi.org/10.3168/jds.2014-8237.
Rajala, P. J., and Y. T. Gröhn. 1998. Disease occurrence and risk factor analysis in Finnish Ayrshire cows. Acta Vet. Scand. 39:1–13.
Rasmussen, L. K., B. L. Nielsen, J. E. Pryce, T. T. Mottram, and R. F. Veerkamp. 1999. Risk factors associated with the incidence of ketosis in dairy cows. Anim. Sci. 68:379–386.
Rathbun, F. M., R. S. Pralle, S. J. Bertics, L. E. Armentano, K. Cho, C. Do, K. A. Weigel, and H. M. White. 2017. Relationships between body condition score change, prior mid-lactation phenotypic residual feed intake, and hyperketonemia onset in transition dairy cows. J. Dairy Sci. 100:3685–3696. https://doi.org/10.3168/jds.2016-12085.
Santschi, D. E., R. Lacroix, J. Durocher, M. Duplessis, R. K. Moore, and D. M. Lefebvre. 2016. Prevalence of elevated milk β-hydroxybutyrate concentrations in Holstein cows measured by Fourier-transform infrared analysis in Dairy Herd Improvement milk samples and association with milk yield and components. J. Dairy Sci. 99:9263–9270. https://doi.org/10.3168/jds.2016-11128.
Schwalm, J. W., R. Waterman, G. E. Shook, and L. H. Schultz. 1972. Blood metabolite interrelationships and changes in mammary gland metabolism during subclinical ketosis. J. Dairy Sci. 55:58–64.
Shetty, N., P. Lovendahl, M. S. Lund, and A. J. Buitenhuis. 2017. Prediction and validation of residual feed intake and dry matter intake in Danish lactating dairy cows using mid-infrared spectroscopy of milk. J. Dairy Sci. 100:253–264. https://doi.org/10.3168/jds.2016-11609.
Suthar, V. S., J. Canelas-Raposo, A. Deniz, and W. Heuwieser. 2013. Prevalence of subclinical ketosis and relationships with postpartum diseases in European dairy cows. J. Dairy Sci. 96:2925–2938. https://doi.org/10.3168/jds.2012-6035.
Tatone, E. H., T. F. Duffield, S. J. LeBlanc, T. J. DeVries, and J. L. Gordon. 2017. Investigating the within-herd prevalence and risk factors for ketosis in dairy cattle in Ontario as diagnosed by the test-day concentration of β-hydroxybutyrate in milk. J. Dairy Sci. 100:1308–1318. https://doi.org/10.3168/jds.2016-11453.
van der Drift, S. G. A., R. Jorritsma, J. T. Schonewille, H. M. Knijn, and J. A. Stegeman. 2012. Routine detection of hyperketonemia in dairy cows using Fourier and acetone in milk in combination with test-day information. J. Dairy Sci. 95:4886–4898. https://doi.org/10.3168/jds.2011-4417.
Van Haelst, Y. N. T., A. Beeckman, A. T. M. van Knegsel, and V. Fievez. 2008. Short communication: Elevated concentrations of C18:1 and long-chain fatty acids in milk fat of multiparous subclinical ketotic cows. J. Dairy Sci. 91:4683–4686. https://doi.org/10.3168/jds.2008-1375.
van Knegsel, A. T. M., S. G. A. van der Drift, M. Horneman, A. P. W. de Roos, B. Kemp, and E. A. M. Graat. 2010. Short communication: Ketone body concentration in milk determined by Fourier transform infrared spectroscopy: Value for detection of hyperketonemia in dairy cows. J. Dairy Sci. 93:3065–3069. https://doi.org/10.3168/jds.2009-2847.
Weigel, K. A., R. S. Pralle, H. Adams, K. Cho, C. Do, and H. M. White. 2017. Prediction of whole-genome risk for selection and management of hyperketonemia in Holstein dairy cattle. J. Anim. Breed. Genet. 134:275–285. https://doi.org/10.1111/jbg.12259.
White, S. L., J. A. Bertrand, M. R. Wade, S. P. Washburn, J. T. Green Jr., and T. C. Jenkins. 2001. Comparison of fatty acid content of milk from Jersey and Holstein cows consuming pasture or a total mixed ration. J. Dairy Sci. 84:2295–2301. https://doi.org/10.3168/jds.S0022-0302(01)74676-0.
Williamson, D. H., J. Mellanby, and H. A. Krebs. 1962. Enzymatic determination of D(-)-β-hydroxybutyric acid and acetoacetic acid in blood. Biochem. J. 82:90–96.