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Prediction of blood β-hydroxybutyrate content and occurrence of hyperketonemia in early-lactation, pasture-grazed dairy cows using milk infrared spectra

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ABSTRACT

The objective of this study was to evaluate the ability of milk infrared spectra to predict blood β-hydroxybutyrate (BHB) concentration for use as a management tool for cow metabolic health on pasture-grazed dairy farms and for large-scale phenotyping for genetic evaluation purposes. The study involved 542 cows (Holstein-Friesian and Holstein-Friesian × Jersey crossbreds), from 2 farms located in the Waikato and Taranaki regions of New Zealand that operated under a seasonal-calving, pasture-based dairy system. Milk infrared spectra were collected once a week during the first 5 wk of lactation. A blood "prick" sample was taken from the ventral labial vein of each cow 3 times a week for the first 5 wk of lactation. The content of BHB in blood was measured immediately using a handheld device. After outlier elimination, 1,910 spectra records and corresponding BHB measures were used for prediction model development. Partial least square regression and partial least squares discriminant analysis were used to develop prediction models for quantitative determination of blood BHB content and for identifying cows with hyperketonemia (HYK). Both quantitative and discriminant predictions were developed using the phenotypes and infrared spectra from two-thirds of the cows (randomly assigned to the calibration set) and tested using the remaining one-third (validation set). A moderate accuracy was obtained for prediction of blood BHB. The coefficient of determination (R^2) of the prediction model in calibration was 0.56, with a root mean squared error of prediction of 0.28 mmol/L and a ratio of performance to deviation, calculated as the ratio of the standard deviation of the partial least squares model calibration set to the standard error of prediction, of 1.50. In the validation set, the R^2 was 0.50, with root mean squared error of prediction values of 0.32 mmol/L, which resulted in a ratio of performance to deviation of 1.39. When the reference test for HYK was defined as blood concentration of BHB ≥1.2 mmol/L, discriminant models indicated that milk infrared spectra correctly classified 76% of the HYK-positive cows and 82% of the HYK-negative cows. The quantitative models were not able to provide accurate estimates, but they could differentiate between high and low BHB concentrations. Furthermore, the discriminant models allowed the classification of cows with reasonable accuracy. This study indicates that the prediction of blood BHB content or occurrence of HYK from milk spectra is possible with moderate accuracy in pasture-grazed cows and could be used during routine milk testing. Applicability of infrared spectroscopy is not likely suited for obtaining accurate BHB measurements at an individual cow level, but discriminant models might be used in the future as herd-level management tools for classification of cows that are at risk of HYK, whereas quantitative models might provide large-scale phenotypes to be used as an indicator trait for breeding cows with improved metabolic health.

Key words: infrared spectroscopy, blood β-hydroxybutyrate, ketosis, prediction model

INTRODUCTION

Hyperketonemia (**HYK**) is an abnormally high concentration of circulating ketone bodies, mainly BHB, which can occur during extreme negative energy balance in early lactating dairy cows (Duffield et al., 2009). Serum BHB concentrations equal or greater than 1.2 or 1.4 mmol/L are the generally accepted reference thresholds to diagnose HYK, and are associated with increased risks of displaced abomasum, metritis, and clinical ketosis (**CK**, Duffield et al., 2009), in addition to decreased conception rates and decreased milk production (Duffield, 2000) in cows managed in housed

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systems. Therefore, these thresholds for HYK are often used to diagnose subclinical ketosis (**SCK**).

Across different dairying systems, observed incidences for SCK, when defined as the percentage of cows that have at least one blood BHB concentration ≥1.2 mmol/L during early lactation, ranged from 11 to 37% in Europe (Suthar et al., 2013) and 40 to 60% in North America (Duffield, 2000; McArt et al., 2012). In pasture-grazed systems in New Zealand, Compton et al. (2015) reported that, on average, 68% of cows tested HYK-positive during the first 5 wk postcalving. Hence, HYK or SCK is common across different dairying systems, and the rates are far higher than the incidence rates reported for CK, in a review of 18 studies, from 0.2 to 17.2% (Pryce et al., 2016).

Hyperketonemia diagnosis is based on BHB in blood, so blood analysis represents the gold standard method for determination. However, due to practical limitations arising from individual blood sampling and sample processing time, the routine testing of all animals at risk using blood tests is unfeasible. These limitations also apply to the more user-friendly, cow-side tools that have been developed to help veterinary practitioners in on-farm HYK diagnosis, based on a rapid assessment of BHB in blood. Implementing a HYK surveillance program using records from routine milk tests, and potentially, in-line milk sensors may be more practical and less labor-intensive. Routine predictions of ketone bodies content in milk can be obtained by infrared (**IR**) spectroscopy analysis of test-day milk samples (Grelet et al., 2016). Even though IR predictions (**IP**) of ketone bodies concentrations in milk have a relatively low accuracy ($R^2 = 0.71$, Grelet et al., 2016), they are moderately heritable and have a moderate to high genetic correlation with CK (Koeck et al., 2014, 2016). In addition, they can predict the occurrence of HYK better than fat-to-protein ratio (van Knegsel et al., 2010).

Commercial calibration equations for the IP of milk ketone bodies have typically been developed for cows managed in housed systems and have not been tested in certain pasture-grazed dairy systems, including New Zealand. Hence, national IP of milk BHB are not yet available. Moreover, due to their differences in seasonal management, whereby cows calve in late winter/early spring and are grazed outdoors for all or part of the year, these systems are likely to benefit from the development of dedicated calibration equations that account for the joint effect of season and lactation stage. Compared with housed systems popular in Europe and North America, cows managed in pasture-grazing systems generally have a relatively lower milk yield, and higher fat and protein concentration, while also having higher circulating BHB concentrations (Roche et al., 2010) due to differences in diet composition and cow genetics. Considering that the average content of milk BHB is below the limit of detection of IR spectrometers (Broutin, 2015) and IP of milk BHB relies on correlated traits (e.g., concentration of fat and protein, lactose, fatty acid profile, and so on), developing dedicated calibration models is particularly important.

An alternative approach to the prediction of milk BHB is to predict blood BHB. By avoiding intermediate steps, prediction errors can be minimized when traits of interest (e.g., HYK) are predicted directly from spectra (Gengler et al., 2016). Depending on the accuracy of IR prediction models, IP might be used to define best practices, adjust feeding and health management, and ultimately improve animal welfare. Under the condition that spectral data are available on a large scale and IP are sufficiently accurate, phenotypes for blood BHB might also allow genetic and genomic evaluations for cow metabolic health traits.

The objective of this study was to evaluate the ability of milk IR spectra to predict the concentration of BHB in blood and the occurrence of HYK in pasture-grazed, early-lactation dairy cows to serve in the future as a tool for large-scale phenotyping for selective breeding purposes and for on-farm management.

MATERIALS AND METHODS

Care and Use of Animals

All research animals were acquired, retained, and used in compliance with national laws and regulations. The Ruakura Animal Ethics Committee (Hamilton, New Zealand) approved all animal manipulations (RAEC#13902) in accordance with the New Zealand Animal Welfare Act (Ministry of Primary Industries, 1999).

Animals and Reference Analysis

This study was part of a larger project investigating treatment of HYK that involved in total 967 cows, from 2 research farms and one university demonstration farm located in the Waikato, Taranaki, and Canterbury regions of New Zealand. Milk spectral data were only available from the Waikato and Taranaki farms (542 cows; Holstein-Friesian and Holstein-Friesian × Jersey cows in both farms).

Both farms operated under a seasonal-calving, pasture-based dairy system. Lactation number of the cows ranged from 1 to 12. A blood "prick" sample was taken using a 29-gauge needle and 0.5-mL syringe (without anticoagulant) from the ventral labial vein of each cow 3 times a week (on Monday, Wednesday, and Friday) for the first 5 wk of lactation. Therefore, each cow was

tested 15 times. The BHB concentration in blood was measured immediately after blood sampling, using a handheld device (FreeStyle Optium Blood Glucose and Ketone Monitoring System, Abbott Diabetes Care Ltd., Maidenhead, Berkshire, UK), as per the manufacturer's instructions. This handheld device has been recently validated previously for use on dairy cows and is known to provide very accurate results (Fiorentin et al., 2017). All blood samples were collected at approximately the same time of the day (0700 h; before the AM milking and before a fresh allocation of pasture and supplementary feed were offered), between June and October 2016.

Cows in each farm were randomly assigned to either a control or a treatment group before calving. Cows in the control group (n = 267) were not treated for HYK, whereas cows in the treatment group (n = 275) were drenched with 300 mL of mono-propylene glycol (equivalent dose 310 g) every time the blood BHB results were between ≥1.2 to <3.0 mmol/L. The treatment was repeated once daily until BHB concentration tested <1.2 mmol/L. However, irrespective of group, if cows tested ≥3.0 mmol/L they were treated for CK with 240 mL of Ketol (Bayer New Zealand Ltd., Glenfield, New Zealand; active ingredients propylene glycol, mineral glycerophosphates, choline, cobalt, and iodide) drenched twice daily for 3 d as per the manufacturer's instructions.

Infrared Spectra Acquisition

Individual milk yields were recorded at each milking (Waikato herd using DeLaval Milk Meter, DeLaval Ltd., Hamilton, New Zealand; Taranaki herd using Westfalia Surge Metatron Milk Meter, GEA Farm Technologies, Cambridge, New Zealand). Milk IR spectra were determined once a week on composite milk samples collected using the herd testing system, for the first 5 wk of lactation. Representative samples from the Monday evening milking and next morning (Tuesday) milking were mixed in relative proportion to milk volume before obtaining spectra. Milk samples were stored at 4°C before milk spectral analysis and samples from the Taranaki herd were also preserved using 0.02% bronopol antimicrobial before refrigerated transport for laboratory analysis. Absorbance spectra were recorded at a central laboratory (DairyNZ Ltd., Hamilton, New Zealand) using a Milko-Scan FT1 (Foss Electric A/S, Hillerød, Denmark) over the spectral range from 5,010 to 925 cm^{-1} within 48 h after sampling. Milk component data were verified by reference techniques for a subset of milk samples for each farm [milk fat by the Röse-Gottlieb method (IDF, 1987), CP by the Kjeldahl technique (Barbano et al., 1991), and lactose by the chloramine-T method (Amin et al., 1982)]. Due to the interference of water absorption, the O–H bending and O–H stretching regions of the spectra (between 1,628 and 1,658 cm^{-1} and between 3,105 and 3,444 cm^{-1}, respectively) were removed before the chemometric analysis (Hewavitharana and Brakel, 1997). Spectra transmittances (T) were transformed to absorbances (A) with the equation A = log(1/T). The number of spectra records per cow ranged from 1 to 5.

Spectra with a global standardized Mahalanobis distance (Shenk and Westerhaus, 1995) greater than 3 (n = 45) were considered outliers and eliminated. After outlier elimination, 1,910 spectra records and relative BHB measures, from 542 cows, were available for calibration. Of these cows, 267 were in the control group and 275 were in the treatment group.

Calibration-Validation Partitions

Prediction models were developed using the records of two-thirds of the cows and validated on the remaining one-third. Cows in the calibration and validation set were randomly selected ensuring that all the records from a cow (through lactation wk 1 to 5) were either in the calibration or the validation subset; thus, the analysis assessed the "across-cow" predictive power of the calibration equations. The calibration-validation procedure described above was replicated 10 times.

Selection of the Reference Values

In preliminary analyses, either Monday, Wednesday, or Friday blood BHB measures were associated with the closest day of measurement of IR spectra and used for model development. As an alternative, the weekly average BHB was also explored. However, slightly higher prediction accuracies (~1.5% higher R^2) were obtained when the concentration of blood BHB measured on Monday and Wednesday (i.e., the closest days to milk spectra collection) were averaged together and associated with the closest spectra record. In addition, the removal of treated animals, in addition to when the BHB measures were taken after an animal had been treated, were also explored, but the results were consistent with those obtained when all records were included in the analysis. Hence, we will discuss only the results obtained using the records from all cows and using Monday and Wednesday averaged blood BHB content as reference values.

Spectral Data Transformation

Several spectra mathematical treatments were compared before chemometric analysis: spectra were trans-

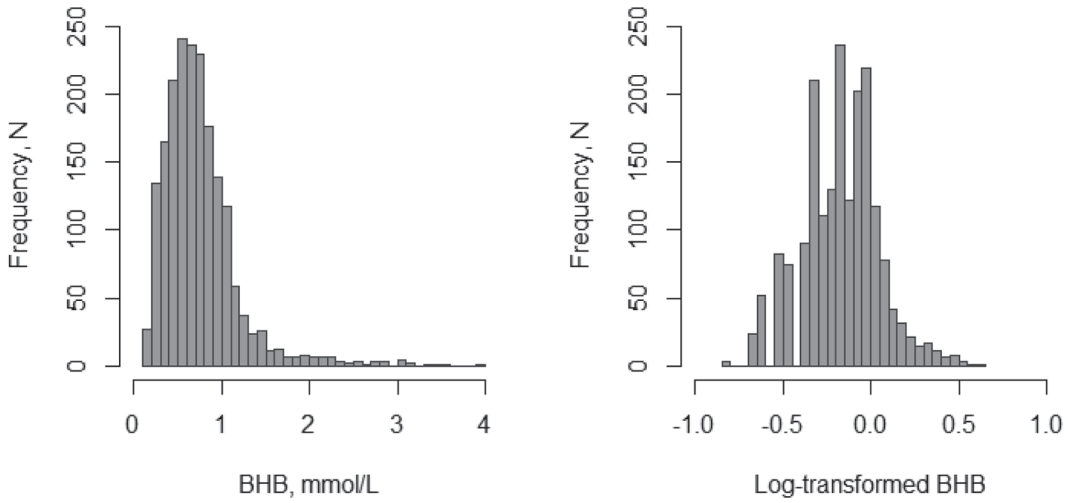

Figure 1. Distribution of blood BHB concentration (average of 2 measurements taken at an interval of 3 d) in the totality of the samples (n = 1,910) before and after $\log_{10}$-transformation.

formed using standard normal variate, multiplicative scatter correction, and extended multiplicative scatter correction, first and second order derivatives. The gaps over which derivatives were calculated ranged from 1 to 10 data points and the smooth ranged from 1 to 8. Preliminary prediction models were also developed using a reduced number of selected spectral variables. The selected spectra regions included 212 wavenumbers in the region covering 968.1 to 1,577.5 cm^{-1}, 1,731.8 to 1,762.6 cm^{-1}, 1,781.9 to 1,808.9 cm^{-1}, and 2,831.0 to 2,966.0 cm^{-1} (Grelet et al., 2016).

The best prediction performance was obtained using the spectra that excluded the water absorbance regions and the noninformative region ranging from 2,966 to 5,010 cm^{-1}, transformed using extended multiplicative scatter correction and a 1st derivative calculated over a window of 5 points. Consequently, 574 spectral variables were used in the final models.

Normalization of the BHB Distribution

Preliminary statistics indicated that BHB values were not normally distributed, with a higher proportion of low values (Figure 1). According to Grelet et al. (2016), when performing calibration models, this type of distribution gives too much weight to the low values, impairing the accuracy in predicting high values. Therefore, following the approach used by those authors, several low value samples in the distribution of BHB phenotypes were randomly removed to obtain a reduced data set that covered the same range of values, but gave less weight to low values. Visual inspections of the data indicated that most of low values were between 0.3 and 0.6 mmol/L. Preliminary prediction models were developed: (1) using the full data set (n = 1,910), (2) after randomly removing 25% of the data with low values (n = 1,780), and (3) after randomly removing 50% of the data with low values (n = 1,650). The values were then $\log_{10}$-transformed to approach a normal distribution. To evaluate the predictive ability of models when applied to a random population, the distribution of the samples in the validation sets was not modified. As the accuracy of validation did not improve when the models were built with a reduced proportion of low BHB values, the final prediction models were developed using all the available records, with no sample removal in calibration. Consequently, the average number of cows for each of the 10 calibration-validation partitions was 360 and 182, respectively, which corresponded, on average, to 1,267 (ranging from 1,250 to 1,283) and 643 (ranging from 627 to 660) spectra records, respectively.

Quantitative Prediction Models

Quantitative models for predicting blood BHB were developed on the calibration sets using partial least squares (**PLS**) regression with a 10-fold cross-validation, implemented in the R (R Development Core Team, 2018) package PLS (Mevik and Wehrens, 2007). Partial least squares regression is the most common multivariate method used in spectroscopy and it has been demonstrated to perform equally well than more innovative methods such as Bayesian regression models (Bonfatti et al., 2017a).

Models were then tested on the corresponding validation sets. The optimal number of PLS components was determined based on first local minimum value in root mean squared error of prediction (**RMSEP**). The RM-

SEP in calibration and validation sets, the coefficient of determination between the predicted and measured values in calibration ($\mathbf{R^2c}$) and validation ($\mathbf{R^2v}$), and the ratio of performance to deviation (**RPD**, i.e., the ratio of the SD of measured BHB values to RMSEP) were calculated and expressed as the average of the values obtained across 10 calibration-validation replicates.

In preliminary analyses, the daily milk yield (L/cow) recorded on the day of spectra acquisition was included with the spectra as an additional predictor in models, but led to only a minor improvement in the prediction accuracy in calibration and had no benefits in validation (data not reported). Hence, milk yield was not included in the subsequent analyses. As an alternative to the use of the IR spectra, prediction models based on milk yield, fat-to-protein ratio, and lactose were also tested, but resulted a poor predictive ability ($R^2 = 0.15$) and are, therefore, not given further consideration.

Discriminant Analysis

Discriminant models aiming to differentiate HYK-positive from HYK-negative cows were developed with partial least squares discriminant analysis (**PLS-DA**; Lê Cao et al., 2011), implemented in the R package mixOmics (Rohart et al., 2017). Duffield et al. (2009) reported an increased health risk and reduced milk production when blood BHB concentrations exceeded either 1.2 or 1.4 mmol/L. Hence, these 2 thresholds were used as a diagnostic reference to discriminate cows with HYK (positives) from non-HYK (negatives) cows in 2 subsequent analyses. Discriminant models were developed and tested on the same calibration-validation sets created for developing and testing the quantitative prediction models.

The accuracy of discriminant models was assessed by producing and calculating the area under the receiver operating characteristic curve (**AUC**) based on a 10-fold cross-validation. The optimal cut-off value for each test variable was defined as the point where the sum between sensitivity and specificity was at a maximum (i.e., equal weighing of false-positive and false-negative test results). The PLS-DA method used in this study implemented in the mixOmics package already uses a prediction threshold based on distances that optimally determine class membership of the samples tested. As such, AUC and ROC are not needed to estimate the performance of the model and are provided only as complementary performance measures. The estimated *P*-value provided is from a Wilcoxon test between the predicted scores of one class and the other.

The statistics of the discriminant models were expressed in terms of sensitivity (the proportion of positives that are correctly classified), specificity (the proportion of negatives that are correctly classified), and global accuracy (total percentage of correct classification). A high specificity can still result in numerous false positive tests when most of the cows tested are actually negative. For this reason, the results were also expressed in terms of positive and negative predictive values (**PPV** and **NPV**, respectively). Positive predictive value is the proportion of positive results that are true positive and is calculated as PPV = number of true positives/(number of true positives + number of false positives). Negative predictive value is the proportion of negative results that are true negatives, calculated as follows: NPV = number of true negative/(number of true negative + number of false negatives). Distributions of observed blood BHB concentrations for each of the test result categories (i.e., true negative, false positive, false negative, and true positive) were also investigated.

Statistical analyses and plots were obtained in R (v. 3.4.4, R Development Core Team, 2018).

RESULTS AND DISCUSSION

Descriptive Statistics of Blood BHB Content and Frequency of HYK and CK

On average, cows produced 21.6 ± 4.8 kg of milk per day, containing 4.41 ± 0.61% of fat and 3.54 ± 0.30% of protein from wk 1 to 5 in lactation. Descriptive statistics for weekly blood BHB content and the frequency of HYK-positive blood samples (defined as number of HYK-positive blood samples, using a threshold of either BHB ≥1.2 or ≥1.4 mmol/L, divided by the total number of averaged Monday and Wednesday blood samples) during this period are reported in Table 1. Mean blood BHB concentrations decreased with the increasing number of week in lactation. The average blood BHB concentration was 0.77 ± 0.43 mmol/L, and ranged from 0.15 to 4 mmol/L. In a study performed by Compton et al. (2014), on a large sample of pasture-grazed, New Zealand dairy cows, the overall mean blood BHB concentration was similar (0.82 ± 0.59 mmol/L, median = 0.7) to our study.

In the current study, using the defined threshold of blood BHB ≥1.2 mmol/L, the frequency of HYK-positive blood samples during the first 5 wk of lactation was, on average, 10.4%. The average frequency of HYK-positive samples calculated using a threshold of 1.4 mmol/L was 6.5%. It is worth noting that the actual incidence of HYK in New Zealand dairy cows is expected to be higher than that reported in this study, as roughly half of the cows were treated for HYK (treatment group) and all cows diagnosed as affected by CK were treated. Furthermore, BHB values for Monday

Table 1. Descriptive statistics for blood BHB concentration (average of 2 measures taken over 3 d) and frequency of blood samples that tested positive for hyperketonemia (HYK; defined as a concentration of blood BHB ≥1.2 or 1.4 mmol/L) across weeks of lactation and parities

Source of variation	n	Blood BHB concentration, mmol/L					HYK frequency, %	
		Median	Mean	SD	Minimum	Maximum	BHB ≥1.2 mmol/L	BHB ≥1.4 mmol/L
DIM								
1–7	212	0.80	0.87	0.40	0.25	3.05	16.04	8.49
8–14	384	0.75	0.83	0.40	0.20	2.65	12.50	6.51
15–21	434	0.70	0.80	0.45	0.20	3.40	11.75	8.29
22–28	495	0.70	0.75	0.45	0.15	3.55	8.28	6.46
29–35	385	0.60	0.66	0.40	0.15	4.00	6.23	3.65
Parity								
1	447	0.70	0.77	0.47	0.15	3.05	12.08	8.05
2–3	721	0.65	0.68	0.34	0.15	3.20	6.52	3.33
4–5	295	0.65	0.74	0.39	0.20	3.40	8.81	6.78
6–7	246	0.88	0.95	0.45	0.30	3.45	17.07	9.76
>7	201	0.80	0.93	0.54	0.25	4.00	14.43	10.45

and Wednesday measures were combined and Friday measures were not included in the current study, which would likely have removed additional positive cases because the median time to spontaneous resolution of HYK is approximately 5 d (McArt et al., 2011). On average, for the cows belonging to the control group, the frequency of HYK-positive samples, defined as BHB ≥1.2 or ≥1.4 mmol/L, was 12.5 and 8.3%, respectively.

The frequency of HYK-positive samples was greatest in the first week of lactation and reduced with increasing week of lactation. This result is consistent with McArt et al. (2012), who reported that the peak daily prevalence of SCK occurred at 5 DIM when 28.9% of housed cows had a positive test when measured 3 times a week during the first 30 DIM. Daily prevalence was defined as the number of cows with BHB ≥1.2 mmol/L on each DIM divided by the total number of cows tested on that DIM. Similarly, Compton et al. (2014) reported that the prevalence of SCK (BHB ≥ 1.2 mmol/L) in pasture-grazed cows was 23.8% at 7 to 12 d postcalving but had decreased to 5.9% when measured again at 35 to 40 d postcalving. In contrast, Compton et al. (2015) reported that the prevalence of SCK in pasture-grazed cows was lowest during 0 to 4 d postcalving (17.9%) relative to weekly measures taken between 7 and 39 d postcalving (range 26.0 to 34.2%).

Mean blood BHB was higher in first and fourth and later parities and at its lowest in parity 2 to 3. Hence, the frequency of HYK-positive samples was lower in cows from parity 2 to 5 compared with cows at first parity and from parities 6 and higher. The greater risk of HYK in older cows is consistent with previous studies conducted in both housed (Duffield et al., 1998) and pasture-grazing (Compton et al., 2015) systems.

On average, cows with blood BHB <1.2 mmol/L produced 21.6 ± 4.8 L/d, with 4.38 ± 0.68 fat % and 3.55 ± 0.30 protein %. Cows with at least one blood BHB ≥1.2 mmol/L had comparable milk yield (21.4 ± 4.8), but slightly higher fat % (4.67 ± 0.59) and lower protein % (3.39 ± 0.30), as expected (Duffield, 2000).

Prediction of Blood BHB Content

The scatter plot of predicted versus measured blood BHB content obtained in one of the validation sets is presented in Figure 2. Results indicate that models were more accurate in predicting the low than the high BHB values, in agreement with Grelet et al. (2016). The relatively low number of samples with high values of BHB is a limiting factor in development of IR prediction models. Hence, as an alternative preliminary approach, a proportion of the samples (25 and 50%) in the calibration sets were excluded from the analysis, to obtain a more balanced distribution of the BHB values. This approach has been used by Grelet et al. (2016) in the IR prediction of milk BHB and NEFA and has been tested by Fleming et al. (2017) for the IR prediction of milk fatty acid profile.

The R^2c increased (up to 0.63, data not reported) as the proportion of samples excluded increased but this led to a reduced R^2v (0.42, data not reported). These results indicate that this approach is not expected to improve the predictive ability of models when they are applied at the population level. Grelet et al. (2016) and Fleming et al. (2017) concluded that artificially modifying the distribution of the variable would be beneficial for the predictive ability of models. However, in both studies, models have been tested on data having the same "modified" distribution of the calibration set. If the models are to be used to predict a large population, the BHB distribution in the new samples are likely to be similar to the full data set, with most samples characterized by very low, or low values. In our study, the models were developed on calibration sets that had a distribution that was artificially modified, but they were then tested on a random sample to mimic the con-

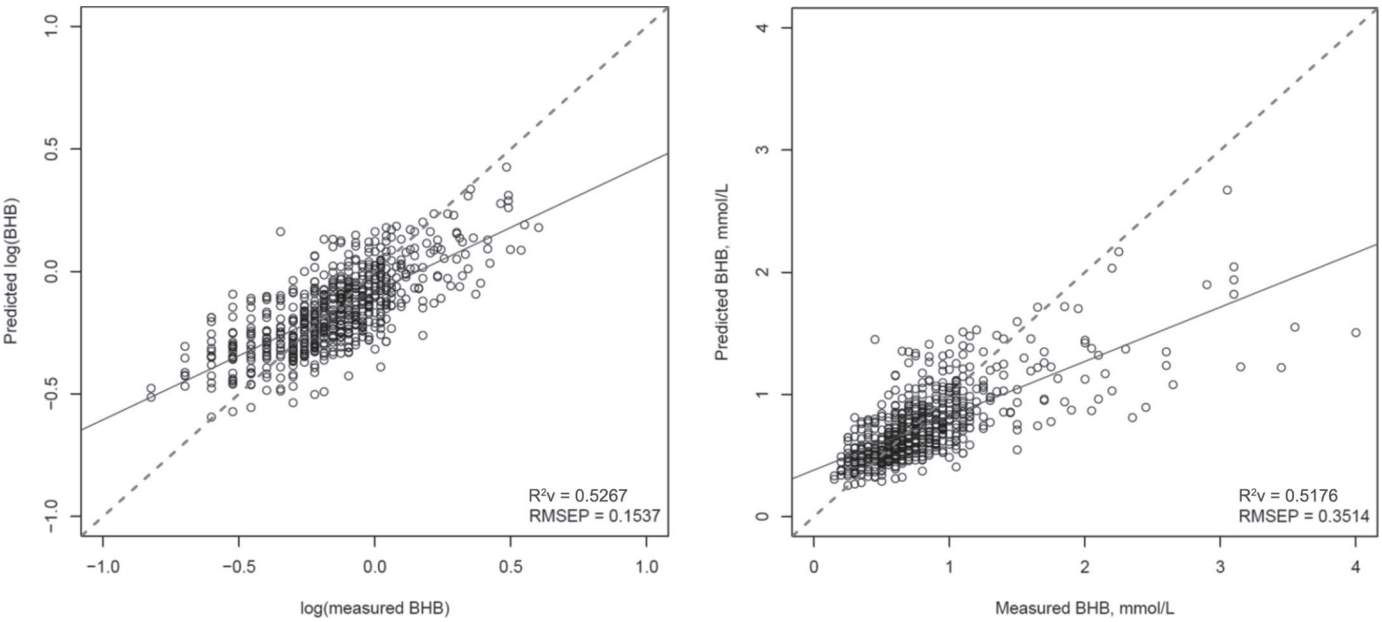

Figure 2. Relationship between measured blood BHB contents and infrared predictions obtained in validation for one of the validation sets, before (left) and after (right) back-transformation of $\log_{10}$-transformed BHB values to millimoles per liter. The dashed line corresponds to the linear regression y = x. The solid line corresponds to the linear regression of infrared predicted BHB on measured blood BHB. R^2v is the R^2 in validation, and RMSEP is the root mean squared error of the prediction model.

ditions that the models will operate under in the future. This can explain the inconsistent results obtained in our study when compared with those in the literature.

Average fitting statistics of final prediction models for blood BHB are reported in Table 2. A moderate accuracy was obtained for prediction of blood BHB. On average, for log(BHB), the R^2c was 0.58 (RPD = 1.56), whereas when predicted values were back-transformed to mmol/L, R^2c was 0.56, with RMSEP = 0.28 mmol/L, and RPD = 1.50. In validation, the R^2 was only slightly lower than in calibration. Specifically, R^2v was 0.52 and 0.50, with RMSEP values of 0.15 and of 0.32 mmol/L, which translated into RPD of 1.43 and 1.39, for log(BHB) and BHB, respectively. These results indicate that the models are not expected to provide accurate quantitative values at the individual cow level, but the moderate R^2v indicate that they could potentially be used to distinguish low and high values, or used to predict aggregate values (i.e., herd average) with reasonable accuracy. Because the prediction error variance of the mean of a group of n samples is $\sqrt{n}$ times smaller than the prediction error variance of an individual sample (Heuer et al., 2001), the IP are expected to be accurate enough to be used as a herd health indicator. For example, in a herd of 100 cows, the prediction error variance of the herd average BHB is expected to be 10 times lower than the prediction error variance of the BHB in individual samples.

Previous studies have focused on using milk IR spectrum to predict indicators of energy status of cows, such as acetone, BHB, and citrate, in milk (Heuer et al., 2001; de Roos et al., 2007; Grelet et al., 2016).

Table 2. Average fitting statistics (SD in parentheses) of quantitative prediction models for blood BHB concentration obtained across 10 calibration-validation partitions[1]

Trait	n	No. of terms	RMSEP	R^2	RPD
Log_{10} (BHB)					
Calibration	1,267 (11)	24 (4.14)	0.14 (0.003)	0.58 (0.027)	1.56 (0.045)
Validation	643 (11)	24 (4.14)	0.15 (0.004)	0.52 (0.033)	1.43 (0.056)
Back-transformed BHB					
Calibration	1,267 (11)	24 (4.14)	0.28 (0.013)	0.56 (0.028)	1.50 (0.048)
Validation	643 (11)	24 (4.14)	0.32 (0.033)	0.50 (0.046)	1.39 (0.068)

[1]n = number of records in the data sets; no. of terms = number of optimal partial least square components; RMSEP = root mean squared error of prediction; RPD = RMSEP/SD of measurements.

However, little information is available in the literature concerning the direct prediction of blood BHB. Broutin (2015) reported an R^2c of 0.54 and a RMSEP of 0.39 mmol/L, but no external validation was reported. Belay et al. (2017a) reported an R^2cv of 0.38 (RMSEP = 0.22 mmol/L) and an R^2v of 0.43 (RMSEP = 0.24 mmol/L). More recently, Luke et al. (2019) reported values of R^2v of 0.48. Hence, the prediction accuracy found in our study is either in line, or higher, than that reported in the previous studies, with a comparable or lower prediction error.

The values of R^2 are relatively low compared with those achieved for prediction of milk ketone bodies (Grelet et al., 2016), but the accuracies can be considered satisfactory considering that blood components were predicted indirectly from milk composition. It should also be noted that blood metabolites can be subject to a considerable variation over time, even within the same day (Oetzel, 2004), thus affecting the reliability of the reference measures, and there might be a time lag between the release of metabolites in blood and modification of milk composition. Besides the biological limitation, an additional technical issue is the fact that reference values produced by the handheld ketone meter are 1-digit values and, consequently, have a discrete variation. This factor offsets the differences in reference BHB values between samples having different spectra, generating prediction errors.

In addition, the data set used in our study included samples from only 2 farms, hence accuracy and robustness of prediction models is likely to improve with the addition of new samples, collected across more herds and seasons, to the calibration set (Blanco Romía and Alcalà Bernàrdez, 2009). While the predominant breeds present in New Zealand dairy cow population (Holstein-Friesian and crossbreds) were well represented in the data set used in this study, the representativeness of farming system types was limited, so the models are not expected to be robust if applied to other diets or geographical regions (Blanco Romía and Alcalà Bernàrdez, 2009). Also, the data were only available for one season, and variation in pasture quality and quantity is known to affect cow performance (Dalley, 2003), so extending the experiment to another season is likely to build confidence in the models developed. Consequently, calibration models should be enriched by the inclusion of additional samples, as described, before being applied on a large scale.

Infrared spectroscopy has a relatively low accuracy in predicting blood BHB and a limitation of practical application of IR models is the frequency of milk recordings. When milk herd testing is practiced monthly, many cows with HYK will be missed (ideally, for routine detection of HYK, milk recordings should be performed weekly). However, IR spectroscopy is still a very attractive option for routine ketosis diagnosis, due to the opportunity of prompt and inexpensive upgrading of the equipment with additional prediction equations for new traits. It has been demonstrated that even low accuracy milk IP can become useful in the context of animal breeding because of the generally high genetic correlation between measured traits and their IP (Bonfatti et al., 2017b) and IR spectroscopy might generate large scale and easily accessible phenotypes that would not be available otherwise. In a recent study, Belay et al. (2017b) demonstrated that, using even a comparatively low accuracy model (R^2cv = 0.38) that was developed using Polish Holstein cows, the IP of blood BHB correctly classified more than 77% of ketotic Norwegian Red cows correctly, using confirmation by veterinary intervention. In addition, BHB predictions were moderately heritable (h^2 ranged from 0.25 to 0.37 across lactation stages), and genetically correlated with CK (0.47), milk yield (0.28), and protein content (−0.37). This means that effects of different breeds and environments might not necessarily prevent the use of prediction models in a different breed and environment. Application of commercial calibration equations for the IP of milk ketone bodies (or of models developed in other countries for the IP of blood BHB content), to New Zealand milk samples would allow comparison of the predictive ability of models developed using different populations. In addition, reference data from different countries might be shared in future to create more robust equations.

Prediction of HYK Occurrence

The number of true positives, true negatives, false positives, and false negatives obtained by discriminant models is reported in Table 3, as well as the sensitivity, specificity, overall accuracy, PPV, NPV, and AUC. When the reference test for HYK was defined as blood concentration of BHB ≥1.2 mmol/L, sensitivity and specificity in calibration were respectively around 82 and 84%, meaning that 82 and 84% of the HYK-positive cows and HYK-negative cows, respectively, were classified as such. The global accuracy (i.e., the total proportion of correct classified records) was 84%. In validation, the global accuracy was 82 and 76% of the HYK-positive cows and 82% of the HYK-negative cows were classified as such. Hence, while the quantitative models developed could differentiate between high and low BHB values, the discriminant models developed here allow the classification of cows with reasonable accuracy.

From a management perspective, it is of interest to correctly identify hyperketonemic cows, as they have

increased risks of metabolic and reproductive disorders (Duffield et al., 2009), but a high specificity does not necessarily translate to a low number of false positive tests. In calibration, the PPV was 37%, meaning that only 37% of the cows that were predicted as being "affected," were actually hyperketonemic. This proportion slightly decreased in validation (35%), leading to a proportion of false positives (expressed on the number of cows predicted as positive) of 63%. At a threshold of 1.4 mmol/L, despite the higher sensitivity and specificity, the PPV decreased to 32 and 28% in calibration and validation, respectively. This is likely due to the lower prevalence of HYK at a threshold of 1.4 mmol/L, compared with the prevalence at a threshold of 1.2 mmol/L. Conversely, the NPV was always greater than 97%.

Plasma BHB concentrations of cows that were false positively or false negatively misclassified by the discriminant model were compared with those of correctly classified as positive or negative cows (results not reported). Mean plasma BHB concentrations of misclassified records were closer to the threshold value of 1.2 or 1.4 mmol/L than those of cows correctly classified as either HYK-positive or HYK-negative. Of the false-positives obtained, independent of the threshold used to classify cows, 50% had BHB ≥0.95 mmol/L and 75% had had BHB ≥0.75 mmol/L.

In the literature, models that use milk IR spectra for classification of hyperketonemic cows have mostly been developed defining HYK based on the concentration of ketone bodies in milk (Hansen, 1999; Heuer et al., 2001; de Roos et al., 2007). If compared with our results, the sensitivity, specificity, and PPV reported in these studies is generally very high. However, the determination of BHB in blood is considered the reference test for SCK (Duffield, 2000) and these methods have not been validated for their ability to detect cows with HYK using blood BHB concentration as the diagnostic reference criterion.

Only few studies (van Knegsel et al., 2010; van der Drift et al., 2012) reported classification models based on IP and blood BHB as a diagnostic method for HYP and only 2 of those (Gelé et al., 2015; Pralle et al., 2018) used the full IR spectra for classifying cows. Excluding the results reported by van der Drift et al. (2012), because their model was not validated on an independent set of samples, sensitivity values in these studies ranged from 0.80 (van Knegsel et al., 2010) to 0.83% (Pralle et al., 2018), whereas specificity ranged from 0.69 (Gelé et al., 2015) to 81% (Pralle et al., 2018), in line with our results. A common finding, consistent across studies, is the low PPV, ranging from less than 20% (van Knegsel et al., 2010) to 48% (Gelé et al., 2015). This is also in line with our results, meaning that the application of models would result in unnecessary treatment of a significant proportion of healthy cows.

It is worth noting that the PPV, albeit of clinical relevance, is dependent on the prevalence of the disease (i.e., it increases at increasing proportions of positive tests). Hence, differences in prevalence (deriving by factors such as breed, farming system, parity, feeding management, production level of the cows, HYK detection method, and so on) can affect model performance. The prevalence of HYK in the study with the best performance in terms of PPV (Gelé et al., 2015) was much higher (35%) than that reported by van Knegsel et al.

Table 3. Performance in classification, obtained in calibration and external validation, for the discrimination of cows affected by hyperketonemia (HYK)[1]

	Threshold for diagnosis of HYK			
	BHB ≥1.2 mmol/L		BHB ≥1.4 mmol/L	
Item	Calibration	Validation	Calibration	Validation
Records, no.	1,267 ± 11	643 ± 11	1,267 ± 11	643 ± 11
Prevalence, %	10.14 ± 0.76	10.82 ± 1.50	6.29 ± 0.75	7.05 ± 1.42
Model components,[2] no.	8 ± 2	8 ± 2	10 ± 2	10 ± 2
True positives, no.	105 ± 9	53 ± 8	68 ± 10	35 ± 9
True negatives, no.	960 ± 16	473 ± 20	1,040 ± 23	510 ± 15
False positives, no.	179 ± 17	100 ± 11	148 ± 15	87 ± 10
False negatives, no.	23 ± 3	17 ± 4	12 ± 3	11 ± 4
Sensitivity, %	81.90 ± 2.47	76.16 ± 4.83	85.17 ± 4.57	76.29 ± 11.21
Specificity, %	84.27 ± 1.38	82.48 ± 2.12	87.54 ± 1.32	85.44 ± 1.65
Global accuracy, %	84.03 ± 1.31	81.81 ± 1.59	87.40 ± 1.32	84.85 ± 1.17
Positive predicted value, %	37.08 ± 3.43	34.56 ± 4.61	31.53 ± 4.11	28.23 ± 5.60
Negative predicted value, %	97.64 ± 0.00	98.89 ± 0.00	98.01 ± 0.01	98.01 ± 0.01
Area under the curve, %	91.63 ± 1.16	87.99 ± 1.39	94.78 ± 1.11	90.94 ± 1.81

[1]Values correspond to the average ± SD obtained for 10 calibration-validation partitions.

[2]Number of components used by the discriminant model.

(2010) and van der Drift et al. (2012; 7–13 and 11%, respectively), and much higher than that observed in our study (10.4%, if considering a threshold of 1.2 mmol/L of BHB). Hence, given the relatively low frequency of HYK in our sample and the sole use of spectral variables as predictors, our results can be considered promising.

On farm, the percentage of cows classified as affected by HYK might be used as an evaluation parameter for cow metabolic health, for example, for diet or intervention management. Validation of IP of BHB with measures of BHB in blood using data from different farms with more variation in cow characteristics and diets could help assess the value of prediction models. However, the practical utility of calibration models relies not only on accuracy of prediction, but on the relationship of the predicted BHB values (or of the predicted class) with cow profitability. In New Zealand dairy cows, concentration of blood BHB ≥1.2 mmol/L during early lactation was associated with decreased 6-wk pregnancy rate (Compton et al., 2015); however, more in-depth investigations of the effect of treating high BHB concentration on production and reproduction parameters are needed. Determining phenotypic and genetic associations between the IP and milk production and reproduction traits will be particularly important for evaluating the potential of IP of blood BHB as a genetic or management tool, to improve cow metabolic health through selective breeding or through on-farm practices.

CONCLUSIONS

The prediction of blood BHB content from milk is possible and moderately accurate and can potentially be used as a herd-level management tool or for genetic selection purposes in pasture-grazed systems. Applicability of IR spectroscopy is not likely suited for obtaining accurate BHB measurements at an individual cow level, but at this level of accuracy, because of the generally high genetic correlation between measured traits and their IP, it might be used by breeding organizations as an indicator trait for cow metabolic health in genetic selection programs. The number of samples and farms should be increased in the future to maximize the variability of the calibration set and increase model robustness. Other blood metabolites could also be analyzed with the aim of predicting cow metabolic status using information from different sources. To evaluate the usefulness of IP as indicator traits of blood BHB content in future selective breeding programs, genetic parameters of the IR predicted blood BHB and its relationship with productive and reproductive performance should also be estimated.

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REFERENCES

Amin, D., K. Y. Saleem, and W. A. Bashir. 1982. Titrimetric determination of chloramine-T and some aldoses by amplification reactions. Talanta 29:694–696.

Barbano, D. M., J. M. Lynch, and J. R. Fleming. 1991. Direct and indirect determination of true protein content of milk by Kjeldahl analysis: Collaborative study. J. Assoc. Off. Anal. Chem. Int. 74:281–288.

Belay, T. K., B. S. Dagnachew, Z. M. Kowalski, and T. Ådnøy. 2017a. An attempt at predicting blood β-hydroxybutyrate from Fourier-transform mid-infrared spectra of milk using multivariate mixed models in Polish dairy cattle. J. Dairy Sci. 100:6312–6326. https://doi.org/10.3168/jds.2016-12252.

Belay, T. K., M. Svendsen, Z. M. Kowalski, and T. Ådnøy. 2017b. Genetic parameters of blood β-hydroxybutyrate predicted from milk infrared spectra and clinical ketosis, and their associations with milk production traits in Norwegian Red cows. J. Dairy Sci. 100:6298–6311. https://doi.org/10.3168/jds.2016-12458.

Blanco Romía, M., and M. Alcalà Bernàrdez. 2009. Multivariate calibration for quantitative analysis. Pages 70–71 in Infrared Spectroscopy for Food Quality Analysis and Control. D.-W. Sun, ed. Academic Press, London, UK. https://doi.org/10.1016/B978-0-12-374136-3.X0001-6.

Bonfatti, V., F. Tiezzi, F. Miglior, and P. Carnier. 2017a. Comparison of Bayesian regression models and partial least squares regression for the development of infrared prediction equations. J. Dairy Sci. 100:7306–7319. https://doi.org/10.3168/jds.2016-12203.

Bonfatti, V., D. Vicario, A. Lugo, and P. Carnier. 2017b. Genetic parameters of measures and population-wide infrared predictions of 92 traits describing the fine composition and technological properties of milk in Italian Simmental cattle. J. Dairy Sci. 100:5526–5540. https://doi.org/10.3168/jds.2016-11667.

Broutin, P. 2015. Determination of the concentration of a component in one fluid of an animal by spectroscopic analysis of another fluid. U.S. Patent Application No. 15/029,163.

Compton, C. W. R., S. McDougall, L. Young, and M. A. Bryan. 2014. Prevalence of subclinical ketosis in mainly pasture-grazed dairy cows in New Zealand in early lactation. N. Z. Vet. J. 62:30–37. https://doi.org/10.1080/00480169.2013.823829.

Compton, C. W. R., L. Young, and S. McDougall. 2015. Subclinical ketosis in post-partum dairy cows fed a predominantly pasture-based diet: Defining cut-points for diagnosis using concentrations of beta-hydroxybutyrate in blood and determining prevalence. N. Z. Vet. J. 63:241–248. https://doi.org/10.1080/00480169.2014.999841.

Dalley, D. 2003. Sharing dairy industry research for all grassland farmers. Pages 31–34 in Proc. Joint Conf. Grassland Society Victoria and Grassland Society NSW. Albury, NSW, Australia.

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.

Duffield, T. F., D. Sandals, K. E. Leslie, K. Lissemore, B. W. McBride, J. H. Lumsden, P. Dick, and R. Bagg. 1998. Efficacy of monensin for the prevention of subclinical ketosis in lactating dairy cows. J. Dairy Sci. 81:2866–2873. https://doi.org/10.3168/jds.S0022-0302(98)75846-1.

Duffield, T. 2000. Subclinical ketosis in lactating dairy cattle. Vet. Clin. North Am. Food Anim. Pract. 16:231–253.

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.

Fiorentin, E., A. Tiecher, C. Menegat, C. Soares, A. Aires, R. Rocha, and F. H. D. Gonzalez. 2017. Accuracy of two hand-held electronic devices for determination of blood β-hydroxybutyrate in dairy cows. Rev. Bras. Saúde Prod. Anim. 18:439–445. https://doi.org/10.1590/s1519-99402017000300004.

Fleming, A., F. S. Schenkel, J. Chen, F. Malchiodi, V. Bonfatti, R. A. Ali, B. Mallard, M. Corredig, and F. Miglior. 2017. Prediction of milk fatty acid content with mid-infrared spectroscopy in Canadian dairy cattle using differently distributed model development sets. J. Dairy Sci. 100:5073–5081. https://doi.org/10.3168/jds.2016-12102.

Gelé, M., M. Ferrand-Calmels, L. Brun-Lafleur, A. Werner, and F. Gollé-Leidreiter. 2015. Predicting the risk of ketosis using mid infrared spectrometry. ICAR Tech. Ser. 19:19–24.

Gengler, N., H. Soyeurt, F. Dehareng, C. Bastin, F. Colinet, H. Hammami, M. L. Vanrobays, A. Lainé, S. Vanderick, C. Grelet, A. Vanlierde, E. Froidmont, and P. Dardenne. 2016. Capitalizing on fine milk composition for breeding and management of dairy cows. J. Dairy Sci. 99:4071–4079. https://doi.org/10.3168/jds.2015-10140.

Grelet, C., C. Bastin, M. Gelé, J. B. Davière, M. Johan, A. Werner, R. Reding, J. A. Fernandez Pierna, F. G. Colinet, P. Dardenne, N. Gengler, H. Soyeurt, and F. Dehareng. 2016. Development of Fourier transform mid-infrared calibrations to predict acetone, β-hydroxybutyrate and citrate contents in bovine milk through a European dairy network. J. Dairy Sci. 99:4816–4825. https://doi.org/10.3168/jds.2015-10477.

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.

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.

Hewavitharana, A. K., and B. V. Brakel. 1997. Fourier transform infrared spectrometric method for rapid determination of casein in raw milk. Analyst (Lond.) 122:701–704. https://doi.org/10.1039/A700953D.

IDF (International Dairy Federation). 1987. Milk: Determination of fat content—Röse-Gottlieb gravimetric method (reference method). IDF Standard FIL-IDF 1C:1987 (IDF, Brussels, Belgium.).

Koeck, A., J. Jamrozik, G. Kistemaker, F. Schenkel, R. Moore, D. Lefebvre, D. Kelton, and F. Miglior. 2016. Genetic and phenotypic associations of milk β-hydroxybutyrate with ketosis in Canadian Holsteins. Can. J. Anim. Sci. 96:302–305. https://doi.org/10.1139/cjas-2015-0206.

Koeck, A., J. Jamrozik, F. Schenkel, R. Moore, D. Lefebvre, D. Kelton, and F. Miglior. 2014. Genetic analysis of milk β-hydroxybutyrate and its association with fat-to-protein ratio, body condition score, clinical ketosis, and displaced abomasum in early first lactation of Canadian Holsteins. J. Dairy Sci. 97:7286–7292. https://doi.org/10.3168/jds.2014-8405.

Lê Cao, K.-A., S. Boitard, and P. Besse. 2011. Sparse PLS discriminant analysis: Biologically relevant feature selection and graphical displays for multiclass problems. BMC Bioinformatics 12:253–286. https://doi.org/10.1186/1471-2105-12-253.

Luke, T. D., S. Rochfort, W. Wales, V. Bonfatti, L. Marett, and J. Pryce. 2019. Metabolic profiling of early lactation dairy cows using milk mid-infrared spectra. J. Dairy Sci. 102:1747–1760. https://doi.org/10.3168/jds.2018-15103.

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/10.3168/jds.2011-4463.

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.

Mevik, B. H., and R. Wehrens. 2007. The PLS package: Principal component and partial least squares regression in R. J. Stat. Softw. 18:1–24. https://doi.org/10.18637/jss.v018.i02.

Ministry of Primary Industries. 1999. New Zealand Animal Welfare Act. Part 6. Use of animals in research, testing, and teaching. Accessed Jan. 4, 2019. http://www.legislation.govt.nz/act/public/1999/0142/latest/DLM49664.html.

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.

Pralle, R. S., K. W. Weigel, and H. M. White. 2018. Predicting blood β-hydroxybutyrate using milk Fourier transform infrared spectrum, milk composition, and producer-reported variables with multiple linear regression, partial least squares regression, and artificial neural network. J. Dairy Sci. 101:4378–4387. https://doi.org/10.3168/jds.2017-14076.

Pryce, J. E., K. P. Gaddis, A. Koeck, C. Bastin, M. Abdelsayed, N. Gengler, F. Miglior, B. Heringstad, C. Egger-Danner, and K. Stock. 2016. Invited review: Opportunities for genetic improvement of metabolic diseases. J. Dairy Sci. 99:6855–6873. https://doi.org/10.3168/jds.2016-10854.

R Development Core Team. 2018. The GNU Project. The R Project for Statistical Computing. Accessed Jan. 25, 2019. http://www.rproject.org/.

Roche, J. R., J. K. Kay, C. V. C. Phyn, S. Meier, J. M. Lee, and C. R. Burke. 2010. Dietary structural to nonfiber carbohydrate concentration during the transition period in grazing dairy cows. J. Dairy Sci. 93:3671–3683. https://doi.org/10.3168/jds.2009-2868.

Rohart, F., B. Gautier, A. Singh, and K.-A. Lê Cao. 2017. mixOmics: An R package for 'omics feature selection and multiple data integration. PLOS Comput. Biol. 13:e1005752. https://doi.org/10.1371/journal.pcbi.1005752.

Shenk, J. S., and M. O. Westerhaus. 1995. Forage analysis by near infrared spectroscopy. Pages 111–120 in Forages. Vol. II. The Science of Grassland Agriculture. 5th ed. R. F. Barnes, D. A. Miller, and C. J. Nelson, ed. Iowa State University Press, Ames.

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.

van der Drift, S. g., R. Jorritsma, J. Schonewille, H. Knijn, and J. Stegeman. 2012. Routine detection of hyperketonemia in dairy cows using Fourier transform infrared spectroscopy analysis of β-hydroxybutyrate and acetone in milk in combination with testday information. J. Dairy Sci. 95:4886–4898. https://doi.org/10.3168/jds.2011-4417.

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 the detection of hyperketonemia in dairy cows. J. Dairy Sci. 93:3065–3069. https://doi.org/10.3168/jds.2009-2847.