Prediction of blood beta-hydroxybutyrate content in early-lactation New Zealand dairy cows using milk infrared spectra

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Summary

The objective of this study was to evaluate the ability of mid-infrared predictions of blood BHB concentration to serve as a tool for large-scale phenotyping and management tool in New Zealand dairy farms. The data were on 553 cows (Holsteins and Holstein x Jersey crossbreds), from 2 farms located in the Waikato and Taranaki regions of New Zealand, operated under a seasonal-calving, pasture-based dairy system. Milk infrared spectra were collected once a week on all cows. 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 â-hydroxybutyrate (**BHB**) in blood was measured immediately using a hand-held device. All blood samples were collected at approximately the same time of the day (7 am, before a fresh allocation of pasture and supplementary feed were offered), between June and October 2016. Concentrations of blood BHB measured on the day before and after the milking when the spectra data were acquired were averaged and used for developing prediction models. After outlier elimination, 1,910 spectra records and relative BHB measures were available for calibration.

Calibration models were developed by PLS regression using two-thirds of the cows (corresponding to 1,297 spectra records) and validated on the remaining one-third. Cows in the calibration and validation set were randomly selected. A moderate accuracy was obtained for prediction of blood BHB. The R^2 of calibration was 0.58, with a ratio of performance to deviation (**RPD**), calculated as the ratio of the SD of the PLS model calibration set to the SE of prediction, of 1.54. In validation, the R^2 was 0.49 with RPD = 1.39. The relatively low number of samples with high values of BHB is a limiting factor in the development of infrared prediction models. Hence, as an alternative approach, part of the samples in the calibration set were excluded from the analysis, in order to obtain a more balanced distribution of the BHB values. The subset of samples excluded from the calibration set ranged from 25 to 50%. The R^2 in calibration increased (up to 0.63) as the proportion of samples excluded

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increased, but this led to a reduced R^2 in validation (0.42), indicating that this approach is not expected to improve the predictive ability of models when they are applied at the population level.

This study has shown that the prediction of blood BHB content from milk is possible and moderately accurate and can be potentially used as management tool at farm level. To evaluate the role of infrared predictions as indicator traits of blood BHB content in future selective breeding programs, genetic parameters of the infrared predicted blood BHB need to be estimated.

Keywords: Infrared spectroscopy, blood â-hydroxybutyrate, ketosis, prediction model.

Introduction

In New Zealand dairy cows, hyperketonaemia (defined as blood β-hydroxybutyrate (**BHB)** concentration ≥ 1.2 mmol/L) is common during the postpartum period with an estimated herd-level incidence of 68% during the first 5 wk of lactation (Compton *et al.*, 2015). As hyperketonaemia is associated with increased occurrence of clinical ketosis, other health disorders, and reduced fertility (Compton *et al.*, 2015), reliable diagnostic methods and strategies to reduce hyperketonaemia are needed.

Currently available commercial calibration equations for the infrared (**IR**) prediction of milk ketone bodies have never been tested in New Zealand. Hence, IR prediction of milk BHB are not yet available. Moreover, due to its pasture-based dairy farming system, where cows are grazed outdoors all year round, New Zealand is likely to benefit from the development of dedicated calibration equations. Considering that the average content of milk BHB is below the limit of detection of IR spectrometers (Broutin, 2015) and prediction of milk BHB, therefore, relies on correlated traits (e.g., concentration of fat and protein, lactose, fatty acid profile etc.), an alternative approach is to use milk IR spectra to predict blood BHB concentration instead of milk BHB content.

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

Material and methods

The study involved 553 cows (Holsteins and Holstein x Jersey crossbreds), from 2 seasonal-calving, pasture-based dairy farms located in the Waikato and Taranaki regions of New Zealand. Milk IR spectra were collected once a week (on PM/AM composite samples) for all cows using a Milko-Scan FT1 (Foss Electric A/S, Hilleroed, Denmark).

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 concentration of BHB in blood was measured immediately using a hand-held device (FreeStyle Optimum™ Blood Glucose Monitoring System with Blood β-Ketone Test Strips, Abbott Diabetes Care Ltd., UK). All blood samples were collected at approximately the same time of the day (7 am, before a fresh allocation of pasture and supplementary feed were offered), between June and October 2016. Concentrations of blood BHB measured at the milking prior to and the day after milk spectral data collection were averaged and used for developing prediction models. The number of spectra records per cow ranged from 1 to 5.

The noise or non-informative regions of the spectra between 1,628 and 1,658 cm^{-1}, 3,105 cm^{-1} and 3,444 cm^{-1}, and 2,966 to 5,010 cm^{-1} were removed before the chemometric analysis. Spectra with a global Mahalanobis distance greater than 3 (N = 45) were considered outliers and eliminated. After outlier elimination, 1,910 spectra records and corresponding BHB measures, from 542 cows, were available for calibration. Spectra were transformed using extended multiplicative scatter correction and a 1st derivative calculated over a window of 5 points. Calibration models were developed by PLS regression with a 10-fold cross-validation, implemented in the R package PLS (Mevik and Wehrens, 2007) using two-thirds of the cows (corresponding to 1,297 spectra records) and validated on the remaining one-third. Cows in the calibration and validation set were randomly selected. The procedure used to create the subsets guaranteed that all the records from a cow were in either the calibration or the validation subset.

Preliminary statistics indicated that blood BHB concentrations were not normally distributed, with a higher proportion of low concentrations. Visual inspections of the data indicated that most low BHB concentrations were between 0.3 and 0.6 mmol/L. Prediction models were developed using the full calibration sets, or after randomly removing 25% and 50% of the data with low concentrations, following the approach used by Grelet *et al.* (2016). The values were then log-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 set was not modified.

The root mean squared error of prediction (**RMSEP**) in calibration and validation, the coefficient of determination between the predicted and measured concentrations in calibration (**R^2c**) and validation (**R^2v**), and the ratio of performance to deviation (**RPD**, i.e. the ratio of the SD of measured BHB concentrations to RMSEP) were calculated.

Results and discussion

The average blood BHB concentration was 0.8 ± 0.4 mmol/L, and ranged from 0.15 to 4.0 mmol/L. With the defined threshold of blood BHB ≥ 1.2 mmol/L, the prevalence of hyperketonemia during the first 5 wk of lactation was, on average, 10.4%.

Fitting statistics of the prediction model for log-transformed blood BHB concentrations are reported in Table 1. In calibration, the R^2c was 0.58 with a RMSEP = 0.14, whereas, in validation, the R^2v was 0.49 with a RMSEP = 0.16, which translated into an RPD

Table 1. Average fitting statistics (SD in parentheses) of quantitative predictions models for log-transformed blood BHB concentration obtained in calibration and validation[1].

Dataset[2]	N	#terms	RMSEP	R^2	RPD
Full					
Calibration	1,297	24	0.14	0.58	1.54
Validation	613	24	0.16	0.49	1.39
25% of low conc. removed					
Calibration	1,100	24	0.15	0.61	1.60
Validation	613	24	0.15	0.43	1.27
50% of low conc. removed					
Calibration	902	24	0.16	0.63	1.65
Validation	613	24	0.16	0.42	1.23

[1]N: number of records in the datasets; #terms: number of optimal partial least square components; RMSEP: root mean squared error of prediction; RPD = RMSEP/SD of measurements.
[2]Prediction models were developed using the full calibration set, or after randomly removing 25% and 50% of the data with BHB concentrations were between 0.3 and 0.6 mmol/L.

of 1.39. These results indicate that the models are not expected to provide accurate quantitative values at the individual cow level, but the moderate R^2v indicates that they could potentially be used to distinguish low and high BHB concentrations, or used to predict aggregate values (i.e., herd average) with reasonable accuracy. The prediction accuracy in our study is either similar to, or higher, than that reported in previous studies (Broutin, 2015; Belay *et al.*, 2017), with a comparable or lower prediction error. When part of the samples (25 and 50%) in the calibration set were excluded from the analysis to obtain a more balanced distribution of the BHB concentrations, the R^2c increased (by 3 to 5%) as the proportion of samples excluded increased, but this reduced the R^2v (by 6 to 7%). Hence, this approach is not expected to improve the predictive ability of models when they are applied at the population level.

The values of R^2v in this study are relatively low if compared with those achieved for prediction of milk ketone bodies (Grelet *et al.*, 2016), but we consider the results to be satisfactory as blood components were predicted from milk spectra. In addition, the dataset used in our study included samples from 2 farms and one season only, hence accuracy and robustness of prediction models can likely improve with additional calibration samples.

Conclusions

This study indicates that the prediction of blood BHB concentrations from milk spectra is possible and moderately accurate. Further data will likely improve the robustness and accuracy of prediction models. To evaluate the role of IR predictions as indicator traits of blood BHB concentrations in future selective breeding programs, genetic parameters of the IR predicted blood BHB need to be estimated.

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