



Beta-hydroxybutyrate and non-esterified fatty acids prediction using milk Fourier-transform infrared spectroscopy

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Negative energy balance during the transition period predisposes dairy cattle to numerous metabolic conditions. Fourier-transform infrared spectroscopy (FTIR) presents one potential data source for estimating metabolic parameters used for the detection of cows suffering from these disorders. We created prediction models for blood BHB and blood NEFA using milk FTIR and production information from 622 milk samples subjected to FTIR measurements. The resulting R^2 for prediction of blood BHB was $0.5627 \pm 0.2610(\text{SD})$ and R^2 for blood NEFA prediction was $0.5093 \pm 0.2473(\text{SD})$. Balanced accuracies for detection of blood BHB greater than or equal to 1.2 mmol/L and for blood NEFA greater than or equal to 0.7 mmol/L were 83% and 73% respectively. Additionally, we predicted metabolic disorders in cows by applying the predicted blood BHB, predicted blood NEFA, and milk fat to protein quotient to an external dataset.

Keywords: Prediction modeling, metabolic disorders.

Abstract

Negative energy balance (NEB) following parturition predisposes dairy cattle to numerous metabolic disorders. While many detection methods for metabolic disorders exist, they suffer from poor accuracy, high costs, or intensive labor requirements. Fourier-Transform Infrared spectroscopy (FTIR) of milk may improve both understanding and detection of metabolic disorders, including those not detected by hyperketonemia alone. This prediction tool has the potential to allow for accurate prediction of biomarkers associated with negative energy balance, beta-hydroxybutyrate (BHB), and non-esterified fatty acids (NEFA), using a routine milk sample. The ability to predict multiple biomarkers from a single sample would allow better characterization of metabolic disorders in dairy cows.

Not all dairy cows experiencing adverse negative energy balance have elevated blood BHBA, the most common on-farm metric for evaluation. Ospina *et al.*, 2013 reported that blood NEFA and blood BHB are not well correlated when measured on the same day. These affected cows are at risk of remaining undetected in an exclusively blood BHB focused testing strategy.

Introduction

We used FTIR, milk production data, and ElasticNet regression to create prediction models for both blood BHB and blood NEFA. Using a combination of these we predicted the metabolic status of cows <60 DIM for an external dataset.

Materials and methods

Nine German dairy farms contributed data from December 2014 to December 2016 for this study. Farms visits occurred as often as once weekly and at least once monthly. Cows were dried off seven weeks before the expected calving date. Days in milk (DIM) targeted for sampling were between 0 and 60 days. Milk samples were taken between 8:00-10:00 AM using proportional milk samplers. A 10ml aliquot of milk was sampled and preserved using 1-2ml of bronopol (2-Bromo-2-nitropropane1,3-diol). Samples were transported at 4°C to regional milk testing facilities within one day of collection and analyzed using a MilkoScan FT-6000 (FossAnalytical A/S, Hillerød, Denmark). Milk FTIR absorbance variables were recorded for 1,060 wavenumbers.

A total of 622 observations from 478 cows had blood BHBA, blood NEFA, and milk FTIR samples collected. Blood samples were obtained following milking from randomly selected cows including milk samples from the same day.

Statistical analysis, variable selection, and editing

All statistical analyses were performed using R software version 3.6.3 (R Core Team (2020)). FTIR wavenumber variables were removed if greater than 15% of observations were missing values for a specific wavenumber. Wavenumber variables for values 900 –1,060 were removed as these 237 observations did not have these values recorded in the dataset. Following the removal of all observations with a missing value (n=134), the dataset contained 622 observations of 910 variables.

The IR spectra were transformed using the second derivative. The transformed spectra were reduced to 212 wavelength variables known to be informative (Grelet *et al.*, 2016). Model input was DIM, lactation group, milk production, and the 212 wavenumbers. Blood BHBA and blood NEFA were log-transformed after visualization using a histogram.

ElasticNet regression and classification for blood BHB and blood NEFA

The ElasticNet (ENET) is a regression method that combines ridge regression and lasso regression to apply regularization aimed at shrinking model coefficients and to reduce coefficient variance (Hoerl and Kennard, 1970; James *et al.*, 2013; Tibshirani, 1996). ENET is fit to the data using two parameters λ and α , and 7-fold cross-validation.

ENET in a logistic regression setting was also used to predict the binary outcome for above or below blood BHB and blood NEFA threshold values. The threshold value for elevated blood BHB was set at > 1.2 mmol/L and > 0.7 mmol/L for blood NEFA. In addition to the preprocessing described above, training data were balanced using Synthetic Minority Oversampling. The fitting of the ElasticNet classification model followed the same procedure as for the regression models.

We evaluated our ENET regression models using an external dataset. The RMSE of predictions and Pearson's correlation coefficients between predicted and observed values were calculated. In addition to the prediction of metabolites independently, we predicted the overall metabolic status of the cow associated with the respective samples as either 'healthy' or risk for 'metabolic disorder'. Cows were identified as metabolically disordered if predicted blood BHB was greater than or equal to 1.2 mmol/L, blood NEFA greater than or equal to 0.7 mmol/L, or fat-protein-quotient (FPQ) greater than or equal to 1.4. Predicted metabolic status was then compared to true metabolic status as determined by measured blood BHB and blood NEFA resulting in a confusion matrix from which diagnostic test parameters were derived.

External validation and metabolic disorder prediction

Cows were sampled from 1 to 3 times. The median number of samples per farm was 55 (range: 2-180). A total of 152 cows were in their first lactation and 470 cows were in their second or greater lactation (median=3, range 1-10). A total of 563 Holstein and 59 Flekvieh samples were collected.

Results and discussion

Descriptive statistics

Cross-validated R² values for blood BHB 0.5627 ± 0.2610(SD) and RMSE of 0.3873 ± 0.1380(SD). The blood BHB classification had a balanced accuracy of 87% (80% – 91%), sensitivity of 90% (81% – 96%) and a specificity of 83% (80% – 86%). PPV and NPV were 43% (35% - 51%) and 98% (97% - 98%) respectively. Blood NEFA prediction RMSE was 0.4825 ± 0.9260(SD) and R² was 0.5093 ± 0.2473(SD). The balanced accuracy of blood NEFA predictions was 73% (68% – 79). These results are comparable to recent publications by Pralle *et al.*, 2018, who reported sensitivities of 76 to 81% and specificities of 71% to 81% for prediction of blood BHB levels greater than 1.2 mmol/L. The blood NEFA prediction results are similar to Luke *et al.*, 2019 and Tremblay *et al.*, 2019 who reported a sensitivity of 73%, and 77% respectively. These results show ENET is a good modeling algorithm for the prediction of blood BHB and blood NEFA.

ENET for prediction of blood BHB and blood NEFA

Validation of our ENET predictive model was performed using the Qcheck dataset with n=9660 observations to account for any overfitting bias. The resulting RMSE was 0.4018 (95% CI 0.3958 - 0.4082) for log-transformed blood BHB and 0.4043 (95% CI 0.3937 - 0.4159) for log-transformed blood NEFA prediction. Figures 1 and 2 display observed values compared to predicted values.

External validation and metabolic disorder prediction

True observed blood BHB and true blood NEFA had a Pearson's correlation coefficient of 0.31 (P>0.001) while predicted blood BHB and predicted blood NEFA had a correlation coefficient of 0.69 (P>0.001). This increased correlation between predicted values of NEFA and BHB may be due to our dataset size.

Prediction accuracy for metabolic disorder classification using predicted blood BHB, predicted blood NEFA, and milk FPQ was 94% (9081 / 9660). Sensitivity was 94% (3354 / 3542) and specificity was 94% (5727 / 6118). By using a combination of blood BHB, blood NEFA, and FPQ, this model enables the identification of cows suffering from hyperketonemia in addition to those with normal blood BHB levels suffering from the consequences of negative energy balance. By identifying these at-risk cows, we can direct necessary medical care to them before they lose massive amounts of body weight and develop more severe conditions resulting in death.

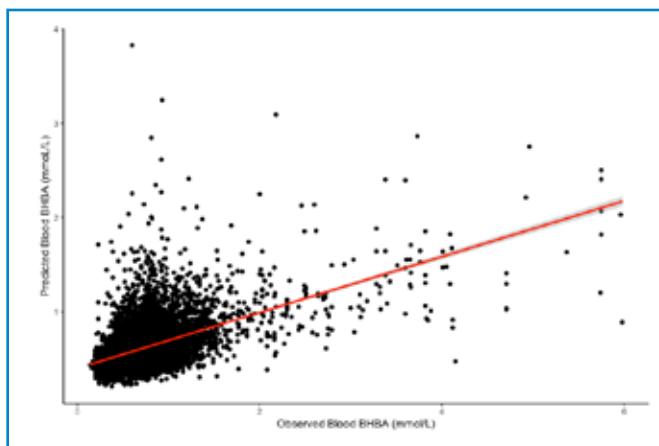


Figure 1. Observed versus predicted values of blood BHBA for ElasticNet regression ($n=9,660$) using milk Fourier-transformed infrared spectroscopy and production variables (milk yield, DIM, lactation group).

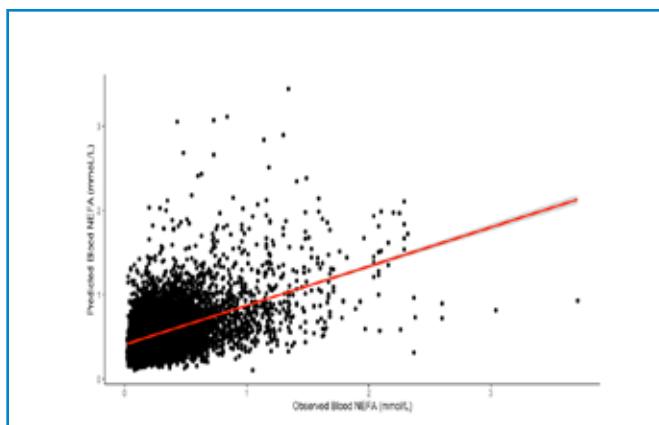


Figure 2. Observed versus predicted values of blood NEFA for ElasticNet regression ($n=9,660$) using milk Fourier-transformed infrared spectroscopy and production variables (milk yield, DIM, lactation group).

Conclusion

Metabolic disorders during the transition period remain a primary issue of concern for dairy cows. Milk FTIR measurement is one source of data for the detection of cows suffering from metabolic disorders with the benefits of obtaining multiple biomarkers from a single milk sample. Prediction of metabolic disorders using FTIR data can be accomplished by combining the predictions into a categorization into cows with and without metabolic disorders. This categorization results in rapid identification of these high-risk cows post-calving. Continued research into prediction modeling with regards to methods and input is warranted.

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Acknowledgments

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