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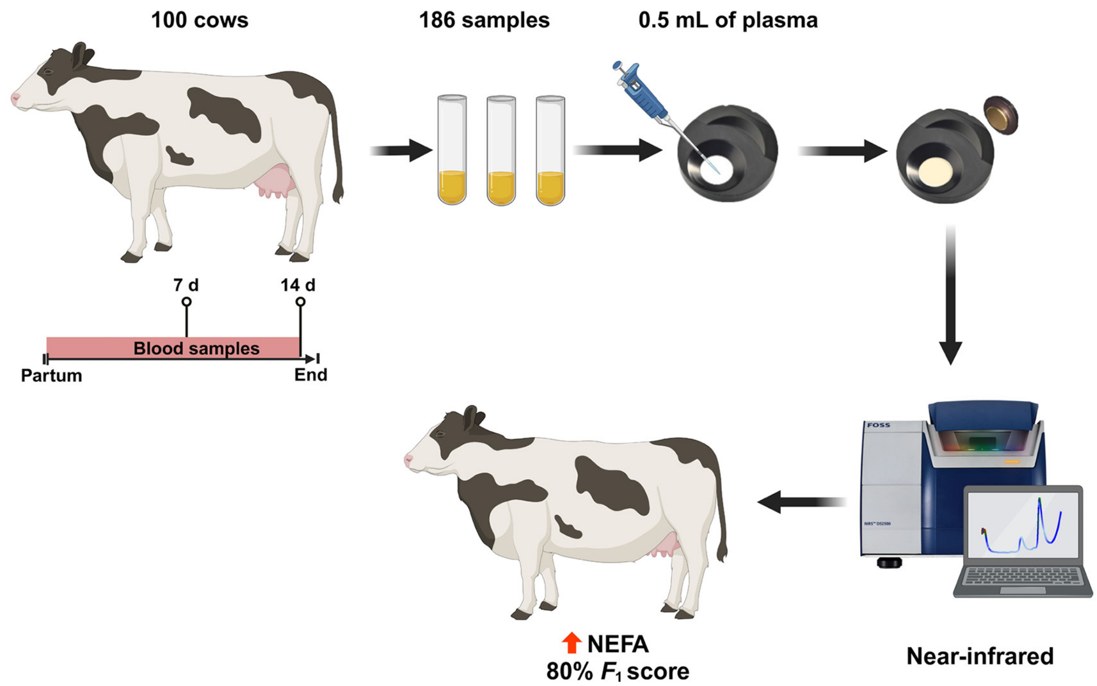
Short Communication
Health, Welfare, and Behavior

Near-infrared spectroscopy analysis of blood plasma for predicting nonesterified fatty acid concentrations in dairy cows

Guilherme L. Menezes,[1] Tiago Bresolin,[2] Rafael Ferreira,[1] Henry T. Holdorf,[1] Sebastian I. Arriola Apelo,[1] Heather M. White,[1] and Joao R. R. Dórea[1,3]*

Graphical Abstract



Summary

Dairy cows under negative energy balance mobilize body fat reserves. Plasma nonesterified fatty acid (NEFA) concentration is commonly used as a biomarker to indicate heightened illness risk. This study aimed to identify cows with NEFA levels greater than 0.60 mEq/L using near-infrared spectra analysis of plasma. Cows with NEFA ≥0.60 mEq/L were detected with a 79.8% F_1 score.

Highlights

- Spectral preprocessing techniques increase the F_1 score of the prediction results.
- Spectral analysis detects NEFA 0.60 mEq/L with 80.8% sensitivity.
- Spectral analysis detects NEFA 0.70 mEq/L with 80.8% sensitivity.
- Blood spectral data are associated with a negative energy balance of dairy cows.

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Abstract: During the transition period, dairy cows are often exposed to negative energy balance (NEB), leading to lipid mobilization from adipose tissue into nonesterified fatty acids (NEFA), a common indicator of heightened illness risk. This study aimed to use blood near-infrared (NIR) spectra data to classify NEB into high or low categories, based on early-lactation cow NEFA thresholds. We collected a total of 186 plasma samples from 100 Holstein cows. The samples were categorized into critical thresholds, based on previous literature, of ≥0.60 and ≥0.70 mEq/L for identifying high NEB. Spectral data were preprocessed before the development of the predictive modes, which included the implementation of multiplicative scatter correction, standard normal variate (SNV), and first and second derivatives. The classification was performed using partial least square discriminant analyses (PLS-DA), and predictive performance was assessed using leave-one-out cross-validation. Predictive quality for each class was evaluated through specificity, precision, sensitivity, and F_1 score. The study showed promising results, with the SNV technique achieving higher F_1 scores. The model found 72.7% specificity, 78.9% precision, 80.8% sensitivity, and 79.8% F_1 score to classify animals with NEFA levels of ≥0.60 mEq/L, and 82.1% specificity, 78.7% precision, 80.8% sensitivity, and 79.7% F_1 score to classify animals with NEFA levels ≥0.70 mEq/L. These results indicate that NIR spectroscopy could serve as a tool for detecting cows under severe NEB, also showing potential for broader application across the entire transition period, as the spectral signal carried relevant information regarding cow metabolism. Furthermore, the combination of predictors derived from plasma spectra and other cow-level information can lead to more accurate disease alerts, given their relationship with the NEB.

Most of the health problems in dairy cows occur during the transition period, which is a time marked by significant changes in their body functions, metabolism, and inflammation responses (Horst et al., 2021). It is commonplace during this period, often due to an energy deficit, for fat mobilization to occur, which leads to the production of nonesterified fatty acids (**NEFA**; LeBlanc et al., 2005; Fiore et al., 2020; Lisuzzo et al., 2022). Higher concentrations of this metabolite during the pre- and postpartum phases are associated with various diseases such as hyperketonemia, metritis, abomasal displacement, and mastitis. These conditions could, consequently, escalate involuntary culling rates within the farm (LeBlanc et al., 2005; Nicola et al., 2022) and reduce herd productivity.

To avoid this problem, critical thresholds have been proposed using NEFA concentrations as an indicator of negative energy balance (**NEB**). During the postpartum period, concentrations exceeding 0.6 mEq/L have been associated with an increased risk of developing clinical ketosis and subsequent culling (Seifi et al., 2011). Similarly, Ospina et al. (2010) identified NEFA concentrations above 0.6 and 0.7 mEq/L postpartum as a critical threshold due to the heightened risk of developing clinical ketosis and displaced abomasum, respectively. Additionally, high NEFA levels are also often related to fatty acid mobilization and hepatic lipidosis (Fiore et al., 2018). Despite their usefulness as health indicators, classical NEFA analyses using blood samples conducted in laboratories can be time consuming and expensive. This can limit their applicability on commercial farms (Benedet et al., 2019). In this context, several studies have been performed using mid-infrared spectra of milk (Grelet et al., 2019; Aernouts et al., 2020) because of its ease of collection, cost effectiveness, and scalability. Although this method presents substantial advantages in animal health monitoring, it is limited to lactating cows.

Thresholds for NEFA concentration before calving offer a chance to examine these markers before lactation (LeBlanc et al., 2005). Such an early examination can support more accurate decisions with fewer production losses (Macmillan et al., 2020; Nicola et al., 2022). Therefore, monitoring NEFA concentrations both before and after calving could be essential for evaluating disease risk and enhancing cow health during the transition period. To monitor NEFA concentration during the transition period without extensive resource allocation toward conventional laboratory analyses, the use of NIR spectra of plasma could be a useful tool for identifying NEFA concentrations. These samples are easy to collect during routine procedures, inexpensive, and could be used to monitor both nonlactating and lactating cows throughout the transition period. In this study, the aim was to investigate the utilization of NIR spectroscopy with plasma spectra from early-lactation cows to classify NEFA levels as high or low based on defined alarm thresholds. This approach holds promise as a potential tool for monitoring NEFA throughout the entire transition period.

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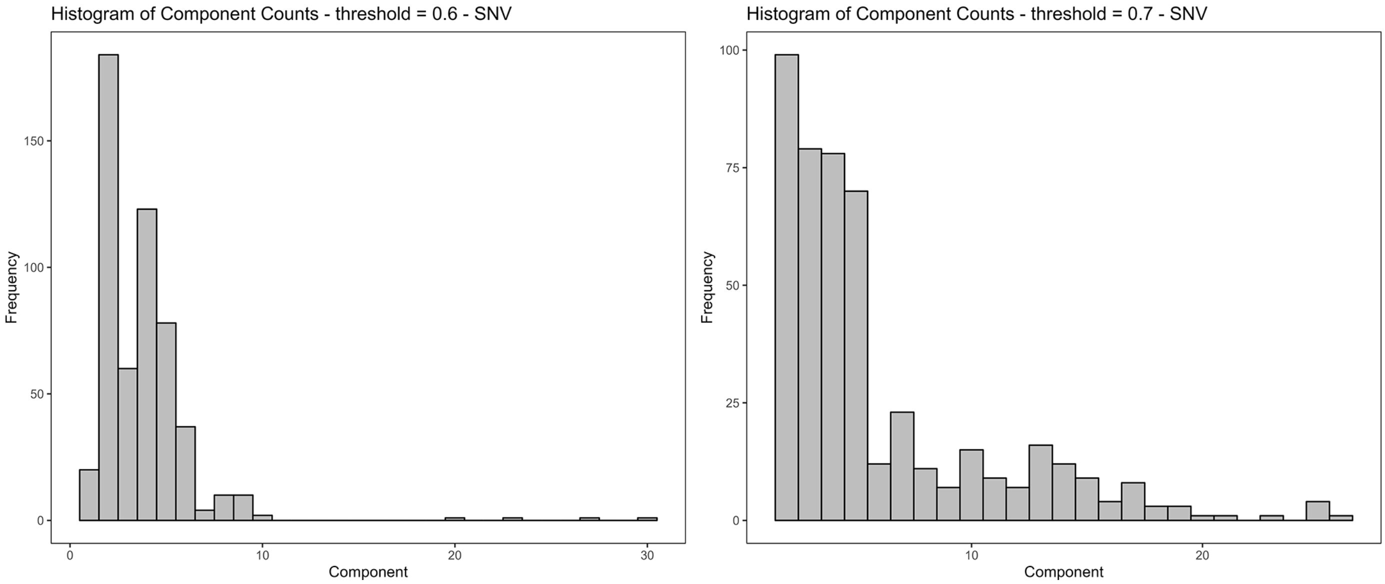

Figure 1. Number of components used in partial least square discriminant analysis for classification using the leave-one-out cross-validation approach. SNV = standard normal variate.

All experimental procedures in this study received ethical approval from the Institutional Animal Care and Use Committee at the University of Wisconsin–Madison. A total of 186 samples were collected from 100 cows during the early-lactation period (7 and 14 DIM) at the Emmons Blaine Dairy Cattle Research Center (Arlington, WI). The cows, diets, and sampling were described in detail previously (Holdorf et al., 2023). The cows were fed a TMR once daily at 0700 h, and the feed bunk was adjusted as needed to ensure it remained full. Before morning feeding, approximately 6-mL blood samples were collected from a tail vessel in tubes containing sodium fluoride and potassium oxalate, centrifuged at 2,000 × g for 15 min at 4°C, and isolated plasma was stored at −20°C until analysis.

Quantification of NEFA was performed using Catachem reagents (V514–0B) with a modified protocol utilizing a NEFA standard (276–76491) from Fujifilm Wako Chemicals USA based on the methodology described by Martin et al. (2021). The protocol used 12 μL of fluid plasma per replicate. For NIR analysis, 0.5 mL of fluid plasma from the same sample used for NEFA quantification was dispensed into a slurry cup equipped with a 0.5-mm gold reflector. All samples were subjected to analysis using FOSS DS2500 spectrometers (Foss, Hillerod, Denmark), resulting in the generation of a single spectrum for each plasma sample. The Foss NIR spectrum comprises 4,200 data points, which reflect the absorption of infrared light across a wavelength range of 400 to 2,500 nm within the plasma sample.

The raw dataset was preprocessed using scatter-correlation methods and spectral derivatives. Scatter-correlation analysis involved the use of multiplicative scatter correction (**MSC**) and standard normal variate (**SNV**) techniques. Spectral derivatives were also analyzed, specifically the first and second derivatives. All transformations were carried out using the software Spectragryph 1.2. All datasets, including the raw data, were used for predictions, and the preprocessing method that yielded the highest F_1 score was chosen to classify NEFA at alarm levels.

A partial least square discriminant analysis (**PLS-DA**) was used to classify NEFA concentrations in plasma samples categorized based on critical thresholds (≥0.60 and ≥0.70 mEq/L) to identify cows with high NEFA levels, as described by Ospina et al. (2010). The PLS-DA algorithm extracts latent variables, known as factors, which capture the underlying structure and linear relationship between the predictors and the response variable (e.g., NEFA). To address the imbalanced data, which included 36 high and 150 low NEFA samples for the ≥0.60 mEq/L threshold, and 26 high and 160 low NEFA samples for the ≥0.70 mEq/L, a random selection process was employed to choose 20% of the low NEFA concentration samples for training and validation of the NEFA classification. The down-sampling process, which is a common technique to balance datasets (Japkowicz, 2000; Mountassir et al., 2012), was repeated 10 times.

A leave-one-out cross-validation (**LOOCV**) approach was used for data analysis. In this approach, all samples from a specific cow were set aside for validation, whereas the remaining samples were used to develop the PLS-DA classification model. The number of factors used to build the model using remaining samples was determined through a 10-fold cross-validation, resulting in the selection of a classification model with high accuracy. During this process, a maximum of 30 components was defined. The cross-validation process yielded multiple iterations with varying numbers of components, as depicted in Figure 1. The variable importance was determined using a LOOCV approach (Figure 2). The numbers of components and variable importance represented in Figures 1 and 2 indicate the results obtained from the dataset using the chosen preprocessing technique with lower F_1 score.

To evaluate the predictive quality of PLS-DA for classification, specificity [1], precision [2], sensitivity [3], and F_1 score [4] were calculated after the 10 rounds, using the following equations:

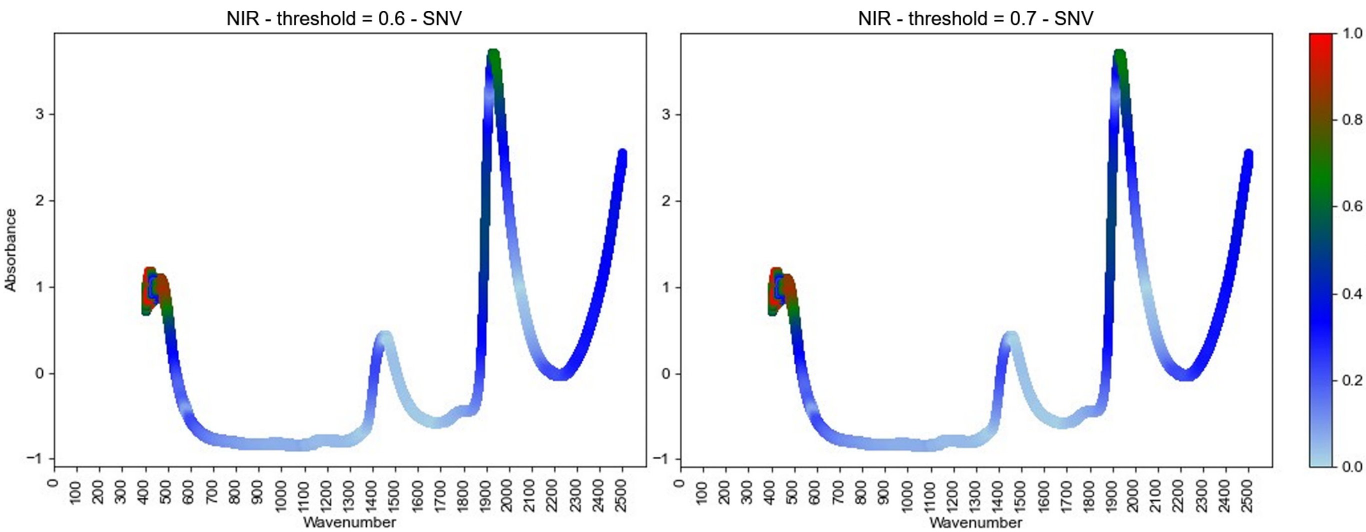

Figure 2. Absorbance and wavenumbers (nm) were obtained from plasma samples using near-infrared (NIR) spectroscopy (n = 186). The variable importance (VI) is represented on a color scale ranging from light (blue), indicating lower importance, to dark (red), indicating higher importance. The pair of graphs illustrates the VI based on the standard normal variate (SNV). The graphs in the first and second columns use ≥0.60 and 0.70 mEq/L as thresholds, respectively.

$$\text{specificity} = \frac{\text{TN}}{\text{TN} + \text{FP}}, \quad [1]$$

$$\text{precision} = \frac{\text{TP}}{\text{TP} + \text{FP}}, \quad [2]$$

$$\text{sensitivity} = \frac{\text{TP}}{\text{TP} + \text{FN}}, \quad [3]$$

$$F_1 = \frac{2\text{TP}}{2\text{TP} + \text{FP} + \text{FN}}, \quad [4]$$

where TP = true positives; TN = true negatives; FP = false positives; and FN = false negatives. All analyses were conducted using the statistical software R (R Core Team, 2019).

Our study aimed to explore the usefulness of NIR spectra as an affordable and cost-effective method for categorizing cows with elevated critical NEFA thresholds. This method could serve as a simple diagnostic tool for monitoring health status and the results indicated promising classification results. All preprocessing methods exhibited similar results. However, the preprocessing technique using SNV resulted in a slight increase in sensitivity and F_1 score of the prediction results (Table 1). This improvement in sensitivity and F_1 score may be attributed to noise mitigation. With SNV, each spectrum is individually adjusted to have a mean of 0 and a standard deviation of 1, as described by Rinnan et al. (2009). The normalization process helps reduce the influence of variations in signal intensity among spectra, which may result in improved predictive performance.

When the threshold for high NEFA was set at 0.6 mEq/L, the model demonstrated specificity, precision, sensitivity, and F_1 score values of 72.7%, 78.9%, 80.8%, and 79.8%, respectively. When the NEFA levels were set at 0.7 mEq/L, the specificity, precision, sensitivity, and F_1 score were 82.1%, 78.7%, 80.8%, and 79.7%, respectively. Aernouts et al. (2020) reported similar results for specificity (80.0%) and sensitivity (83.1%) using milk mid-infrared

Table 1. Specificity, precision, sensitivity, and F_1 scores of plasmas near-infrared spectroscopy in predicting nonesterified fatty acid (NEFA) concentrations[1]

Data	Threshold (mEq/L)	Specificity (%)	Precision (%)	Sensitivity (%)	F_1 score (%)
SNV	0.6	72.7	78.9	80.8	79.8
	0.7	82.1	78.7	80.8	79.7
Raw data	0.6	74.5	78.8	75.6	77.2
	0.7	84.0	79.7	76.9	78.3
First derivative	0.6	74.4	79.5	77.7	78.6
	0.7	83.0	81.4	82.3	81.8
Second derivative	0.6	71.7	76.5	73.3	74.9
	0.7	81.2	76.2	73.9	75.0
MSC	0.6	72.7	78.8	80.5	79.7
	0.7	80.9	77.4	80.8	79.1

[1]High classifications were evaluated using NEFA thresholds of 0.6 or 0.7 mEq/L; SNV = standard normal variate; MSC = multiplicative scatter correction.

technology to classify NEFA levels >0.6 mmol/L. These findings demonstrate the possibility of accurately identifying animals with elevated NEFA levels through routine blood sample analysis.

Creating alerts based on health thresholds before clinical illness on dairy farms is crucial, primarily due to the significant costs associated with treatment and potential productivity losses. McArt et al. (2015) estimated that the average losses associated with hyperketonemia, displaced abomasum, and metritis amounted to $289, $707, and $396, respectively. This study also revealed that during early lactation, the incidence of hyperketonemia in dairy cows was 32%. Among cows with displaced abomasum, 88% of cases showed hyperketonemia, and among cows with metritis, 70% of cases presented hyperketonemia. In the present study, other factors not considered in the model, such as body condition, changes in body composition score during the transition period, number of lactations, and milk production (Rathbun et al., 2017), could improve the predictive accuracy in classifying alarm levels for NEFA samples ≥0.60 mEq/L. In this context, the use of NIR spectroscopy can serve as a potential tool to identify animals at high risk of developing diseases, which can reduce financial losses in dairy farms.

The present results suggest a potential association between certain components found in plasma spectra and NEB. This may allow direct prediction of diseases such as hyperketonemia, metritis, abomasal displacement, and mastitis. Recently, this indirect prediction approach has been used to address challenges in predicting individual DMI at the herd level, using mid-infrared data obtained from raw milk. In such approach, the spectral data are used as a predictor of phenotypes not necessarily present in the sample (e.g., milk fat), but indirectly related such as feed intake and milk spectra. Models incorporating spectra have shown improvements in predicting DMI, with R^2 values of 0.70 and 0.81 and root mean square errors of prediction of 2.15 and 1.52 kg/d (Dórea et al., 2018; Lahart et al., 2019).

The improvement observed in these cases may be attributed to the presence of specific milk compounds that are associated with the phenotype of interest, such as fatty acids, proteins, and lactose. These components have been associated with both the quantity and quality of ingested forage and the forage-to-concentrate ratio (Aguerre et al., 2011; Elgersma, 2015). The quality and quantity of forage, as well as the concentrate ratio, directly affect DMI, considering fill limitations (Mertens and Grant, 2020). Therefore, due to the relationship found between plasma spectra and NEB, the combination of predictors derived from plasma spectra with other cow-level information can potentially improve the predictive quality of severe cases of NEB, thereby enabling more accurate generation of disease alerts.

In future applications, as the blood centrifugation process for plasma separation can be used on farms, the monitoring process can be facilitated with the use of portable spectrometers. This technology has already been employed in various applications such as feed and nutrition evaluation, milk analysis, and the assessment of manure's chemical composition (Malley et al., 2005; Santos et al., 2013; Bell et al., 2018). Although the present study used a benchtop NIR instrument to extract the spectral signal, the features with the greatest variable importance are concentrated at approximately 600 nm of wavenumbers (Figure 2). This indicates possibilities for applications using portable spectrophotometers developed with silicon-based detectors (~400 to ~1,050 nm) that are very cost effective and can potentially be integrated into smartphones and cameras (Crocombe, 2018). However, the data presented in this study do not allow for the exploration of portable NIRS use, and suggest future studies for the application of portable NIRS.

Our findings suggest that NIR spectroscopy has the potential to be a valuable alternative for identifying cows in early lactation with high NEFA thresholds, offering significant advantages for dairy farms by efficiently identifying cows at risk of diseases such as hyperketonemia, lipomobilization, hepatic lipidosis, displaced abomasum, and metritis. This could lead to reduced financial losses and improved animal welfare. Future studies should evaluate the effectiveness of this technique in a large number of animals and herds, as well as in dry cows during the transition period. As suggested by Nicola et al. (2022), the threshold value of ≥0.26 could be used as a risk factor for the development of diseases in prepartum. This evaluation may also extend to other metabolites, such as BHB.

Although future datasets should include samples from cows fed varied diets, taken at different times of day, and during different physiological states and energy balances, it is crucial to note that factors influencing plasma NEFA concentrations would likely be mirrored in the spectral data. Using the same samples for NIR scanning and NEFA analysis to calibrate the models can increase the consistency of results across different scenarios. Additionally, given the relationship found between plasma spectra and NEB, the combination of predictors derived from plasma spectra with other cow-level information can potentially improve the predictive quality of severe cases of NEB, enabling more accurate generation of disease alerts.

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Notes

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