MID-INFRARED MILK TESTING FOR EVALUATION OF HEALTH STATUS IN DAIRY COWS

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INTRODUCTION

Feeding and managing dairy cows for optimal health, productivity, and fertility is critical for our dairy industry. One of the challenges we face is early detection and treatment of health issues, especially for our early lactation (i.e., fresh) cows. Milk composition is recognized as being a relatively good source of health and metabolic information given that there is interaction between circulating blood and milk synthesis outcome (Gengler et al., 2016).

Mid-infrared (MIR) spectroscopy is used for routine payment testing of milk and provides information about the major components (i.e., fat, protein, and lactose) found in milk. Traditionally, information such as the fat to protein ratio, has been used to make feeding and management decisions. There is tremendous interest in moving beyond using MIR spectroscopy of milk for only major components. It has the potential to identify minor (fine) milk components and health issues in dairy cows (De Marchi et al., 2014; Gengler et al., 2016; Barbano et al., 2018). Also, changes in milk detected through the MIR spectra allows blood biomarkers not found in milk to be predicted. Blood testing is invasive, requires labor, and is costly. Milk is readily available with harvest occurring two or three times daily in most herds. Research needs to focus on automated and cost-effective methods for biomarker quantification and use of MIR spectroscopy is one possible approach.

USE OF MILK MID-INFRARED SPECTRA TO DETECT HEALTH ISSUES IN DAIRY COWS

Metabolic Status

In early lactation, dairy cows experience negative energy balance that can affect their health, productivity, and fertility. Studies have been conducted to determine the feasibility of using MIR spectra of milk to indicate the energy status of cows by predicting energy intake and balance, dry matter intake, and residual feed intake (McParland et al., 2011, 2012, and 2014; Shetty et al., 2017). The models of McParland et al. (2011, 2012, and 2014) in general were moderately accurate during cross-validation but were not accurate during external validation because of the limited variability in the dataset population (McParland and Berry, 2016). Shetty et al. (2017) had better success in predicting dry matter intake with confinement housed dairy cows, especially when milk yield and body weight were modeled with the MIR spectral data. Interestingly, the milk fat region of the spectra was responsible for the majority of variation in dry matter intake.

A more viable option to understand the metabolic status of cows, especially during early lactation, is the use of biomarkers related to negative energy balance and ketosis. Many biomarkers require a sample to be collected invasively (e.g. drawing blood) and are expensive and time consuming to analyze in a laboratory. Cow-side tests for biomarkers in urine, milk, or blood are available, but can be labor intensive for dairy producers. The use of MIR spectrometry may allow some biomarkers to be measured easily, quickly, and cost effectively. Milk beta-hydroxybutryate (BHB) and milk acetone are biomarkers that have been most extensively researched and implemented to date.

Hyperketonemia (i.e., ketosis) is determined by measuring blood BHB. Given the correlation between blood BHB and milk BHB, researchers (de Roos et al., 2007) developed a prediction model for milk BHB and ketosis monitoring. Although the relationship between the blood-based method and the MIR predicted method for BHB was moderate ($r = 0.79$), they suggest the MIR prediction of milk BHB could be part of a useful screen tool for subclinical ketosis. The MIR predictions of milk BHB and acetone were better (80% sensitivity) at predicting hyperketonemia than the traditionally used milk fat to protein ratio (66% sensitivity; van Knegsel et al., 2010). The use of a milk BHB threshold of ≥ 0.20 mmol/L had excellent accuracy to detect hyperketonemia in dairy cows and its use was proposed in herd-level surveillance programs (Denis-Robichaud et al., 2014).

Santschi et al. (2016) found that testing milk for BHB with models developed by de Roos et al. (2007) and Denis-Robichaud et al. (2014) was a fast and inexpensive approach to determining herd-level prevalence and that prevalence was affected by parity of the cow and season of calving. They noted that a major limitation of the testing was the frequency of sampling with monthly DHI milk samples. The use of the de Roos et al. (2007) milk BHB and acetone models was found to be not suitable for individual detection of cows with ketosis due to the length of the test-day interval and the poor predictive performance (van der Drift et al., 2012). Inclusion of cow test-day information improved marginally the model performance (van der Drift et al., 2012). Similarly, Chandler et al. (2018) found that including cow test-day milk and performance variables in logistic and multiple linear regression models improved the ability to predict hyperketonemia at a herd-level. Grelet et al. (2016) took a novel approach to modeling milk BHB and acetone by using cows of different breeds, countries, and management systems along with milk spectral data from different analytical equipment. They found R^2 of cross-validation of 0.71 for BHB and 0.73 for acetone which is considered moderately accurate. Thus, it appears that milk BHB can be determined by MIR analysis as part of a Dairy Herd Improvement testing program thereby providing a fast and inexpensive alternative to blood BHB analysis.

The possibility of using MIR spectra from milk to predict blood biomarkers (i.e., metabolites and hormones) related to metabolic status is gaining interest. This is a rather novel approach as the concentration of a biomarker in a fluid (i.e., blood) is determined by the spectroscopic analysis of another fluid (i.e., milk) that may not even contain the biomarker of interest. Pralle et al. (2018) predicted blood BHB using milk MIR spectra,

milk composition, and producer-reported variables. The models were very good (0.83 ≤ AUC ≤ 0.90) but not excellent at determining hyperketonemia. The researchers noted that none of the models achieved the sensitivity and specificity of cow-side blood tests that are available currently. Before the Pralle et al. (2018) study, the prediction of blood BHB in dairy cows from milk MIR spectra was poor with several models evaluated and all yielding R^2 in the range 0.21 to 0.38 on cross-validation (Belay et al., 2017).

Grelet et al. (2018) used MIR spectra from milk to predict blood components such as glucose, insulin-like growth factor 1 (IGF-1), nonesterified fatty acids (NEFA), and BHB. Unfortunately, the quantitative models were weak for glucose, IGF-1, NEFA, and BHB with a R^2 of cross-validation of 0.44, 0.61, 0.39, and 0.70, respectively. Therefore, quantitative values could not be predicted accurately. However, Grelet et al. (2018) suggested that the models allowed for screening of individual milk samples for high or low values that represented a healthy, a moderately impacted, and an imbalanced metabolic status. This approach was more promising with discriminant models correctly identifying up to 74% of the samples. Luke et al. (2018) also found it difficult to use milk MIR analysis to predict biomarkers of metabolic health and nutritional status of early lactation cows quantitatively. They found a good estimation of blood urea nitrogen (R^2 = 0.89), but moderate estimations of serum NEFA (R^2 = 0.46) and BHB (R^2 = 0.49), and poor estimations of serum albumin (R^2 = 0.26), globulin (R^2 = 0.20), calcium (R^2 = 0.18), and magnesium (R^2 = 0.17). Interestingly, the researchers noted the importance of including data from as many herds as possible in the calibration data set to improve performance with the validation data sets.

We (Barbano et al., 2015) developed a model that predicted blood NEFA directly from the milk MIR spectra. This was a unique approach since it was not by calculation from milk fatty acid data as others have proposed with gas chromatography (Jorjong et al., 2014). The milk-predicted blood NEFA model worked well for Holstein cows during the first 3 wk of lactation. This model is being used at Miner Institute to evaluate fresh cows at a frequency of 1 milking/d. Figure 1A shows as expected the decrease in NEFA concentration as days in milk (DIM) increase and the difference between primiparous and multiparous cows. Milk-predicted blood NEFA concentration is monitored over time (Figure 1B) and can be used to make nutritional and management decisions. The milk-predicted blood NEFA model is being applied to milk collected inline from pens of fresh cows across the US and analyzed at Miner Institute. This information is helping nutritionists identify nutritional and management opportunities for their dairy producers. We find that monitoring groups of milk fatty acids determined by MIR analysis (Wojciechowski and Barbano, 2016; Woolpert et al., 2016; Figure 2), such as de novo fatty acids and preformed fatty acids, is beneficial for individual fresh cows (Barbano et al., 2018) or pens of fresh cows. These metrics are related to body weight change and health of the cows.

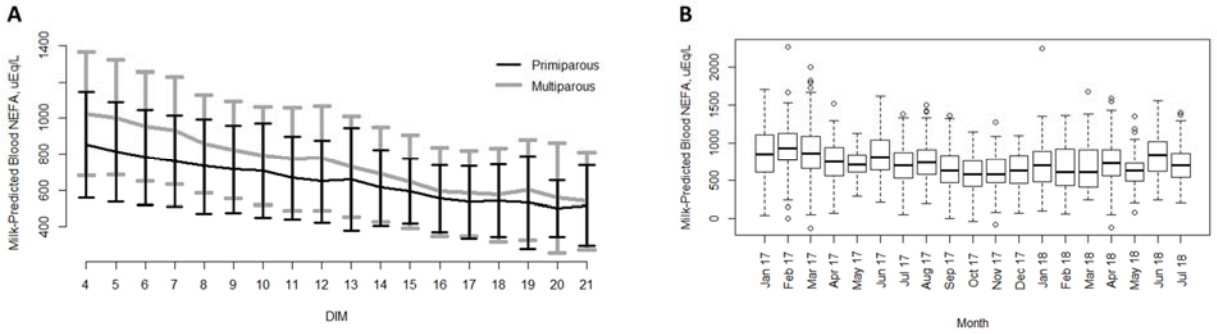

Figure 1. Concentration of milk-predicted blood nonesterified fatty acids (NEFA) for Holstein cows fed a total mixed ration during the first 21 days in milk (DIM) summarized by DIM and parity (A) or monthly average (B).

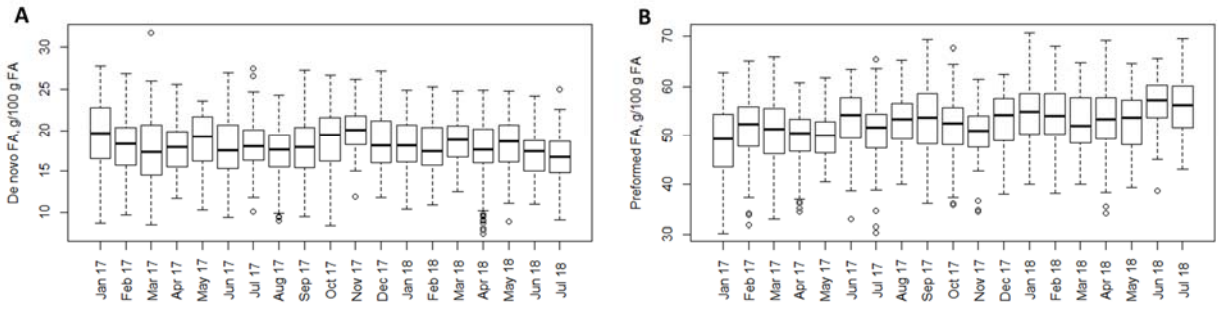

Figure 2. Concentrations of de novo fatty acids (A) and preformed fatty acids (B) for Holstein cows fed a total mixed ration during the first 21 days in milk.

One area in which milk composition is potentially useful is in providing warning of impending health events before clinical signs are visible. We (Pape et al., 2018) examined the relationship between changes in milk composition based on MIR analysis and the onset of ketosis or displaced abomasum (DA) in fresh cows. The approach used was to test the extent to which machine learning models could differentiate between milk samples from cows that went on to experience either ketosis or a DA and milk samples from cows that did not at approximately the same date and DIM (Figures 3 and 4). Milk-predicted blood NEFA, preformed fatty acids, and the fat/protein ratio were all elevated leading up to DA and ketosis, while de novo fatty acids was depressed. For both DA and ketosis, milk-estimated blood NEFA, de novo fatty acids, preformed fatty acids, and the fat/protein ratio all provided strong predictive power. Milk BHB did not. Our models yielded an area under the curve of ~0.8 from 5 to 0 d in advance of the event, with predictive power generally increasing up to an area under the curve of ~0.89 on the day of the health event. Overall, this suggests that there is a great deal of potential for improving early warning of health events like ketosis and DA and enabling better preventative treatment. The next step is to implement an early warning system in real-time. So far, we have developed a pilot version of such a system for the Miner Institute herd, providing general-purpose alerts for health issues in fresh cows to the farm staff. We are evaluating its usefulness currently.

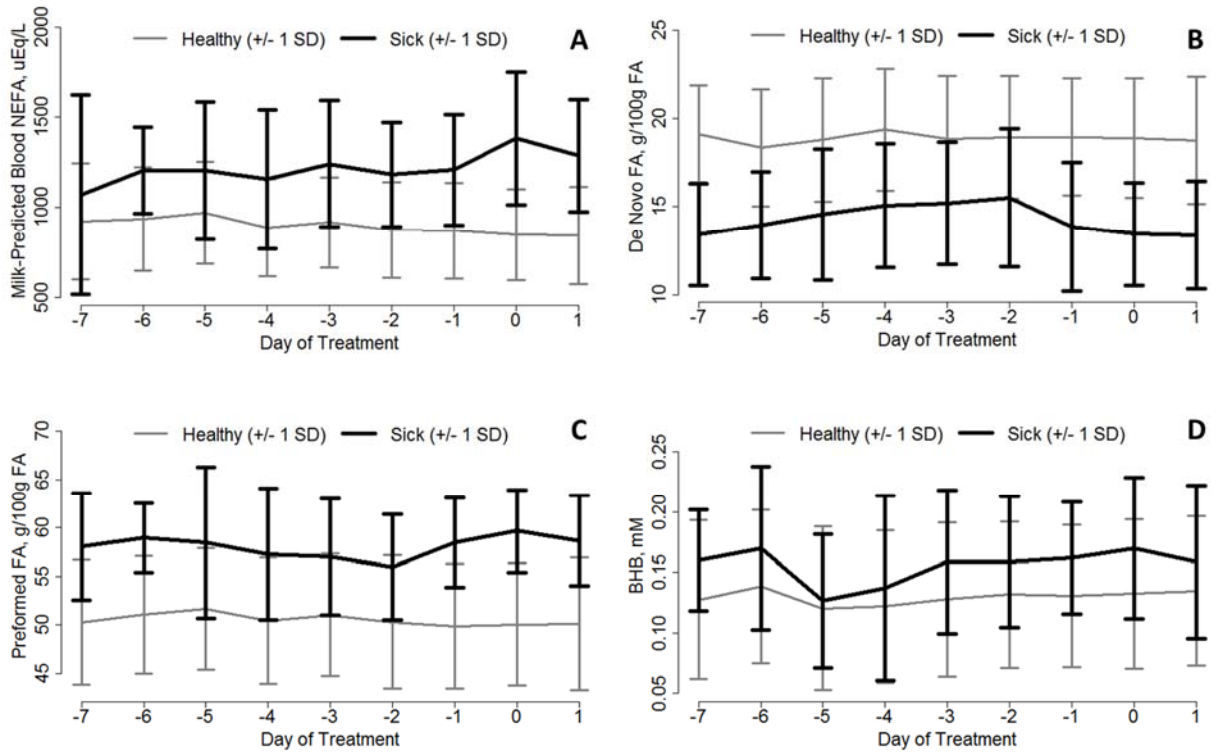

Figure 3. Changes in milk-predicted blood nonesterified fatty acids (NEFA; A), milk de novo fatty acids (FA; B), milk preformed FA (C), and milk BHB (D) for lactating cows that had ketosis or were healthy and matched by date and days in milk to cows with ketosis.

Immunological Biomarkers and Inflammation

German researchers (Zoldan et al., 2017) used healthy and diseased (e.g mastitis or systemic) Holstein cows to identify and validate immunogenic biomarkers in milk that indicated inflammatory diseases outside of the mammary gland. They demonstrated with receiver operator characteristic analysis (with a 94% specificity) that haptoglobin (82% sensitivity), secretory component (59% sensitivity), lactoferrin (55% sensitivity), and vascular endothelial growth factor (67% sensitivity) showed the highest discriminatory capability for healthy and diseased cows. Haptoglobin was the best single-use biomarker based on multinomial logistic regression. This is not surprising as acute phase proteins are generally considered appropriate biomarkers for inflammation. In milk, haptoglobin, serum amyloid A, and lactoferrin are often identified as biomarkers for mastitis.

We have evaluated several regression models to predict inflammation based on blood acute phase protein from milk MIR spectra from dairy cows. We matched serum haptoglobin and serum amyloid A data with milk composition and spectral data for 420 Holstein cows that were either healthy, clinically ill, or possibly experiencing a subclinical health issue. The coefficient of determination from cross-validation of the models was poor for serum haptoglobin (<0.4) and serum amyloid A (<0.3). Some of the issue with predicting blood acute phase proteins from milk are related to a lack of validated methods

that accurately measure the biomarkers of interest. Given that a dairy producer is probably not interested in the actual value of acute phase proteins, but is interested to identify if a cow is has having an inflammatory response or not, we used classification models to categorize cows into low (10th, 25th, and 33rd) and high percentiles (90th, 75th, and 66th) for each acute phase protein. The receiver operating characteristic area under the curve for the different models ranged from 0.81 to 0.90 for serum haptoglobin and 0.85 to 0.95 for serum amyloid A. Using another approach, the cows were classified into low and high serum haptoglobin categories based on a percentile threshold. This approach resulted in receiver operator characteristic area under the curve values between 0.7 and 0.9 when thresholds of 50 to 90 percentile were used. These models indicate to us that cows that are experiencing systemic inflammation can be identified through milk sample analysis.

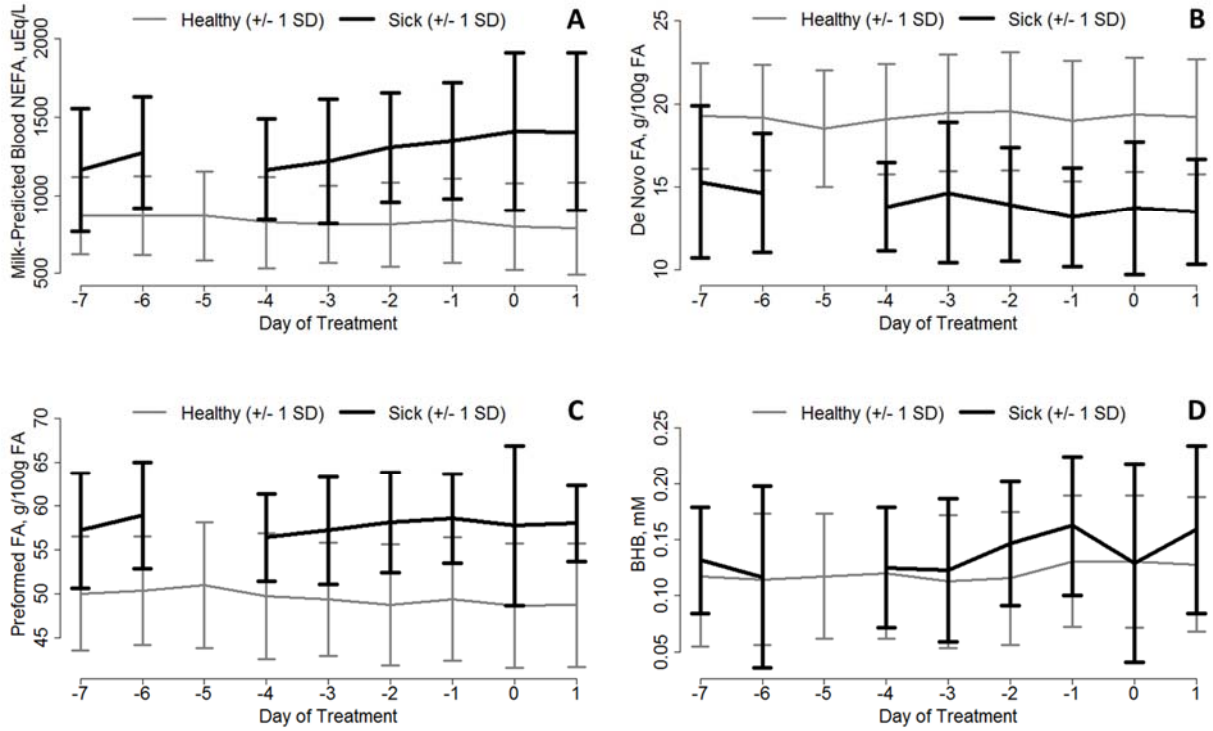

Figure 4. Changes in milk-predicted blood nonesterified fatty acids (NEFA; A), milk de novo fatty acids (FA; B), milk preformed FA (C), and milk BHB (D) for cows that had displaced abomasum or were healthy and matched by date and days in milk to cows with a displaced abomasum.

Subacute Ruminal Acidosis (SARA)

The ratio of milk fat to protein was found to not be specific enough to detect low ruminal pH values in early and mid-lactation dairy cows (Guegan et al., 2015). This is not surprising given that 1) ruminal samples were collected by an oro-ruminal probe method that can have saliva contamination, and 2) the relationship between SARA and milk fat content is inconsistent in several studies. Many factors can affect fat content such as

breed, season, and days in milk. Interestingly, the daily ruminal pH mean and variation have been shown to be related to specific milk fatty acid changes (measured by gas chromatography) in Holstein-Friesian cows (Colman et al., 2012). The susceptibility of dairy cows to SARA was shown to be reflected in milk fatty acid proportions measured by gas chromatography with C18:1 trans-10 as primary and C15:0 and C18:1 trans 11 as secondary indicators (Jing et al., 2018).

In general, a decrease in ruminal pH will result in changes in ruminal biohydrogenation, C18 biohydrogenation intermediates, and microbial populations (source of odd and branched chain fatty acids) that alter the milk fatty acid profile. Thus, Giger-Reverdin et al. (2018) viewed milk fatty acid composition as a good candidate to detect SARA in dairy goats. They used two methods to determine milk fatty acid composition: gas chromatography (expensive and time-consuming method) and MIR spectroscopy analysis (inexpensive and quick method). An index of short and medium chain fatty acids (≤ C13) to long chain fatty acids based on gas chromatography was positively related ($r = 0.60$) to ruminal pH. However, the index calculated from MIR analysis was not correlated as the MIR method overestimated the short and medium chain fatty acids and underestimated the long chain fatty acids. They concluded that MIR analysis was not a viable method for prediction of SARA until the inaccuracy in the prediction of fatty acids is addressed.

Recently, Luke et al. (2018) suggested that a number of fatty acids have been proposed as potential biomarkers of SARA and that MIR analysis can predict some fatty acids with good accuracy so MIR spectra may be able to be used to predict ruminal pH and SARA. They evaluated several pH metrics: mean pH, time below pH 6, area under the pH curve between milkings and pH at the time of milk sample collection along with milk spectra from ruminally cannulated dairy cows. The partial least squares models to predict pH metrics from milk spectra had poor to moderate accuracy with R^2 values between 0.22 and 0.59. Discriminant analysis models categorized cows as either having or not having SARA with a sensitivity of 81% and a specificity of 72%. The researchers concluded that the MIR analysis of milk shows promise as a useful tool for monitoring ruminal pH in lactating dairy cows.

Mastitis

It is well-documented (review by Brandt et al., 2010) that milk composition changes with subclinical and clinical mastitis. Laine et al. (2014) suggested that use of MIR analysis of milk might provide indicators of mammary health that could allow for earlier detection of mastitis and identification of cows with chronic infections while being quick and cost-effective. Traditionally, somatic cell count determined by flow cytometry has been used to monitor mammary health. Laine et al. (2014) found that milk composition described by MIR predicted components or the MIR spectrum was different before and during mastitis. They identified lactoferrin, urea, sodium, and titratable acidity as early indicators of mastitis. However, they did not investigate the sensitivity and specificity of those indicators to predict mastitis.

Lameness

Early detection and treatment of lameness is important to support animal welfare. Lameness is associated with physiological changes such as inflammation and modified feeding behavior that potentially alter the milk composition. Mineur et al. (2017) found that categorizing all types of lameness together was not useful in predicting lameness from milk MIR spectra. The sensitivity and specificity were generally in the 60 to 70% range indicating too many cows were not being identified as lame. However, sensitivity and specificity increased generally to the 80 to 90% range when lameness issues like heelhorn erosion or whiteline disease were grouped separately from other types of lameness and modeled. The researchers indicated that future work requires the use of data from varied cows and herds and validation steps before implementing this technology on a large scale.

CHALLENGES WITH MODEL DEVELOPMENT AND IMPLEMENTATION

The use of milk MIR spectra to detect health issues in dairy cows is promising. However, there are some challenges to wider implementation. Currently, application of MIR spectra to detect health issues is limited primarily to milk BHB for the determination of prevalence of hyperketonemia at the herd-level. Typically, the testing is conducted as part of the monthly DHI milk-testing program. The frequency of testing needs to be at a much higher frequency for the detection and treatment of individual cows (Barbano et al., 2018). In the future, development of on-farm MIR milk analyzers in needed to allow high frequency sampling and the generation of real-time actionable alerts.

The accuracy of a prediction model based on milk MIR spectra depends on the precision of measurement of the validated method of the biomarker of interest. This may be a larger issue for blood-based biomarkers compared with traditional milk components. Laboratories analyzing biomarkers need to have methods validated and ideally participate in an external quality assurance program (i.e., interlaboratory proficiency tests) to justify the effort in developing milk prediction models for commercial use. Model development is not about the quantity of milk spectra one uses, but is about the quality of milk spectra and balancing the variation of the biomarker of interest and designing the variation into the background chemistry of the modeling dataset.

Models will need to be validated and then implemented by the milk analyzer equipment manufacturers. Implementation may be very time consuming. Calibration samples may be needed by the laboratories. Laboratories will need to determine how to process and report the information while dairy producers and their consultants will need to determine how to use/interpret the information.

SUMMARY

Mid-infrared spectroscopy of milk is a tool to predict health issues in dairy cows. It has the advantage over other methodologies in that it is noninvasive, it is lower-cost, and the sample (i.e., milk) is available multiple times per day. Several studies have

demonstrated the usefulness of milk BHB and acetone in predicting herd-level hyperketonemia (i.e., ketosis). Other studies have identified its use for the prediction of energy balance and metabolic status, inflammation, and digestive issues. The real advantage of the tool will be realized when it can be used to provide real-time information to dairy producers regarding the health and physiological status of their cows.

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