Development and use of Mid Infrared Spectra to measure milk fatty acid parameters and estimated blood NEFA for farm management

D.M. Barbano[1], H.M. Dann[2], C. Melilli[1] and R.J. Grant[2]

[1]Department of Food Science, Cornell University, Ithaca, NY
[2]William H. Miner Agricultural Research Institute, Chazy, NY

Summary

Partial least square (**PLS**) models were developed from Fourier transform mid-infrared (MIR) spectra, externally validated, and are being used commercially in the US for direct measurement of:

- Froups of milk fatty acids [i.e., de novo (**DN**), mixed origin (**MO**), and preformed fatty (**PF**) acids]
- Fatty acid (**FA**) chain length (expressed as carbon number).
- FA unsaturation (expressed as double bonds per FA).
- Estimated blood non-esterified FA (**NEFA**).

Six laboratories in different regions of the US are routinely using the models for bulk tank bovine milk analysis simultaneously with payment testing for individual farms on almost every milk pick up basis. Two research laboratories are testing both bulk tank and individual cow milk samples, while one is also testing sheep and goat milk. There is a high correlation in bulk tank milk of DN (C4 to C15) FA concentration (g/100 g milk) with increased bulk tank milk fat and milk protein percentage. The DN FA are made in the mammary cells from acetate and butyrate produced by the microbial fermentation of carbohydrates in the rumen. The changes in concentration of DN FA in milk reflect efficiency of rumen fermentation and the microbial biomass load (i.e., essential amino acid production) in the rumen. Seasonal variation in bulk tank milk fat and protein content are highly correlated with seasonal variation in milk DN FA. As milk FA chain length and double bonds per FA increase, milk fat decreases, and DN and MO FA synthesis and output per cow per day decreases. Farms with high bulk tank milk double bonds per FA, where the average days in milk of the herd is >120 d, have a much higher incidence of trans FA induced milk fat depression. These FA metrics in combination with milk fat and protein concentration, plus milk weight, MUN, and milk SCC have been used to make decisions to adjust feeding to increase production of grams of fat and protein per cow per day and net income from milk minus feed cost. The estimated blood NEFA and DN FA (expressed as DN as a percentage of total FA) are used in combination to monitor fresh cow metabolic

Corresponding Author: barbano1@aol.com

status for early detection of individual cows that will develop clinical ketosis or displaced abomasum. These milk-based transition cow analytical tools provide an opportunity to intervene earlier thereby improving recovery while reducing the negative impact of these adverse metabolic health events on animal welfare and lactation performance.

Introduction

In 2014 (Barbano *et al.*, 2014), the application of mid-infrared (**MIR**) for rapid milk fatty acid (**FA**) analysis was introduced in a commercial laboratory and positive correlations of bulk tank milk fat test with a higher proportion and concentration of de novo FA in bulk tank milk were reported. The analytical aspects of reference milk FA analysis, PLS model development, and validation statistics were reported by Wojciechowski and Barbano (2016) and Woolpert *et al.* (2016). Briefly, partial least squares (**PLS**) chemometric prediction models for FA were developed from MIR spectra in the Cornell University laboratory using a Delta Instruments Lactoscope (Delta Instruments, Drachten, Netherlands). The form of the FA data from the MIR was structured to report fatty acid values for DM, MO, and PF fatty acids in g/100 g milk and calculated values as the relative proportions of de novo (C4 to C15), mixed origin (C16:0, C16:1, C17:0), and preformed (C18:0 and longer) FA in milk were also provided. The mean FA chain length (carbon number) and degree of unsaturation (double bonds/fatty acid) are chemical structure metrics, not concentration metrics. The ratio of SD of reference values for the modeling set divided by standard error of cross validation (RPD) for DN, MO, and PF are 10.4, 6.2, and 7.3, while the RPD FA chain length and degree of unsaturation are 2.1 and 3.3. Manley (2014) indicated that RPD values greater than 3 are useful for screening, values greater than 5 can be used for quality control, and values greater than 8 for any application. With field experience in testing bulk tank milk from commercial farms, we found that providing this FA information in units of grams per 100 grams of milk was more useful when making feeding and management decisions on whole herd or feeding group basis, while the relative percentages of DN, MO, and PF fatty acids are more useful for transition cow metabolic health diagnostics in combination with the results of PLS model for milk estimated blood non-esterified fatty acids (**NEFA**). This paper will focus on the use of the milk FA information for management of dairy cows at the bulk tank level and report the status of our work on individual cow data with respect to how these milk composition and production parameters change with stage of lactation for primiparous and multiparous cows.

Woolpert *et al.* (2016, 2017) reported the results of two studies to determine feeding and farm management factors influencing milk FA composition and their relationship to bulk tank milk fat and protein content and yield per cow per day. The first study (Woolpert *et al.*, 2016) used 44 commercial dairies that were identified as either predominantly Holstein or Jersey in Vermont and northeastern New York. The yields of milk fat, true protein, and de novo FA per cow per day were higher for high de novo (**HDN**) versus low de novo (**LDN**) farms. The HDN farms had lower free-stall stocking density (cows/stall) than LDN farms. Additionally, tie-stall feeding frequency was higher for HDN than LDN farms. No differences between HDN and LDN farms were detected for dietary dry matter, crude protein, neutral detergent fiber, starch, or percentage of forage in the diet. However, dietary ether extract was lower for HDN than LDN farms. Overall, overcrowded free-stalls, reduced feeding frequency, and greater dietary ether extract content were associated with lower de novo FA synthesis and reduced milk fat and true protein yields on commercial dairy farms in this study.

The difference in income per cow depends on the actual milk price at any point in time. The average fat and protein price for the USDA Federal Milk Order No. 1 for March and April 2014 was $2.10 and $4.62 per lb ($4.62 and $10.17 per kg), respectively. Therefore, at 55 lb (25 kg) of milk per cow per day, the average HDN

farm earned a gross of $5.50 and $7.72 per cow for fat and protein, respectively. The average LDN farm at 55 lb (25 kg) milk per cow per day earned a gross of $5.26 and $7.29 per cow for fat and protein, respectively. These differences for fat and protein between HDN and LDN herds at 55 lb (25 kg) of milk per 100 cows per year would result in a gross income difference of $8,544 for fat and $15,695 for protein.

Woolpert *et al*. (2017) conducted a second study with 39 commercial Holstein herds in Vermont and northeastern NY. No differences in milk (about 70.5 lb (32 kg) /cow/d), fat (2.73 lb (1.24 kg)/cow/d), and true protein (2.2 lb (1.0 kg)/cow/d) yields were detected between HDN and LDN farms, but the percentage of milk fat (3.98 vs 3.78%) and true protein (3.19 vs 3.08%) were both higher on HDN farms. The HDN farms had higher de novo FA, a trend for higher mixed origin FA, and no difference in preformed milk FA daily yield per cow per day. This positive relationship between de novo FA and milk fat and true protein percentage agrees with previous results of Barbano *et al*. (2014) on bulk tank milk composition from 400 commercial dairy farms. The average fat and protein price for USDA Federal Milk Order No. 1 for February through April 2015 (US Department of Agriculture, 2015) was $1.90 and $2.61 per lb ($4.19 and $5.74 per kg), respectively. Therefore, at 66.1 lb (30 kg) of milk per cow per day, the average HDN farm earned a gross of $5.00 and $5.49 per cow for fat and protein, respectively. The average LDN farm at 30 kg of milk per cow per day earned a gross of $4.75 and $5.30 per cow for fat and protein, respectively. These differences for fat and true protein between HDN and LDN herds at 66.1lb (30 kg) of milk would result in gross income differences of $9,125 for fat and $6,935 for true protein per 100 milking cows per year. Management (i.e., frequent feed delivery and increased feed bunk space per cow) and dietary (i.e., adequate physically effective fiber and lower ether extract) factors that differed between these HDN and LDN farms have been shown in earlier studies to affect ruminal function.

Based on data from these studies the following graphs (Figures 1 to 4) for Holstein farms were developed to help farms understand the relationships between bulk tank milk FA composition and bulk tank fat and protein tests.

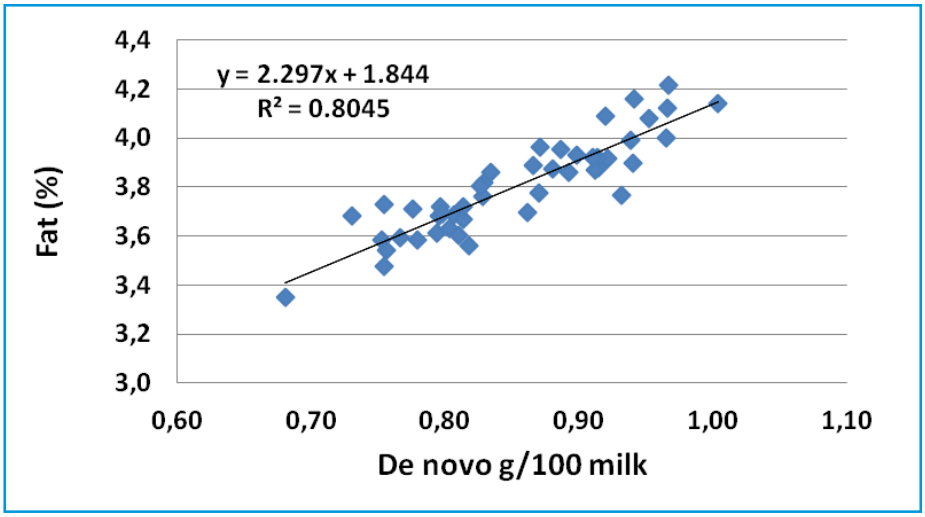

Figure 1. Relationship of bulk tank milk fat test to concentration (g/100 g milk) of de novo FA in milk. In general, a farm needs to have a concentration of de novo FA higher than 0.85 g/100 g milk to achieve a bulk tank fat test higher than 3.75%.

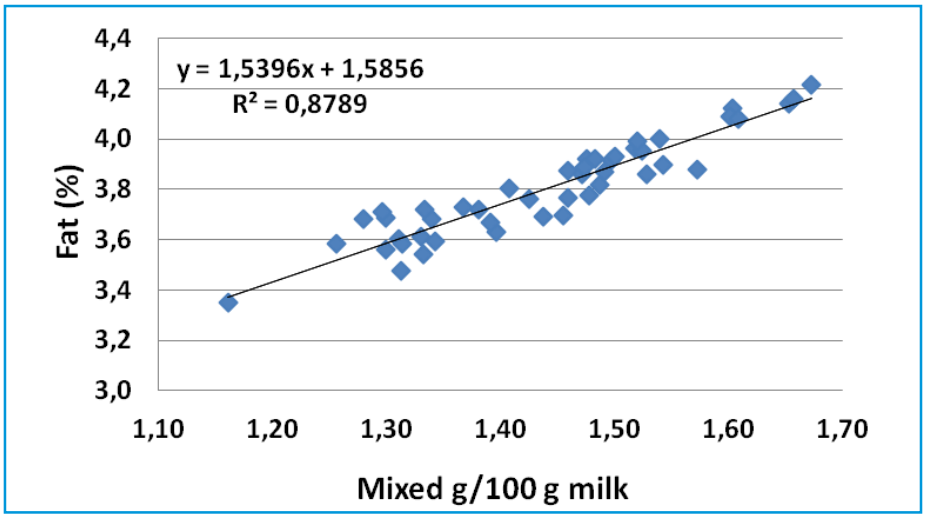

Figure 2. Relationship of bulk tank milk fat test to concentration (g/100 g milk) of mixed origin FA in milk. In general, a farm needs to have a concentration of mixed origin FA higher than 1.40 g/100 g milk to achieve a bulk tank fat test higher than 3.75%.

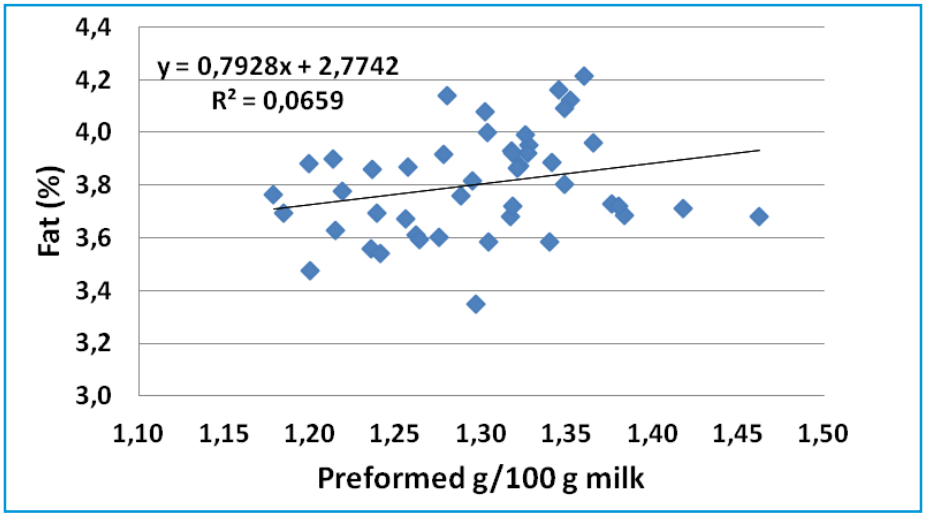

Figure 3. Relationship of bulk tank milk fat test to concentration (g/100 g milk) of preformed FA in milk. In general, the variation in preformed FA concentration in Holstein herds is less than de novo and mixed origin FA and is not well correlated with bulk tank milk fat test.

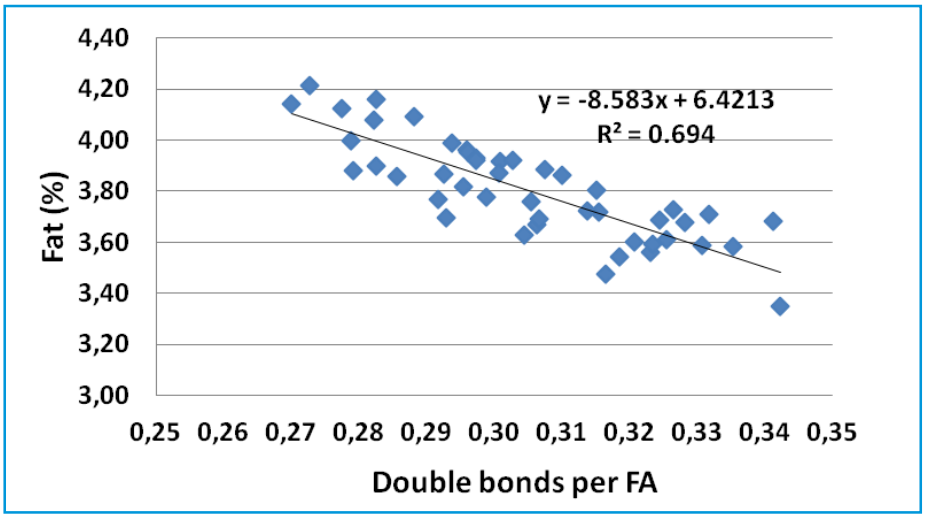

Figure 4. Relationship of bulk tank milk fat FA unsaturation with bulk tank milk fat test. As double bonds per FA increases the bulk tank milk fat test decreases. To achieve a 3.75 % fat test a farm needs to have a double bond per FA of less than 0.31.

Field adoption of routine milk fatty acid analysis

Starting in February of 2016, information on FA composition of bulk tank milk was provided to the individual producers of the St Albans Cooperative (Vermont) along with their payment test data on the same milk samples and in the summer of 2017 Agrimark Cooperative (Springfield, MA) and Cayuga Milk Ingredients (Auburn, NY) have started providing similar data to their producers on the official bulk tank milk samples that are used for milk payment testing. For producers that are not members of those cooperatives, milk samples can be analyzed at commercial laboratory (i.e., Minnesota DHIA) and our research laboratories at Cornell University and Miner Institute. The MIR milk analysis models used for measuring de novo, mixed origin and performed FA and fatty acid chain length (i.e., carbon number) and unsaturation (double bonds per fatty acid) are specific PLS models designed to measure these milk parameters using the MIR equipment produced by Delta Instruments. The values cannot be accurately calculated from other measured fatty acid parameters. The procedures used for development of these herd management MIR PLS models, external validation of the PLS models, and performance statistics for the models have been published (Wojciechowski and Barbano, 2016; Woolpert *et al.*, 2016). Other MIR milk analysis equipment manufacturers may develop similar PLS models to measure these parameters, validate the models and make them available to their customers in the future.

Seasonality of bulk tank milk

Over the past 3 to 4 years we have followed the pattern of seasonality of milk fat and protein in relation to milk FA composition on a group of 40 farms with the St. Albans Cooperative. The data January 2014 through July 2017 are from the routine testing results using MIR-analysis in the St. Albans Cooperative on fresh bulk tank milk samples used for payment testing (Figures 5 to 8).

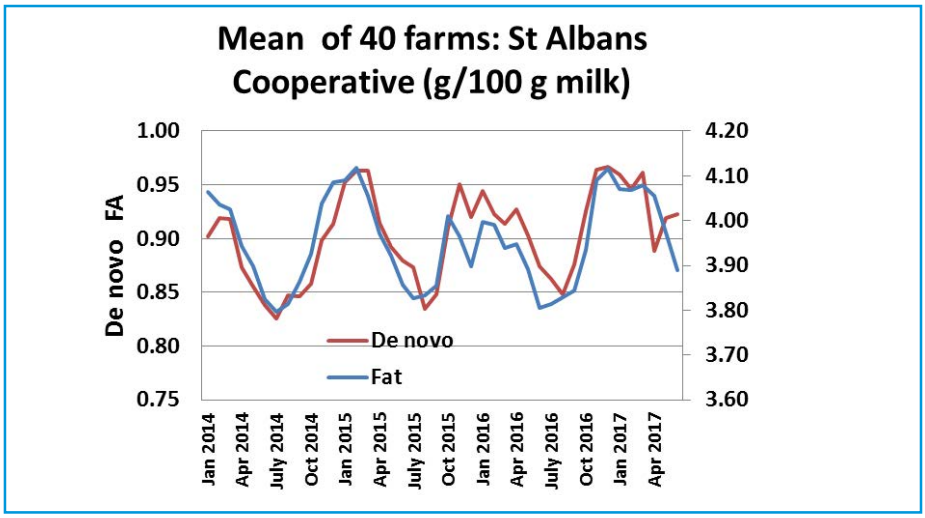

Figure 5. Seasonality of milk fat and de novo FA in milk.

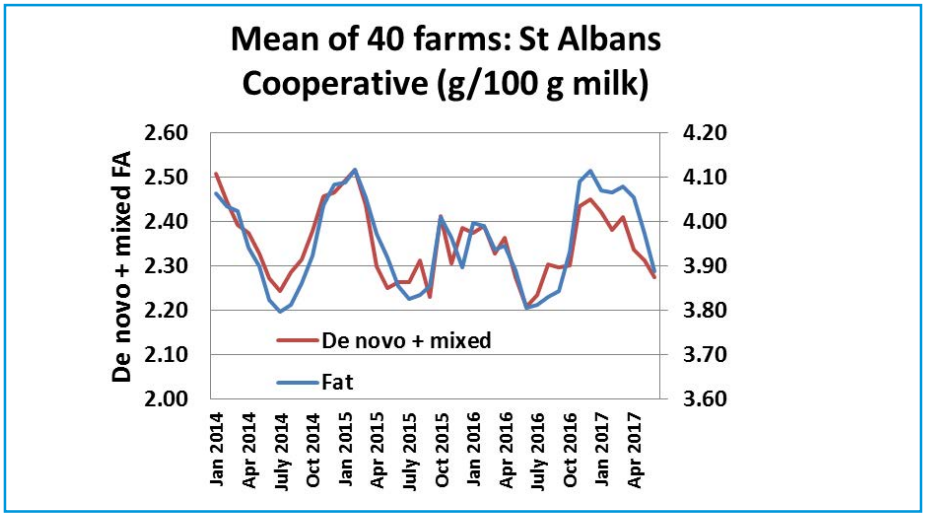

Figure 6. Seasonality of milk fat and de novo + mixed origin FA in milk

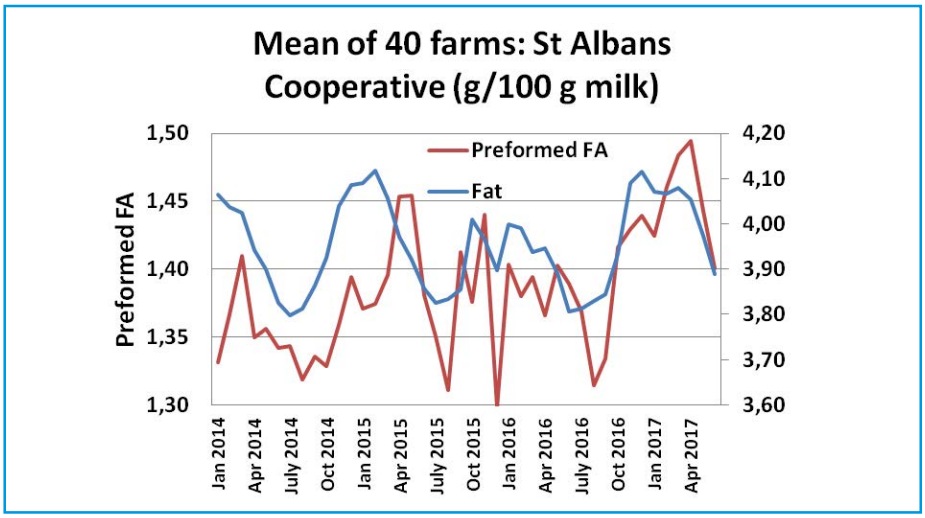

Figure 7. Seasonality of milk fat and preformed FA in milk.

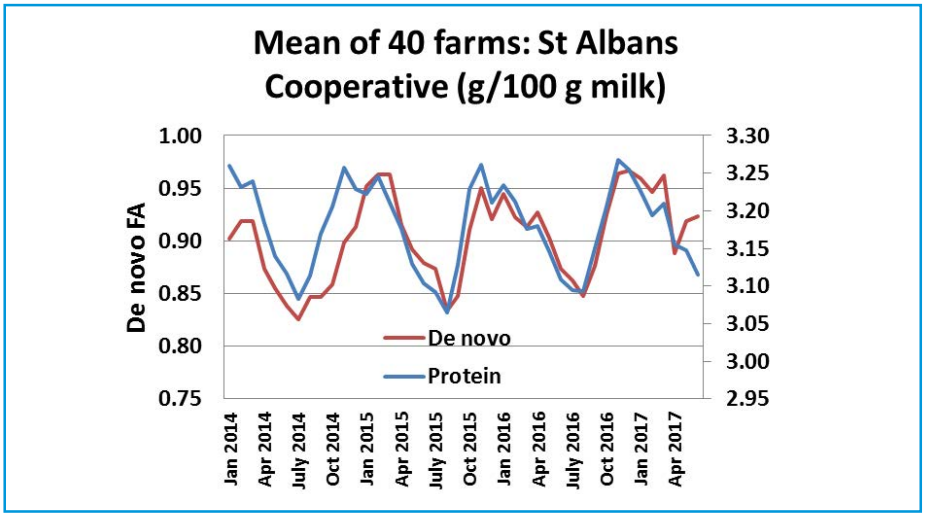

Figure 8. Seasonality of milk protein and de novo FA in milk.

The seasonality of de novo and mixed origin milk FA concentration follows the seasonal pattern of milk fat (Figure 5) and protein (Figure 6) variation while variation in preformed fatty FA in milk does not (Figure 7). Much of the variation in the mixed origin FA concentration is probably due to variation in the portion of the mixed origin FA produced by de novo synthesis from acetate and butyrate from forage digestion in the rumen. These seasonal changes may be related to time and temperature induced changes in the fermentation of corn silage, starch degradability, forage quality and heat stress on the cows.

Herd to herd variation in milk composition in North America

Over the past year, bulk tank milk sampling has been done on a wide range of farms from various regions of the US to confirm if the same milk fat, protein and milk FA composition relationships are observed in bulk tank milks from different regions of the US. These samples were collected daily for 5 to 7 days on each farm, preserved and refrigerated. At the end of the collection period, the milk samples were shipped on ice to Cornell University for MIR analysis and spot-checking FA composition with GLC analysis, particularly to obtain more detail about milk trans FA levels at each farm. There were some grazing herds, organic herds, and very large conventional herds in the population with a wide range of milk production per cow and milk composition.

The findings from these 167 farms were reported at the 2017 Cornell Nutrition Conference (Barbano et. al., 2017). The behavior of bulk tank milk fatty acid composition as it related to bulk tank fat and protein test is shown in Figures 9 and 10 for herds managed and fed over a wider range of types of feeds and management systems than we encountered in our studies of farms in the Northeast US. The relationship between de novo and de novo plus mixed origin observed in bulk tanks milk produced by farms from across the US were similar those found for Holstein herds in the Northeast. A level of about 0.85 g de novo FA per 100 g of milk will achieve about a 3.75% fat test (as seen by comparison of Figure 1 versus Figure 9).This indicated that the milk fatty acid metrics (de novo, mixed, preformed, fatty acid chain length, and

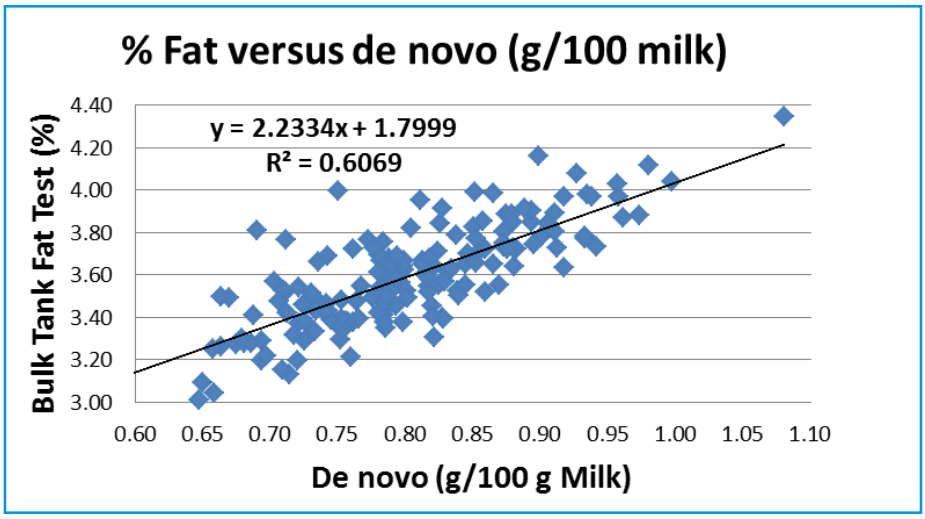

Figure 9. Correlation between bulk tank fat and de novo FA concentration (167 farms).

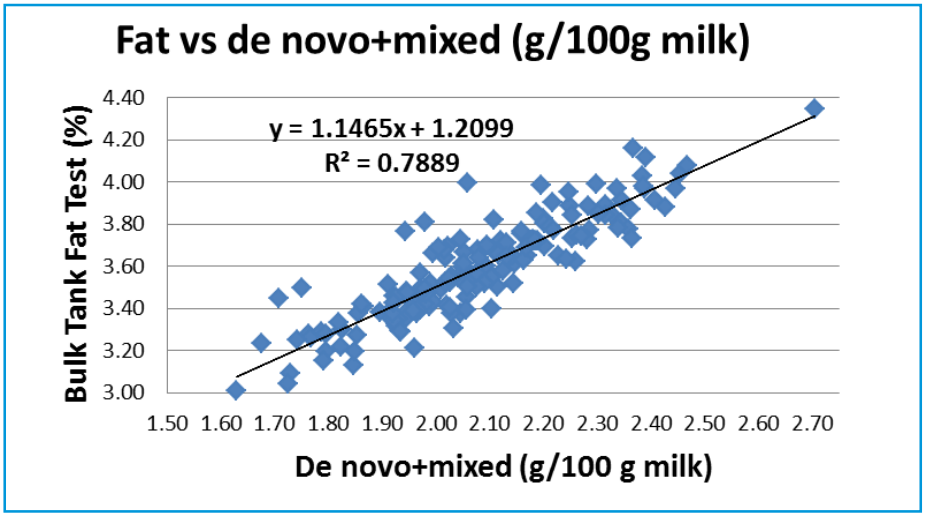

Figure 10. Correlation between bulk tank fat and de novo + mixed origin FA (167 farms).

double bonds per fatty acid) are robust indices for use for herd management and apply over the wide range of condition found across North America. A discussion of interpretation of bulk tank milk fatty acid composition was reported previously (Barbano *et al.*, 2017).

Milk fat and protein output per cow per day are also strongly correlated with total weight of milk produced per day. Those relationships for the 167 farms from across North America are shown below in Figures 11 and 12.

Overall, dairy cows have the potential to produce more grams of fat and protein per day if they produce more milk. The synthesis of lactose and increasing the grams per day output of lactose is needed to produce more pounds of milk per day. Lactose production is highly dependent on glucose metabolism in the cow. To produce more milk per cow, a cow will need to produce more lactose per day, as shown in Figure 13 below. The correlation is very strong. To achieve 90 to 100 lb (40.9 to 45.4 kg) of milk, the cows need to be producing between 1900 and 2100 grams of lactose per day. A major factor that would compete for use of glucose that could be used in support of milk synthesis is an immune response by the animal because of some adverse health event (e.g., leaky gut, mastitis, lower GI viral infection, etc.). Thus, in our field work we are encountering situations where the fatty acid profile and a high bulk tank fat and protein test indicate that rumen function is good, but the weight of milk per day is low (i.e., low lactose output per cow per day) and as a result total weight output per cow per day of fat and protein are not as high as they should be. This is a sign that there is some non-feed/non-rumen fermentation problem that is limiting milk production.

As this new milk testing technology becomes more widely available in the dairy industry it is likely to be used as a herd management tool to test milk from different feeding groups of cows that may have a very different number of days of milk (**DIM**) from one group to another or have a different parity status from one group to another. Both DIM and parity influence milk and milk FA composition. There are large changes in milk FA composition with stage of lactation, particularly during the transition period. When looking at milk composition and FA composition, differences in parity or stage of lactation needs to be taken into account when interpreting data. As a result, we have been collecting data at the Miner Institute to produce lactation curves on all of these milk parameters.

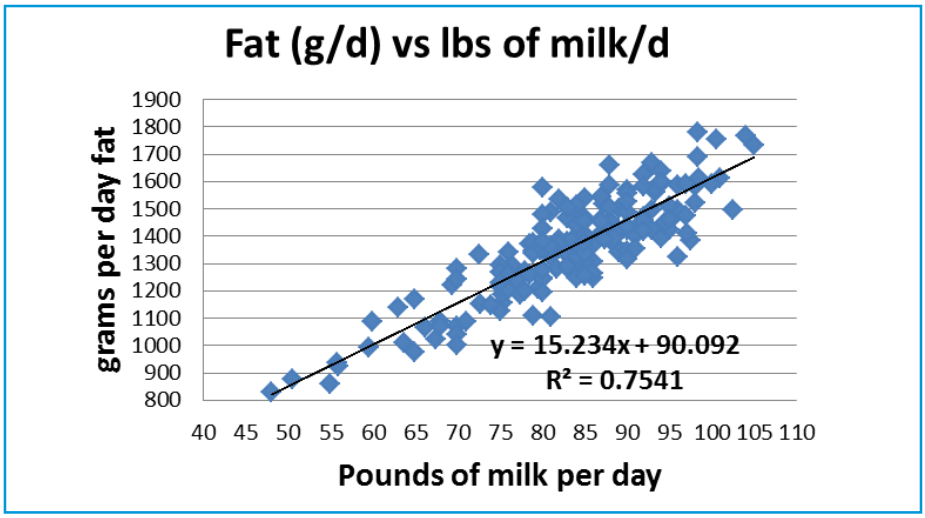

Figure 11. Grams of fat per cow per day and milk production (167 farms).

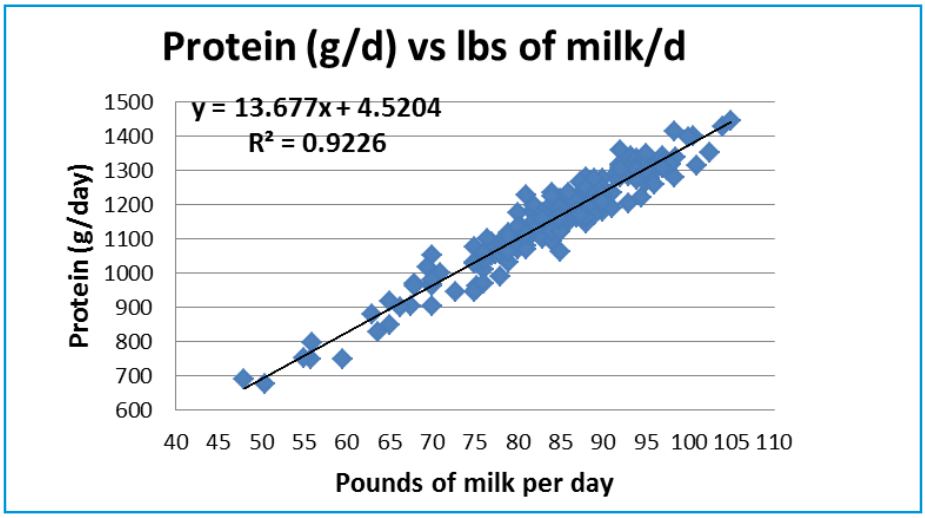

Figure 12. Grams of protein per cow per day and milk production (167 farms).

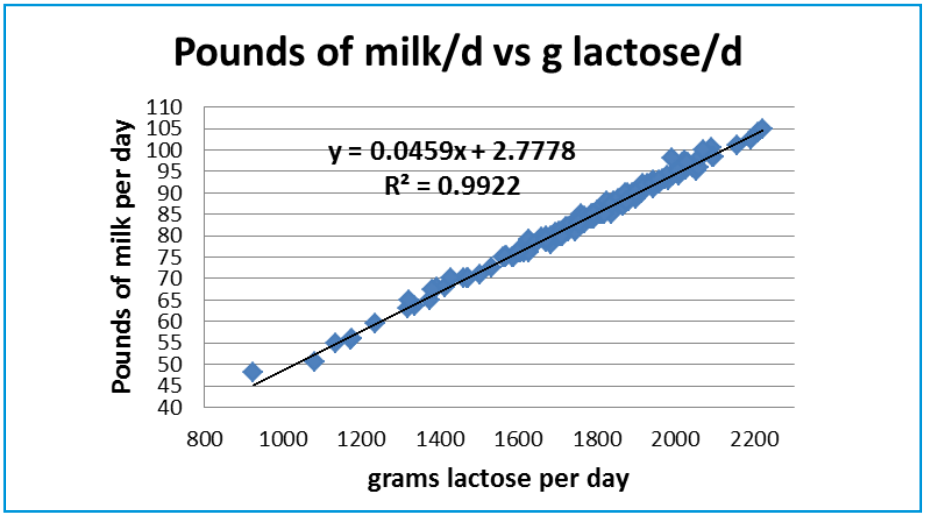

Figure 13. Grams of lactose produced per cow per day and milk production (167 farms).

Stage of lactation affects milk fatty acid composition

For the past 2 to 3 years, we have been conducting an intensive study at Miner Institute with individual cow milk analysis to better understand changes in milk FA across the lactation cycle. The goal is to build stage of lactation curves for all the new milk analysis parameters on both a concentration basis and a daily output per cow basis. Milk is collected from the entire herd (~400 cows) weekly from 3 milking shifts within the same day and analyzed on-site with a high-speed MIR milk analysis system.

As expected, the concentrations of FA in milk changes with DIM. The changes are particularly large in early lactation (i.e., the transition period) when the cow is in negative energy balance. During this period it is normal for the preformed FA to be high and the mixed and de novo FA to be low. However as dry matter intake increases after calving, the milk FA composition should change quickly if the cow's blood NEFA concentration decreases normally. If milk sampling and testing for FA is being done on different groups of cows within a herd, then these stage of lactation changes need to be considered to properly interpret that data. The graphs below (Figures 14 to 17) are stage of lactation data collected from Holstein cows over a period of 2 to 3 years that were milked 3 times per day, had a rolling herd average of ~30,000 lb (12,636 kg) and were fed total mixed rations based on stage of lactation (i.e., fresh, 1st lactation, high and low groups). In general, the diets were typically 50 to 60% forage with at least 2/3 of forage coming from corn silage. Grain mixes typically contained corn grain, soybean meal, commercial soy/canola products, byproducts, rumen inert fat, plus mineral and vitamin supplements. Diets were balanced for lysine and methionine. The change in g/100 g milk of de novo, mixed, and preformed FA with week of lactation is shown in Figure 14 and the relative percentages are shown in Figure 15.

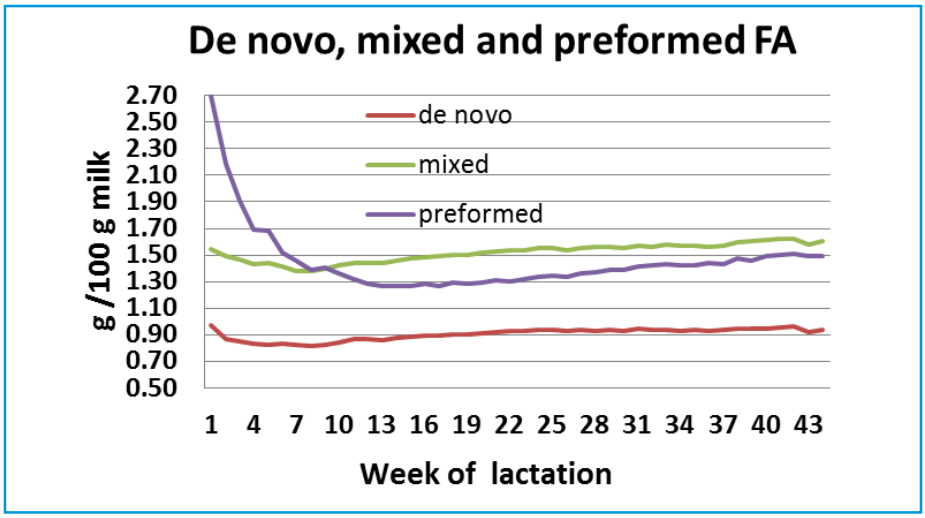

Figure 14. De novo, mixed, and preformed FA (g/100 g milk) over lactation for all cows.

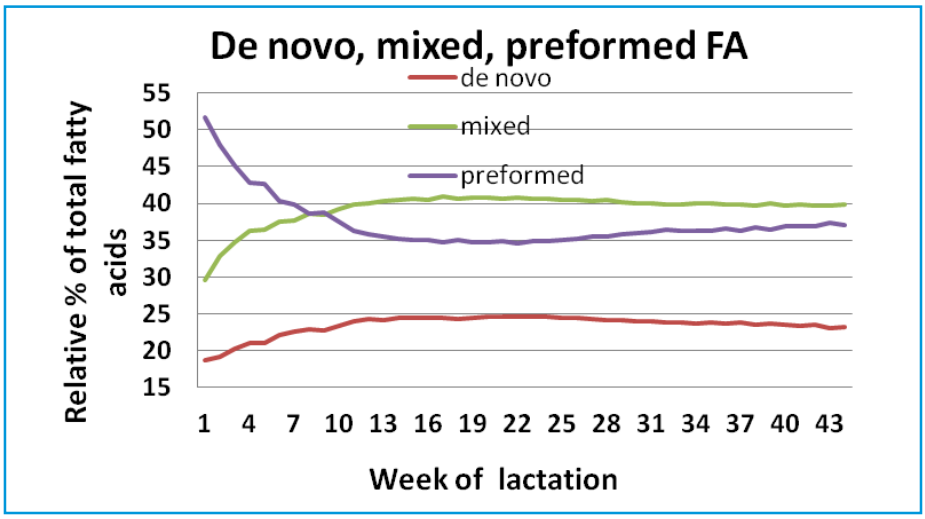

Figure 15. De novo, mixed, and preformed FA (relative %) over lactation for all cows.

There are large changes in milk FA composition during the first 10 weeks of lactation on both a g/100 g milk and relative percentage basis with the preformed FA being high at the beginning of lactation and decreasing to relatively stable levels by about 10 weeks of lactation. When testing milk on larger farms from groups of cows that differ in stage of lactation, these changes in milk FA composition with stage of lactation need to be considered when interpreting data along with information on milk production per cow per day, cow health, milk SCC, feed composition, and dry matter intake.

Interpretation of results from a management point of view becomes even more interesting when the data are converted to grams per day per cow output. Figure 14 represents the average of all cows in the herd, but the stage of lactation graph for

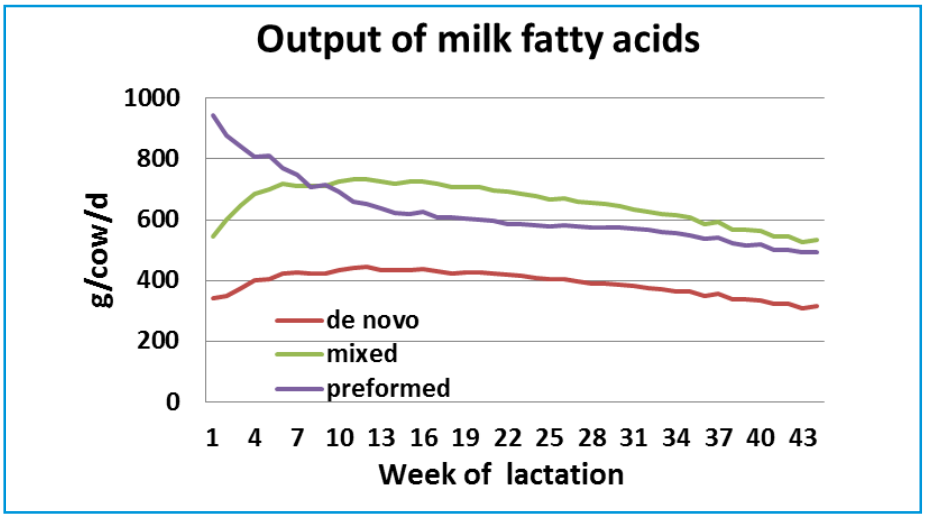

Figure 16. Stage of lactation production graph for all cows (g/cow/day).

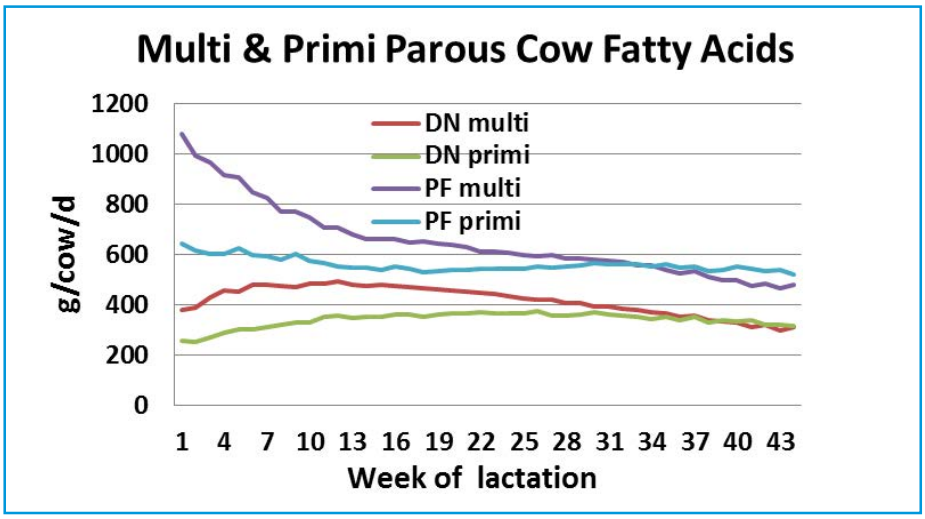

Figure 17. Stage of lactation: de novo (DN) and preformed (PF) fatty acids for primiparous and multiparous cows.

grams per cow per day is very different for first parity versus older cows. When evaluating performance of older versus younger cows, this factor needs to be considered. The difference between multi and primiparous cows for output of de novo and preformed FA per cow per day is shown in Figure 17. The output of all groups of FA in grams per cow per day is much more stable over time for primiparous cows versus older cows. The older cows have much higher preformed FA output per cow per day in early lactation due to body fat mobilization than primiparous cows.

Milk fatty acid data and milk estimated blood NEFA: tools for management of metabolic health during the transition period

To apply milk analysis to transition cow health management the frequency of individual cow milk testing needs to be much higher than what is currently being done with monthly DHIA milk testing. Our research results indicate that there may be an excellent farm management and individual cow health management opportunity with a higher frequency of milk analysis. As shown above, milk fatty acid composition and yield per cow per day changes rapidly especially during the first 10 weeks of lactation when cows are transitioning from negative to positive energy balance. Separately if blood samples are collected and blood non-esterified fatty acid concentration is measured, cows with a high probability of metabolic related disorders (e.g., ketosis, displaced abomasum, etc.) can be identified. Barbano *et al.* (2015) reported a method for estimation of blood NEFA (µEq/L) directly from the MIR milk spectra, not by calculation from fatty acid data. Use of milk estimated blood NEFA is faster, less expensive, and timelier than blood analysis. Are there differences in milk fatty acid profile and milk estimated blood NEFA that can be used to better manage transition cow health? To address this question, we did high frequency (1 milking per day) testing of milk from individual fresh cows at Miner Institute to compare data from healthy cows and cows that had clinical diagnoses of DA and/or ketosis. In general, milk was collected before fresh cow exam/check. The milk information was pared with health data collected and stored in a herd management software program.

Typically, blood samples are collected from early lactation cows that may have a high risk of a metabolic disorder. However, when blood NEFA is estimated from the MIR spectra of milk, it becomes relatively easy to produce a milk estimated blood NEFA lactation curve. The change in milk estimated blood NEFA for primiparous and multiparous cows throughout lactation is shown in Figure 18. In early lactation when cows are in negative energy balance, blood NEFA is high as the cows mobilize body fat to help meet energy requirements of milk production in very early lactation when their dry matter intake is increasing. In general, multiparous cows are mobilizing more body fat in the first few weeks of lactation than primiparous cows.

Displaced Abomasum (DA)

In general the milk estimated blood NEFA was much higher (about 1400 µEq/L) for cows that were clinically diagnosed with a DA than healthy cows (about 800 µEq/L) (Figure 19) and milk de novo fatty acids (Figure 20) were lower for cows with a DA (ca. 13 vs 19% g/100 g fatty acids). Post DA surgery, milk estimated blood NEFA decreased and milk de novo fatty acids increased but did not equal the values for

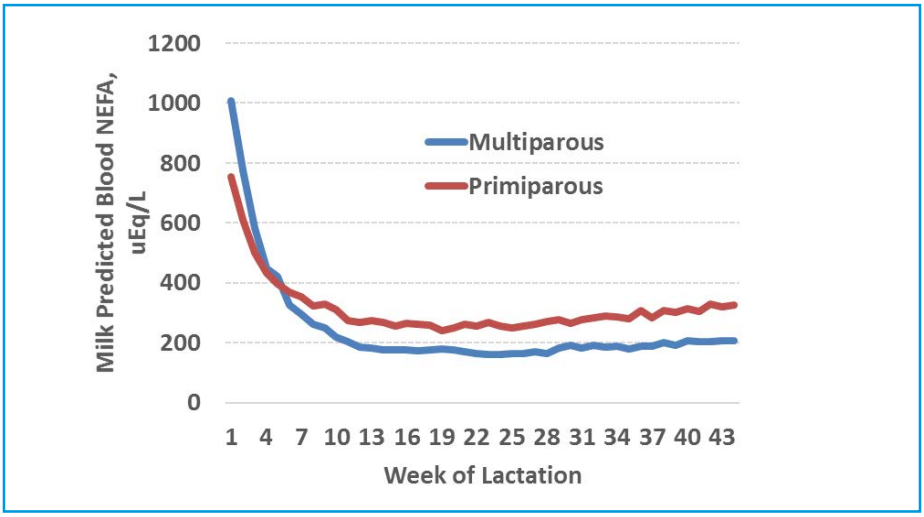

Figure 18. Stage of lactation: milk predicted blood NEFA (µEq/L) for primiparous and multiparous cows.

healthy cows at the same days in milk, indicating that there will probably be some longer term negative effect of the DA event on milk production for that cow as lactation continues.

Ketosis

In general the milk estimated blood NEFA was much higher (about 1400 µEq/L) for cows that were clinically diagnosed with ketosis than healthy cows (about 800 µEq/L) (Figure 21) and milk de novo fatty acids (Figure 22) were lower for cows with ketosis (ca. 13 versus 19% g/100 g fatty acids). Post-ketosis treatment with propylene glycol, milk estimated blood NEFA decreased and milk de novo fatty acids increased but did

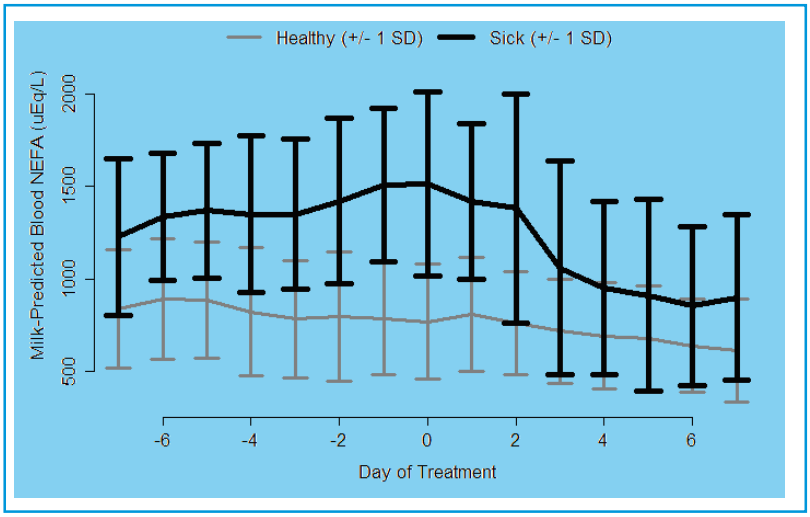

Figure 19. Milk predicted blood NEFA for multiparous cows (n = 47) with a DA versus healthy cows (n = 191) with day zero being the day of treatment. Day 0 = 9.4 days in milk. Healthy cows were matched with DA cows based on days in milk and calving date.

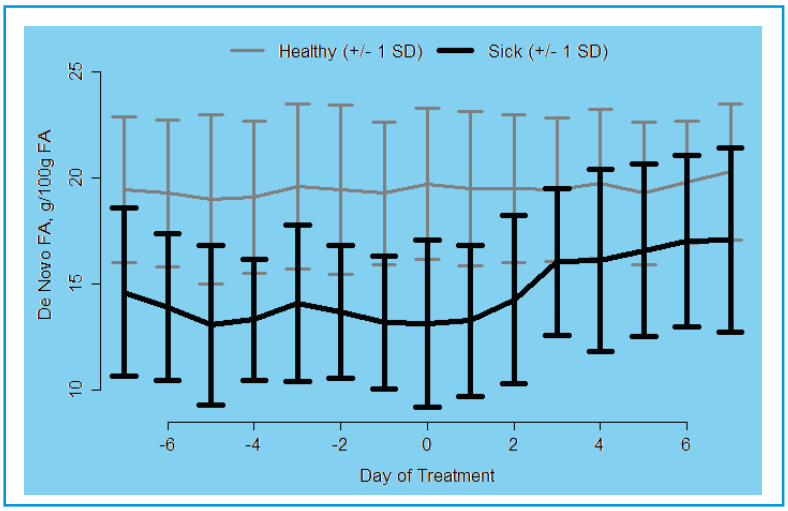

Figure 20. Milk de novo fatty acids relative % (g/100 g fatty acids) for multiparous cows (n = 47) with a DA versus healthy cows (n = 191) with day zero being the day of treatment. Day 0 = 9.4 days in milk. Healthy cows were matched with DA cows based on days in milk and calving date.

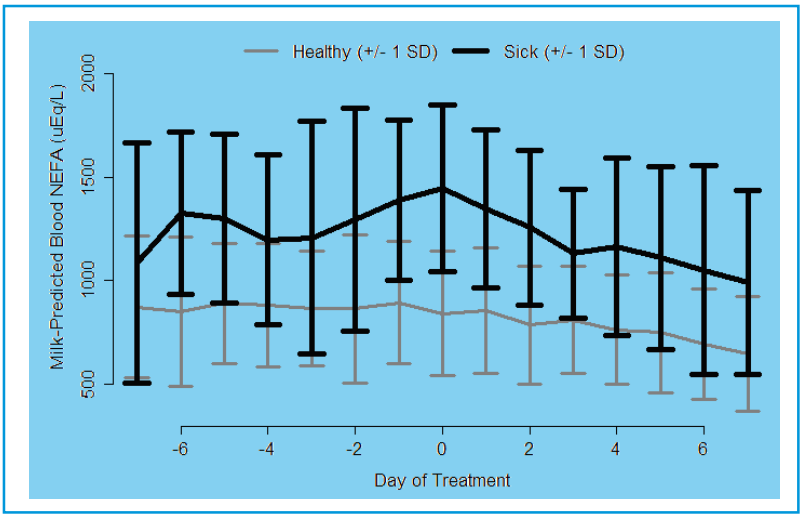

Figure 21. Milk estimated blood NEFA for cows (n = 87) with ketosis versus healthy cows (n = 239) with day zero being the day of treatment. Day 0 = 7.3 days in milk. Healthy cows were matched with ketotic cows based on days in milk and calving date.

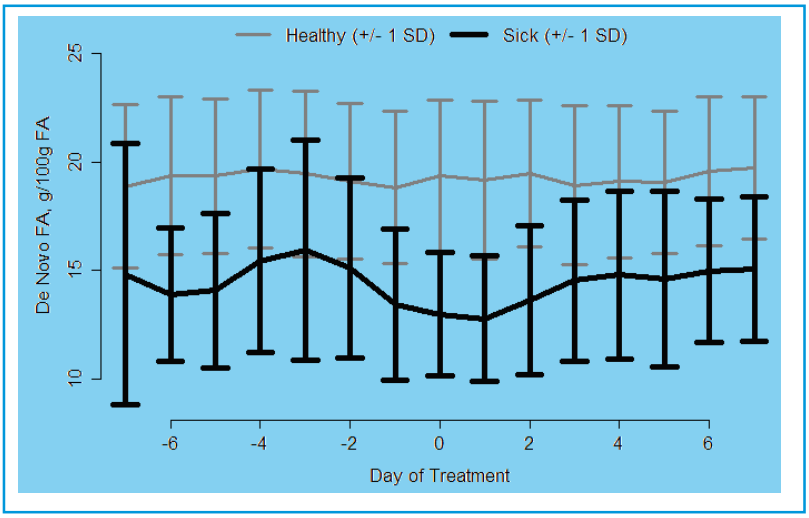

Figure 22. Milk de novo fatty acids relative % (g/100 g fatty acids) for multiparous for cows (n = 87) with ketosis versus healthy cows (n = 239) with day zero being the day of treatment. Day 0 = 7.3 days in milk. Healthy cows were matched with ketotic cows based on days in milk and calving date.

not equal the values for healthy cows at the same days in milk, indicating that there will probably be some longer term negative effect of the ketosis event on milk production for that cow as lactation continues.

Conclusions

Data from routine high frequency (i.e., daily) bulk tank milk component, SCC, and milk FA testing combined with milk weight per cow for whole herd diagnostic analysis of overall nutritional and management status of dairy herds. The testing was done using MIR as part of the routine milk payment testing. The advantage of this approach is that no additional sampling collection cost is required, the instrument that does the milk FA analysis can be the same instrument that produces the milk fat and protein test result, and it does not take any longer to test each milk sample. There would be additional cost to purchase reference milk samples for calibration of the FA parameters for the MIR milk analyzer. The positive correlation between increased de novo fatty acid synthesis and bulk tank milk fat and protein concentration can be used as an indicator of the quality and balance and the rumen fermentation of carbohydrates and if changes in feeding and management are impacting de novo synthesis of milk fat. Seasonal variation in whole herd milk fat and protein concentration was highly correlated with seasonal variation in de novo FA synthesis. Milk FA composition changes with both DIM and differs between primiparous and multiparous cows. Milk fatty acid testing and this diagnostic approach could be applied to testing milk from large feeding groups of cows within the same farm, if representative feeding group milk samples can be collected and tested and the milk produced per cow is known. For feeding group or individual cow milk testing care must be taken to consider the milk weight per cow per day, diet composition, dry matter intake, DIM and parity into the interpretation of the milk composition data.

Data from high frequency MIR milk testing of individual cow milks, particularly during the transition period can be used to identify quickly cows at high risk for displaced abomasum and ketosis before a clinical diagnosis is made oftentimes. The concentration of milk estimated-blood NEFA (µEq/L) was higher and milk de novo fatty acids as percent of total fatty acids (g/100 g fatty acids) was lower than healthy for cows for cows diagnosed with clinical ketosis or displaced abomasum. At the present time based on the current milk analysis tools, we were not able to differentiate whether a cow was going to have ketosis or a displaced abomasum in advance, but further research being done to develop milk analysis tools to differentiate these health events in advance of clinical diagnosis. This may allow development of earlier intervention strategies to reduce the severity of these metabolic disorders and their negative impact on milk production. Mid-infrared analysis of milk from transition cows may be an alternative to blood sampling and testing for management of transition cow health.

Acknowledgments

The authors acknowledge financial support of the Test Procedures Committee of the UDSA Federal Milk Markets (Carrollton, Texas). The technical assistance of St. Albans Cooperative Creamery (St. Albans, Vermont) and staff of Miner Institute for sampling and MIR milk analysis and Delta Instruments (Drachten, The Netherlands) with technical support for development of chemometric models and MIR analysis equipment support.

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