

INFRARED MILK FATTY ACID ANALYSIS: EXPERIENCE IN THE FIELD FOR FARM MANAGEMENT

D. M. Barbano¹, C. Melilli¹, H. Dann², and R. Grant²

¹Department of Food Science, Cornell University, Ithaca, NY

²William H. Miner Agricultural Research Institute, Chazy, NY

INTRODUCTION

In 2014 (Barbano et al., 2014), we introduced the application of mid-infrared (**MIR**) for rapid milk fatty acid (**FA**) analysis and reported positive correlations of bulk tank milk fat test with a higher proportion and concentration of de novo FA in bulk tank milk. The analytical aspects of reference milk FA analysis and model development and validation were reported by Wojciechowski and Barbano (2016) and Woolpert et al. (2016). The form of the FA data from the MIR was structured to provide information on 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, the mean FA chain length (carbon number) and degree of unsaturation (double bonds/fatty acid). With experience in the field testing milk from bulk tank milk from individual on farms we found that providing this FA information in units of grams per 100 grams of milk was more useful. Since that time, we have continued to collect data on milk FA variation in bulk tank milk and it's relationship to feeding and farm management.

Woolpert et al. (2016, 2017) have 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 test and production per cow per day of fat and protein. In the first study (Woolpert et al., 2016) with 44 commercial dairies that were identified as either predominantly Holstein or Jersey in northern Vermont and 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 freestall stocking density (cows/stall) than LDN farms. Additionally, tiestall 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 freestalls, 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 would depend on the actual milk price at any point in time. The average fat and protein price for the Federal Milk Order No. 1 for March and April 2014 was \$4.62 and \$10.17 per kg (\$2.10 and \$4.62 per lb), 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.

A second study (Woopert et al., 2017) with 39 commercial Holstein herds was conducted as a follow up to the previous study. No differences in milk (about 32 kg (70.5 lb) /cow/d), fat (1.24 kg (2.73 lb)/cow/d), and true protein (1.0 kg (2.2 lb)/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%) content were both higher on HDN farms. HDN farms had higher de novo FA, a trend for higher mixed origin, and no difference in preformed milk FA output/cow/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 Federal Milk Order No. 1 for February through April 2015 (US Department of Agriculture, 2015) was \$4.19 and \$5.74 per kg (\$1.90 and \$2.61 per lb), 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.

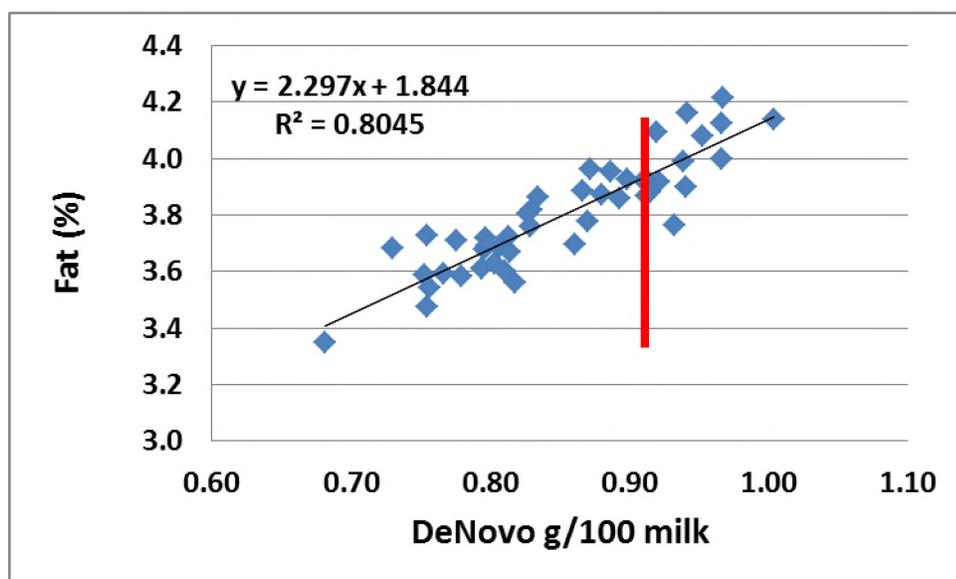


Figure 1. Relationship of bulk tank milk fat test to concentration (g/100 g milk) of de novo FA in milk. In general, a farm needs to have a concentration of de novo FA higher than 0.85 g/100 g milk to achieve a bulk tank fat test higher than 3.75%.

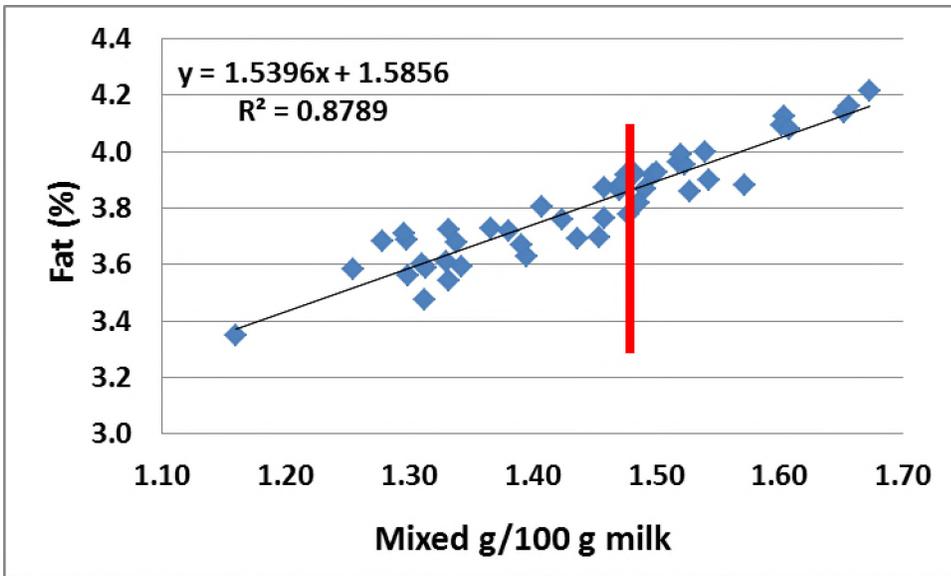


Figure 2. Relationship of bulk tank milk fat test to concentration (g/100 g milk) of mixed origin FA in milk. In general, a farm needs to have a concentration of mixed origin FA higher than 1.40 g/100 g milk to achieve a bulk tank fat test higher than 3.75%.

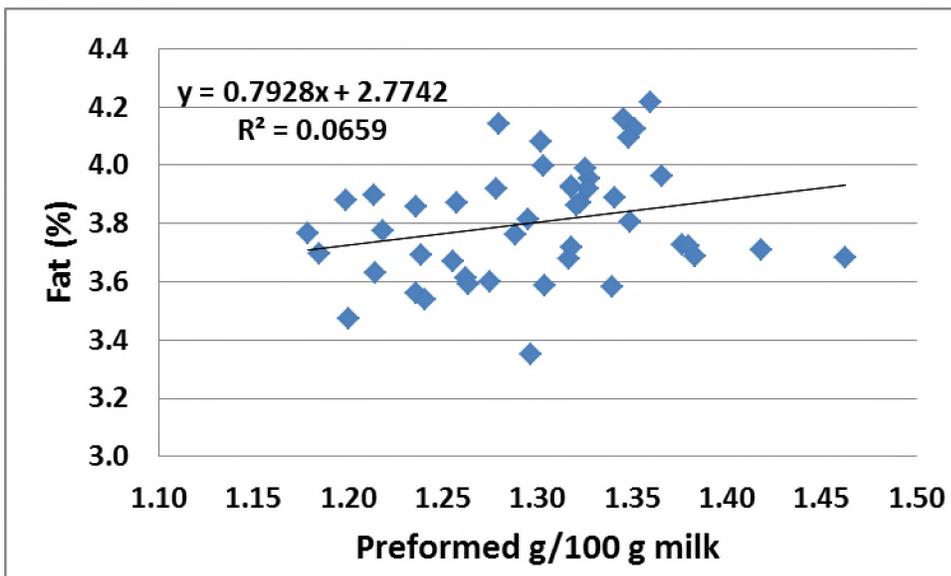


Figure 3. Relationship of bulk tank milk fat test to concentration (g/100 g milk) of preformed FA in milk. In general, the variation in preformed FA concentration in Holstein herds is less than de novo and mixed origin FA and is not well correlated with bulk tank milk fat test.

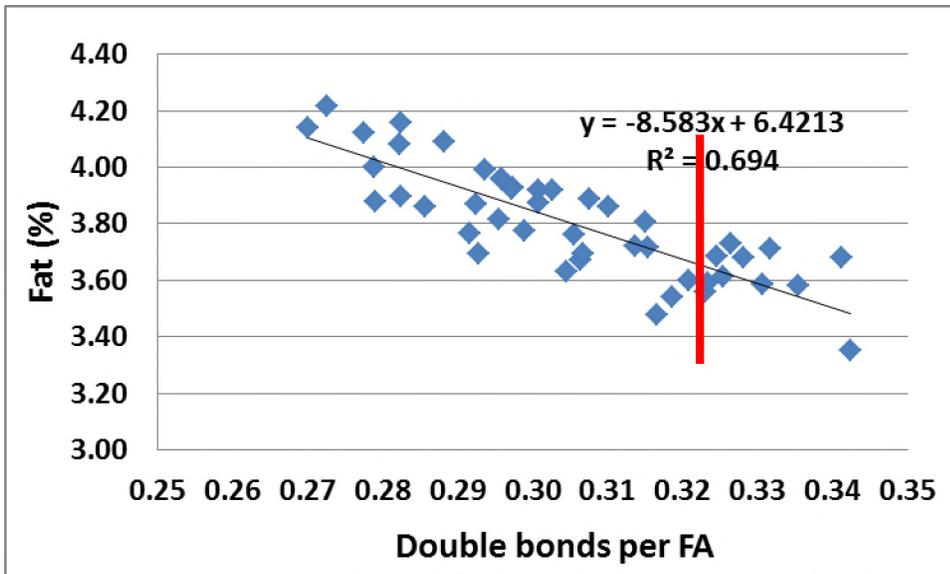


Figure 4. Relationship of bulk tank milk fat FA unsaturation with bulk tank milk fat test. As double bonds per FA increases the bulk tank milk fat test decreases. To achieve a 3.75 % fat test a farm needs to have a double bond per FA of less than 0.31.

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.

In addition, in the last 2 years we have expanded our milk analysis research on FA analysis to individual cow milk samples at Cornell and in collaboration with Miner Institute in Chazy, NY. This paper will focus on the use of milk FA information for feeding 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.

EXPERIMENTAL APPROACH

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) and have been described in detail by Wojciechowski and Barbano (2016). Data collection has continued at the St Albans Cooperative and within farm seasonality patterns of bulk tank milk fat, protein, and milk FA composition has been measured using the routine milk fatty acid analysis by MIR. In addition, in 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.

For individual cow milk analysis we are conducting an intensive study at Miner Institute. We have a high speed MIR milk analysis system on site testing milk from individual cows. The routine fresh milk testing is done one day per week, 3 milkings in a row on each cow in the herd. 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.

RESULTS

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 are from the routine testing results using MIR in the St. Albans Cooperative on fresh bulk tank milk samples used for payment testing.

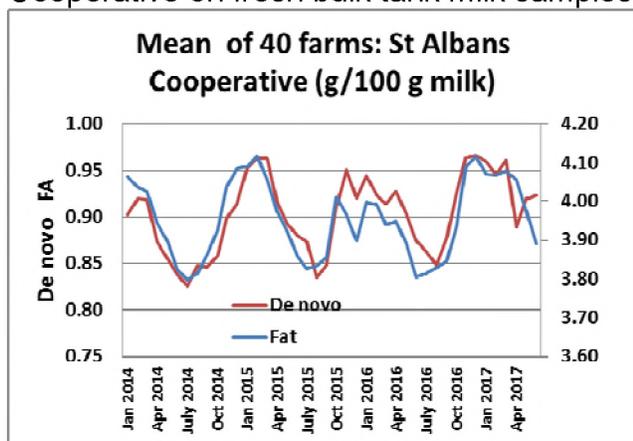


Figure 5. Seasonality of milk fat and de novo FA in milk.

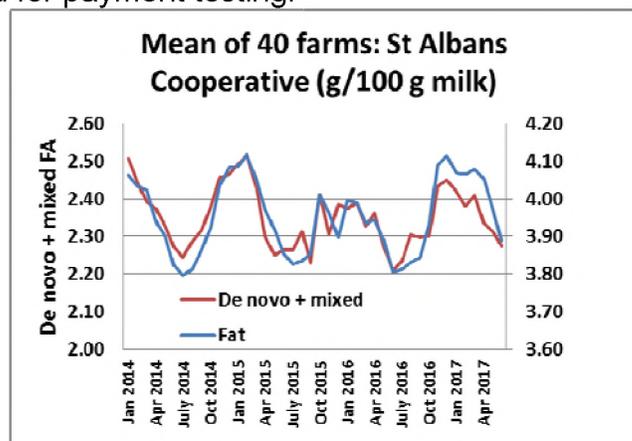


Figure 6. Seasonality of milk fat and de novo + mixed origin FA in milk

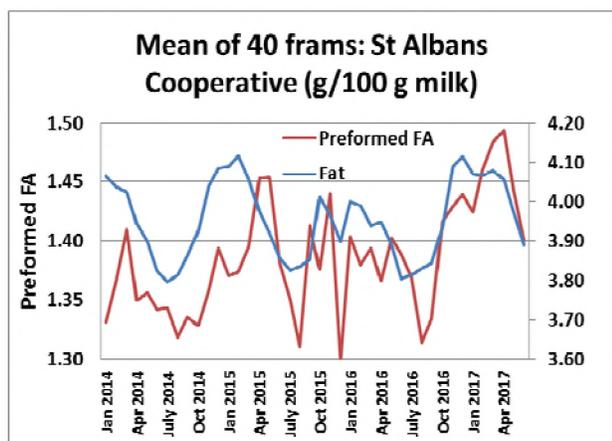


Figure 7. Seasonality of milk fat and preformed FA in milk.

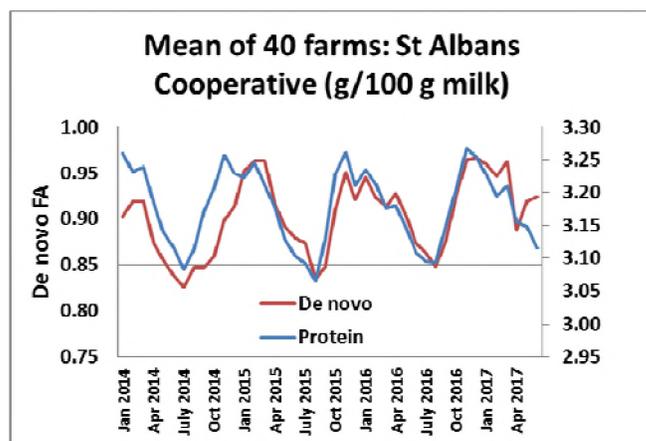


Figure 8. Seasonality of milk protein and de novo FA in milk.

The seasonality of de novo and mixed origin milk FA concentration follows the seasonal pattern of milk fat and protein variation while variation in preformed fatty FA in milk does not. 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.

Herd to herd variation in milk composition in North America.

Over the past year bulk tank milk samples were collected from large and small Holstein farms from different regions of the US. Each bulk tank or tanker within the farm was sampled each day for 5 to 7 day periods and milk samples were sent to the Cornell University laboratory for MIR and GLC analyses. 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 relationship between bulk tank milk composition and FA composition is shown (Figures 9-13) below for 167 farms.

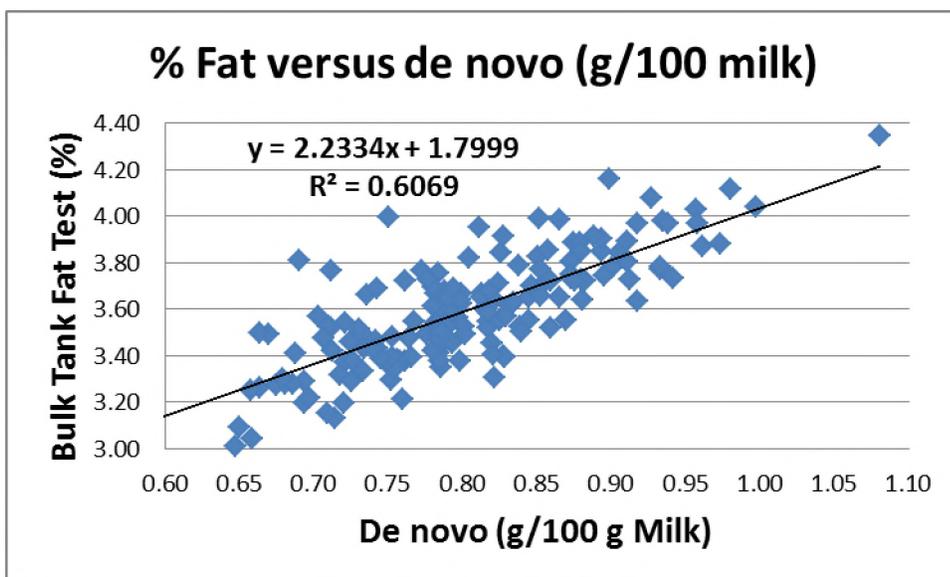


Figure 9. Correlation between bulk tank fat and de novo FA concentration (167 farms).

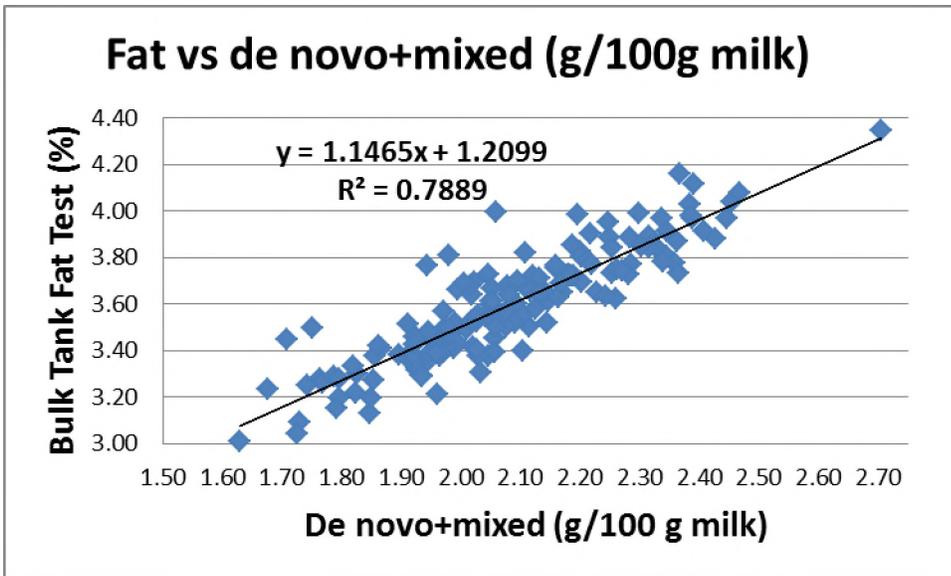


Figure 10. Correlation between bulk tank fat and de novo + mixed origin FA (167 farms).

The relationship between de novo and de novo plus mixed origin observed in bulk tanks milk produced by farms from across the US are 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). The same general relationship is seen in both data sets. Another data set of 500 farms from the Texas/New Mexico area shows similar patterns (data not shown). Milk fat and protein output per cow per day are also strongly correlated with total weight of milk produced per day. Those relationships are shown below.

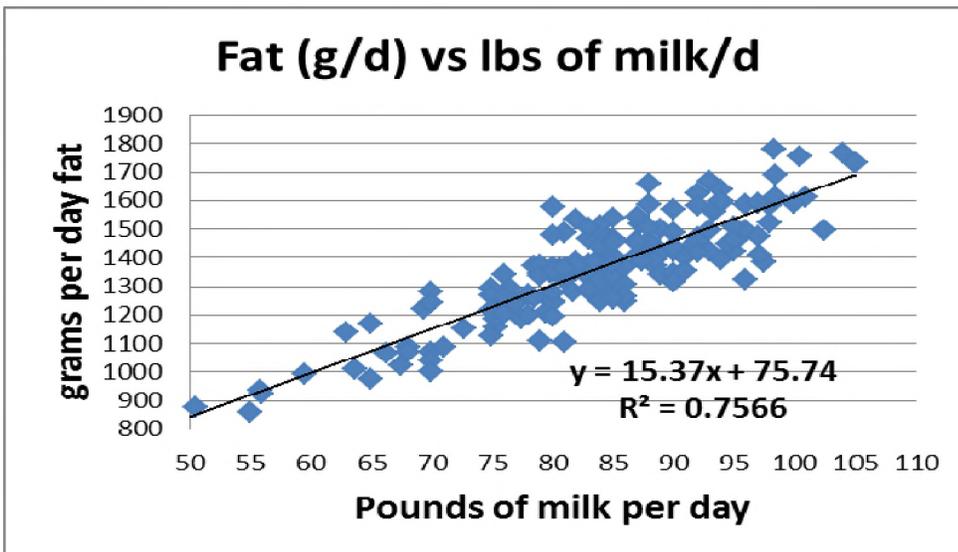


Figure 11. Grams of fat per cow per day and milk production (167 farms).

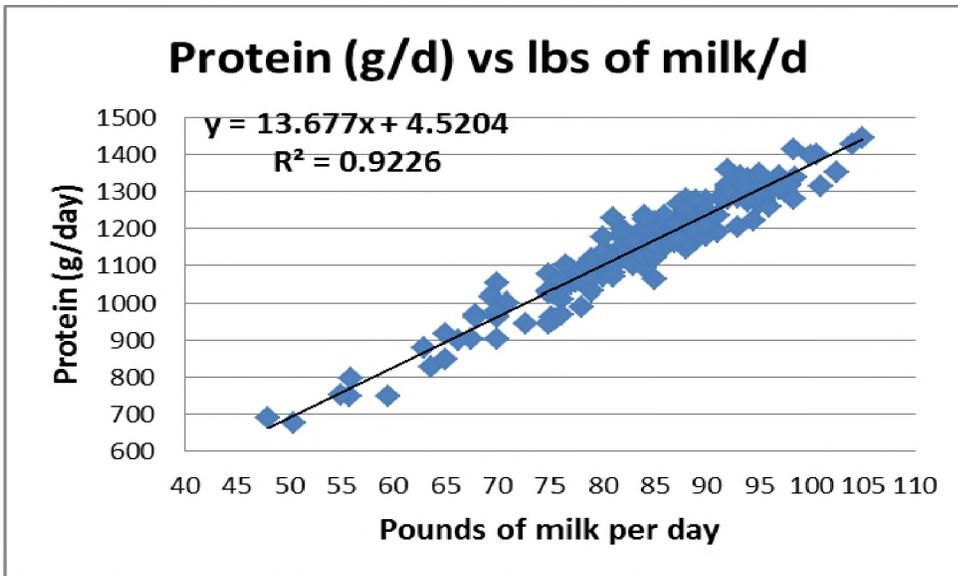


Figure 12. Grams of protein per cow per day and milk production (167 farms).

Overall, dairy cows have the potential to produce more grams of fat and protein per day if they produce more milk. But what drives milk production? The synthesis of lactose and increasing the grams per day output of lactose is fundamental to producing more pounds of milk per day. How often do we think about or look at how much lactose is being produced per cow per day? Does my lab even report a value for lactose and is the lactose value correct? Because there is no payment based on lactose nutritionists may ignore it. Lactose production is highly dependent on glucose metabolism in the cow. If you want to produce more milk per cow, you need to produce more lactose per day, as shown in Figure 13 below. The correlation is very strong. If you want 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. How do we feed for that?

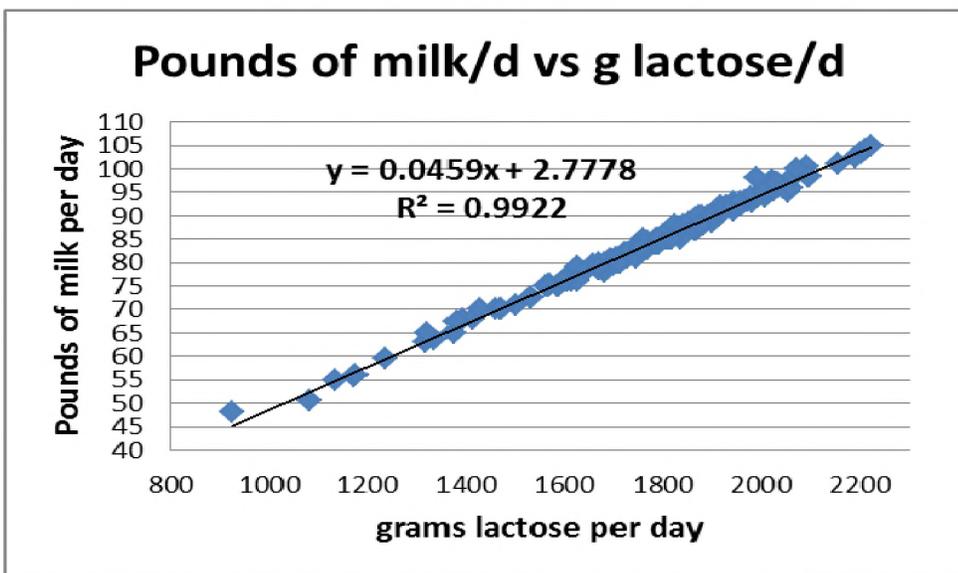


Figure 13. Grams of lactose produced per cow per day and milk production (167 farms).

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.

Stage of Lactation.

The concentrations of FA in milk changes with DIM and the changes are particularly large in early lactation 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 cows over a period of 3 years at the Miner Institute. The Miner Institute Holstein herd milked is 3 times per day. In July 2017 the DHI test results were: RHA of 29,711 lb (13,489 kg) milk, 1261 lb (572 kg) fat, 908 lb (412 kg) protein, 104,000 cells/mL weighted SCC, 94.6 lb (42.95 kg) test day milk/cow, 167 DIM, and 376 cows milking (388 yearly rolling average). Lactating diets are typically 50 to 60% forage with at least 2/3 of forage coming from corn silage. Grain mixes typically contain corn grain, soybean meal, commercial soy/canola products, byproducts, rumen inert fat, plus mineral and vitamin supplements. Diets are 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 for the Miner herd producing an average of about 92 lb (41.77 kg) per cow per day on TMR feeding system. The fat, protein, and lactose content of the milk are shown in Figure 16.

fThere are large changes in milk FA composition during the first 10 weeks of lactation

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.

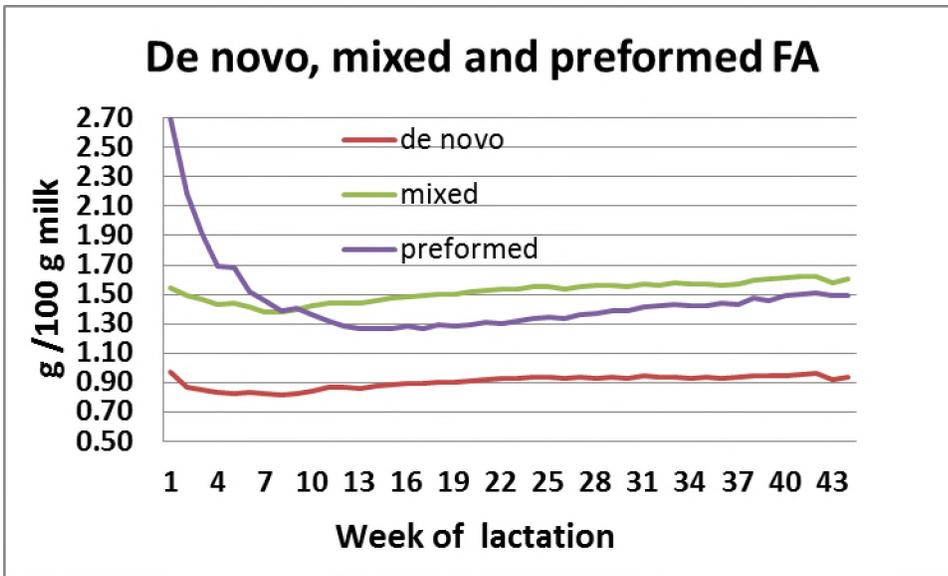


Figure 14. De novo, mixed, and preformed FA (g/100 g milk) over lactation for all cows.

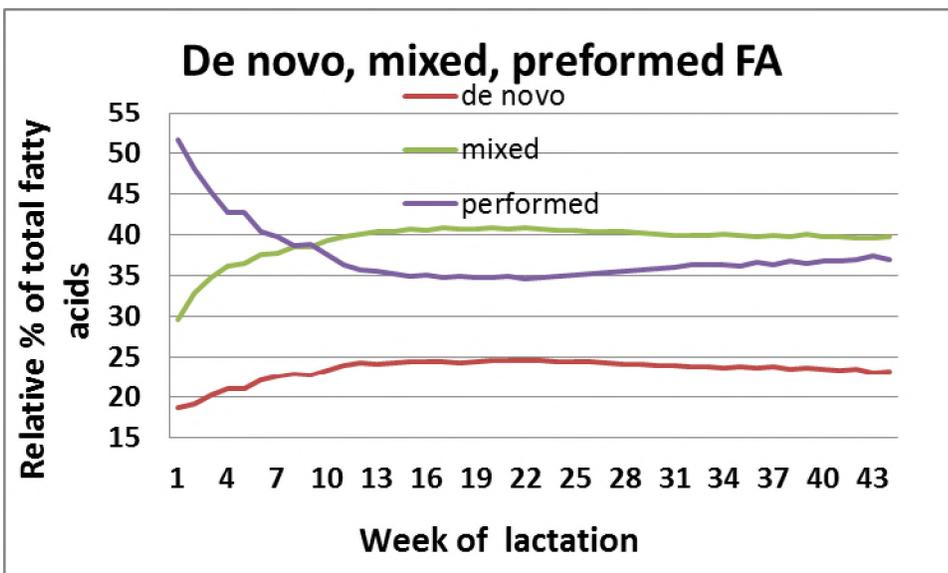


Figure 15. De novo, mixed, and preformed FA (relative %) over lactation for all cows.

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. The weight of FA divided by 0.945 is approximately equal to the fat test (g/100 g milk). This factor assumes that milk fat is about 5.5% by weight glycerol and 94.5% by weight fatty acids. Figure 16 represents the average of all cows in the herd, but the stage of lactation graph for 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.

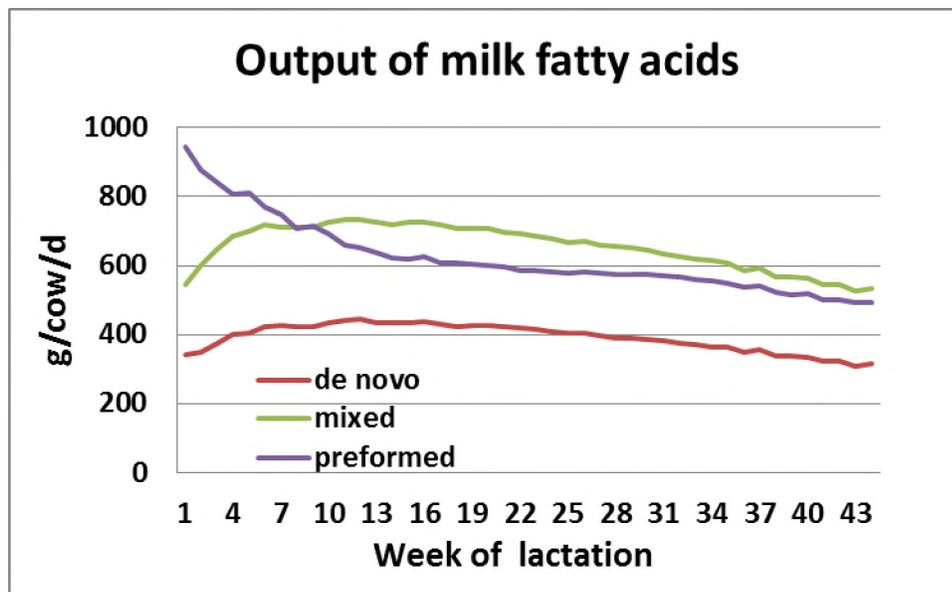


Figure 16. Stage of lactation production graph for all cows (g/cow/day).

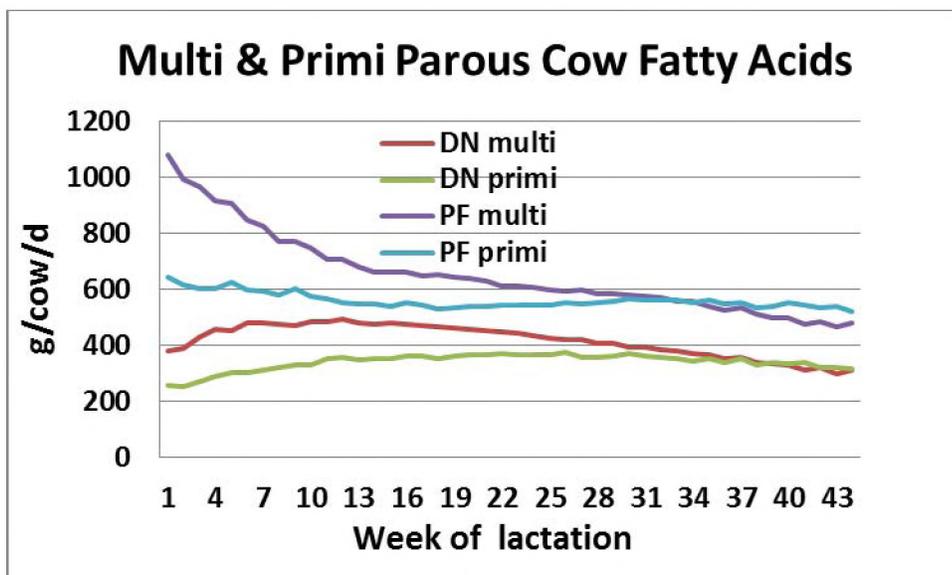


Figure 17. Stage of lactation: de novo and preformed FA for primi and multiparous cows.

Interpretation of Field Data on Bulk Tank Milks: Whole Herd Diagnostic.

Milk FA data will become more commonly available on bulk tank milks as milk payment testing laboratories adopt this new milk testing technology in combination with existing metrics of milk composition and milk quality. Given the factors shown above and the wide range of differences in farm management conditions and feeding, the data need to be interpreted with caution and complete knowledge of the management and ration on each farm is essential. Given those cautions, the new milk analysis data add a powerful new opportunity in precision management of milk production.

In looking at the bulk tank data from the 167 farms (Figures 9 to 13), the following questions and relationships in the data start to become apparent. For milk composition data from an individual farm the following data are useful for the full herd or for groups of cows:

- Milk per cow per day
- Milking frequency (2X or 3X) – milk and component output expected to be 10 to 15% higher on 3X farms
- Milk SCC (cells/mL)
- Milk MUN (mg/dL or mg/100 g milk)
- Milk fat unsaturation (double bonds per fatty acid)
- Milk fat (g/100 g milk and g/day production)
- Milk protein (g/100 g milk and g/day production)
- Milk lactose (g/100 g milk and g/day production)
- Milk de novo fatty acids (g/100 g milk and g/day production)
- Milk mixed origin fatty acids (g/100 g milk and g/day production)
- Milk preformed fatty acids (g/100 g milk and g/day production)

An example of how to look at the data and questions to ask:

Milk somatic cell count: cells/mL. What is the bulk tank milk SCC over a period of time? The bulk tank should be <200,000 cell/mL. If > 300,000 cell/mL, look at the milk lactose in g/100 g milk. If the lactose is 4.65 g/100 g milk or higher, the high bulk tank SCC is likely to be caused by a very small number of individual cows in the herd/group with very high SCC, while if the lactose is low (< 4.60 g/100 g milk) there is probably a more wide spread (i.e., more cows) incidence of cows with mammary infections. If the herd has a wide spread mastitis problem, that problem needs to be addressed first because it is negatively impacting the production of the herd.

Milk urea nitrogen: mg/dL. What is the concentration and day to day variation in MUN? If the MUN is >14 to 16, it is likely that rumen ammonia levels are too high. Lower ration input of dietary degradable protein or increasing available carbohydrates in the ration should be considered depending on the context of the complete ration composition. Another aspect of MUN is to look at the day-to-day variation in MUN within the same farm. MUN decreases rapidly when cows do not have access to feed. Thus, day to day variation in MUN within the same farm is an index of how consistently the farm is keeping feed accessible to cows on a continuous basis (i.e., feed bunk management).

Milk fat unsaturation: double bonds per FA. This is a useful index of what is happening in the rumen, but is less of a driver and more of a correlated outcome of other things that are happening. In general, as double bonds per FA increases milk fat decreases (Figure 4). A rule of thumb based on our observations for Holstein herds is that when the double bonds per FA is > 0.31, the probability of trans FA induced milk fat depression is greatly increased for Holstein milk. A word of caution is that there is a large stage of lactation impact on double bonds per FA and cows in the transition period will have a high double bond per FA without having trans FA induced milk fat depression. Thus, be careful with interpretation of milk fat unsaturation on groups of early lactation cows.

Lactose: grams per cow per day. Making more lactose per day (anhydrous lactose, not lactose by difference) makes more milk per day (see Figure 13). To have a high output of lactose per cow per day, glucose supply, transport, and metabolism need to be working very well. Without increasing lactose production in a Holstein cow, you cannot increase milk. Thus, figuring out how to manage cows to produce lactose is the key to getting more milk per cow per day and the partially correlated higher outputs of fat and protein per cow per day. Factors to consider are the production of propionate produced in the rumen and the undegraded starch that is leaving the rumen and available in the lower gastrointestinal tract. Also, is there some cow health issue (immune system activation) or environmental factor (e.g., heat stress) in the herd that is putting a demand on the glucose supply and reducing the glucose available for milk synthesis?

When milk production per cow per day is low in a Holstein herd, is synthesis of lactose the first thing a dairy nutritionist thinks about? It should be. If a 3X Holstein multiparous cow is going to produce a lactation average of > 85 lb (38.6 kg) of milk per day, she is going to need to produce at least an average of 1800 grams of lactose per day. This is the foundation upon which to build high fat and protein output per cow per day.

De novo and mixed origin fatty acids: g/100 g milk. There is a strong correlation between changes on de novo FA concentration in milk and bulk tank milk fat and protein tests (Figures 1, 2, 5, 6, 9 and 10). It is thought that the basis for the correlation between de novo FA and milk protein (Figure 8) is due to the higher microbial biomass that provides essential amino acids in support of milk protein synthesis in combination with rumen undegradable protein. For multiparous cows, stage of lactation has a large impact on de novo and mixed origin milk FA production. By pass feeding of palm-based fat supplements may also increase the mixed origin FA content of in milk (Piantoni et al., 2013). In general, when de novo (> 0.85 g/100 g milk) and mixed origin FA (>1.35 g/100 g milk) are high, it is an indication that rumen fermentation of carbohydrate is working well and the supply of volatile fatty acids from the rumen is good. This can be the case with either a high or lower level of milk (i.e., lactose) production. Fixing the low lactose production issue will likely allow the cows to maintain high concentration of de novo and mixed origin but increase their per day output of fat and protein given an adequate supply of their precursors.

Preformed fatty acids: g/100 g milk. The preformed FA do not normally vary so much within a herd across time in the bulk tank (1.2 to 1.4 g/100 g milk), unless there is some major change in diet/nutrition. However, it does change dramatically with stage of lactation and it can be very high for multiparous early lactation cows (Figures 14 and 15). As we have more experience with the milk FA metrics in the field, it may lead to strategies of using a different chain length of by-pass fat at different stage of lactation to better support maintenance of body condition and milk production at the appropriate times during lactation.

Fat and protein percent and g/cow per day. For multiparous cows, stage of lactation has a large impact on both parameters. Generally fat and protein in g/ 100 g milk and grams output per cow per day will be higher when de novo and mixed origin FA are high. Focusing on feeding and nutrition factors that support high production per cow per day of de novo and

mixed origin FA and lactose will maximize both milk fat and protein output per cow per day if there is an adequate supply of essential amino acids to support milk protein synthesis.

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 primi 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.

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