NEW MILK ANALYSIS TECHNOLOGIES TO IMPROVE DAIRY CATTLE PERFORMANCE

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INTRODUCTION

Two years ago we introduced the application of new mid-infrared (**mid-IR**) for rapid milk fatty milk analysis (Barbano, et al., 2014) and reported positive correlations of bulk tank milk fat test with a higher proportion and concentration of de novo fatty acids in bulk tank milk. The form of the fatty acid data from the mid-IR was structured to provide information on the relative proportions of de novo (C4 to C14), mixed origin (C16:0, C16:1, C17:0), and preformed (C18:0 and longer) fatty acids in milk. We can also provide that information in units of grams per 100 grams of milk. Since that time, we have continued to collect data on milk fatty acid variation in bulk tank milk and it's relationship to feeding and farm management. A field study of 20 Holstein and 20 Jersey farms was completed in 2014 (Woolpert et al., 2016) and a follow up study of 40 Holstein farms was completed in 2015 (Woolpert, 2016) with the objective of determining farm feeding and management practices relate to milk fatty acid compostion and bulk tank milk fat and protein concentration. Starting in February of 2016, information on milk fatty acid 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.

In addition, in the last 2 years we have expanded our milk analysis research on fatty acid analysis to individual cow milk samples at Cornell and in collarboration with Miner Institute in Chazy, NY. Additional work is in progress in collaboration with Penn State and Michigan State Universities. Today, I will focus on the use of milk fatty acid (**FA**) information for feeding management of dairy cows at the bulk tank level and report the status of our work on individual cow data, particularly transition cows.

EXPERIMENTAL APPROACH

Prior to the current study a group of partiail least squares (**PLS**) chemometric prediction models were developed from mid-IR spectra. The spectra of modified milk calibration samples (Kalylegian et al., 2006a,b), bulk tank milks, and individual cow milks were used in combination with chemical reference chemistry for fat (AOAC, 2000; method 989.05; 33.2.26), total protein (AOAC, 2000; method 991.20; 33.2.11 and nonprotein nitrogen (AOAC, 2000; method 991.21; 33.2.12) with true protein calculated by difference, anhydrous lactose (Lynch et al., 2007) and gas liquid chromatography (Barbano and Sherbon, 1980; Lynch et al., 1992) for FA analysis using a Varian CP-SIL88 capillary column [(100m x 0.25 mm x 0.2 µm film thickness), ID code # CP7489; Varian, Inc., Lake Forest, CA], installed in a Hewlett Packard 6890 GC System

equipped with an automatic liquid sampler and a flame ionization detector (Hewlett Packard Co., Wilmington, DE). A more complete descripiton of the fatty acid analysis methods and PLS model for fatty acid prediction model development was reported by Wojciechowski and Barbano (2016).

A library of chemometric prediction models for the major components in milk and milk FA composition for use on a Lactoscope FTA and Lactoscope CombiScope FTIR 600/300 (Delta Instruments, Drachten, The Netherlands) has been developed. A variety of individual FA and groups of FA were measured. The following individual FA were measured by mid-IR: C16:0; C18:0; C18:1 *cis*9, *cis*12; C18:1 t*rans* 10; andC18:1 *trans* 11. The following groups of FA were measured: total FA; DeNovo (C4:0 to C14:0), mixed origin (C16:0, C16:1, C17:0), preformed (C18:0 and longer); total unsaturated FA, total cis FA; total trans FA; mono unsaturated FA; and poly unsaturated FA. All FA measures produce results from the IR in grams of FA per 100 grams of milk. Some researchers have used the grouping of FA as short, medium, and long chain FA but the exact definition of those groups varies among researchers. The group definitions of de novo, mixed origin, and preformed FA are much more clear and consistent because they are based on the biochemical pathways for FA synthesis and have better potential to be correlated with the biology, metabolism, and feeding of dairy cows.

In addition to the measures of FA concentrations, two fat concentration independent measures of FA structure were also done on each sample: mean FA chain length (expessed as mean carbon number per FA) and mean FA unsaturation (expressed as double bonds per FA). The measure of total FA (not fat) in g/ 100 g of milk is used as a new basis for a more accurate measurement of total fat content in the milk. This approach eliminates most of the weakness of traditional measurses of fat by IR using the Fat A (C=O stretch) and Fat B (C-H stretch) because it compenstates sample by sample for differences in FA composition when trying to estimate the total fat content of the milk in comparison to ether extraction (Kaylegian et al., 2009a,b). The relative proportion of the total FA in milk that are represented by an individual or group of FA can be expressed on a relative basis as a precent of total FA in the sample. Thus, it is possible to produce a simulated gas chromatograph FA analysis of milk fat directly from the same (IR spectra) of milk tested on the IR for fat, protein, and lactose concentration.

The calibration adjustment of the fat, true protein, anydrous lactose and all FA measures on the IR milk analyzer is done once per month using a set of 14 modified milks described by Kaylegian et al. (2006a,b) that has reference values in (g FA per 100 g of milk) for each of the individual or groups of FA measured. The set of calibration samples is produced monthly at Cornell and was used to check the calibrations during the month.

RESULTS

2014 Farm Study (Woolpert et al., 2016)

This study investigated the relationship of management practices, diet characteristics, milk composition, and lactation performance with de novo fatty acid (FA) concentration in bulk tank milk from commercial dairy farms with Holstein, Jersey, and mixed breed cows. It was hypothesized that farms with higher de novo milk FA concentrations would more commonly use management and nutrition practices known to optimize rumen conditions that enhance de novo synthesis of milk FA. Farms (n = 44) located in Vermont and northeastern New York were selected based on a history of high de novo (HDN; 26.18 ± 0.94 g/100g FA; mean ± SD) or low de novo (LDN; 24.19 ± 1.22 g/100g FA) FA in bulk tank milk. Management practices were assessed during one visit to each farm in March or April, 2014. Total mixed ration samples were collected and analyzed for chemical composition using near infrared spectroscopy. There were no differences in days in milk at the farm level.

Yield of milk fat, true protein, and de novo FA per cow per day were higher for HDN versus 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. The difference in income per cow would depend on the actual milk price at any point in time. However, 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, respectively. Therefore, at 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 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 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. This research indicated that overcrowded freestalls, reduced feeding frequency, and greater dietary ether extract content are associated with lower de novo FA synthesis and reduced milk fat and true protein yields on commercial dairy farms.

2015 Farm Study (Woolpert, 2016)

The objective of this study was to evaluate the relationship of management practices and dietary factors with de novo fatty acid concentration in bulk tank milk from commercial dairy farms milking Holstein cows. Farms were selected based on de novo fatty acid concentration during the 6 mo previous to the farm visit and were categorized as high de novo (HDN; 24.61 ± 0.75 g/100 g of FA, mean ± standard deviation; n = 19) or low de novo (LDN; 23.10 ± 0.88 g/100 g of FA; n = 20). Farms were visited once in February, March, or April, 2015 and evaluated based on management and facility design known to affect cow behavior, physical and chemical characteristics of the diet,

and the ration formulation and forage analyses obtained from the farm's nutritionist. The mean milk composition for HDN and LDN farms is shown in Table 1.

No differences in milk, fat, and true protein yields were detected between HDN and LDN farms, but milk fat and true protein content were higher ($P < 0.01$) on HDN farms (Table 1). This positive relationship between de novo FA and milk fat and true protein percentage agrees with previous results of Barbano et al. (2014) who evaluated bulk tank milk composition on over 400 commercial dairy farms. De novo FA expressed as g/100 g of FA and as g/100 g milk were higher ($P < 0.01$) on HDN farms, and preformed FA expressed as g/100 g of FA and as g/100 g milk were lower ($P < 0.01$ and $P = 0.02$, respectively) on HDN farms. These results are consistent with previous research (Woolpert et al., 2016) that indicated that HDN farms have higher milk fat and true protein content in bulk tank milk. De novo FA yield, expressed as g/d, was higher ($P < 0.01$) for HDN farms with no difference detected in milk yield ($P = 0.91$) suggesting that cows on HDN farms synthesized more de novo FA. However, milk weights per cow were not measured directly, but were estimated indirectly based on the number of cows milking on the day of the farm visit and the average bulk tank milk shipped per day during the month of the farm visit. Thus, the uncertainty in milk weight data was higher than the uncertainty in milk composition data. Consequently, further research is needed under conditions where milk weight per cow per day can be accurately measured, along with milk composition, to determine whether greater de novo FA synthesis is always associated with greater milk fat and true protein yields.

There were no differences in farm size, time away from the pen for milking, days in milk, or body condition score for HDN versus LDN farms. No differences between HDN and LDN farms in milk, fat, or true protein yield were detected; however, milk fat and protein content and de novo fatty acid yield per day were higher for HDN farms, as was gross income per unit of milk sold.

The relationships between various milk fatty acid parameters across 40 farms and bulk tank milk fat test are shown in the Figures 1 thru 5 below.

Table 1. Least squares means of milk composition factors for high de novo (HDN) and low de novo (LDN) farms for the month of the farm visit.

Item	HDN	LDN	SEM	*P* value
Milk yield, kg/d	31.9	32.1	0.9	0.91
Fat, %	3.98	3.78	0.04	<0.01
Fat, kg/d	1.27	1.21	0.03	0.25
De novo fatty acids[1]				
g/100 g milk	0.99	0.86	0.01	<0.01
g/100 g FA	25.99	23.78	0.22	<0.01
g/d	315.6	276.2	9.5	<0.01
Mixed fatty acids[2]				
g/100 g milk	1.48	1.35	0.02	<0.01
g/100 g FA	38.86	37.36	0.37	<0.01
g/d	472.0	434.2	15.2	0.08
Preformed fatty acids[3]				
g/100 g milk	1.32	1.38	0.02	0.02
g/100 g FA	34.60	38.21	0.50	<0.01
g/d	419.0	439.3	10.4	0.17
True protein, %	3.19	3.08	0.02	<0.01
True protein yield, kg/d	1.02	0.99	0.03	0.44
MUN, mg/dL	12.1	12.9	0.5	0.25
Anhydrous lactose, %	4.65	4.66	0.02	0.66
Anhydrous lactose, kg/d	1.46	1.51	0.05	0.51

[1] C4 to C14.
[2] C16, C16:1, and C17.
[3] Greater than or equal to C18.

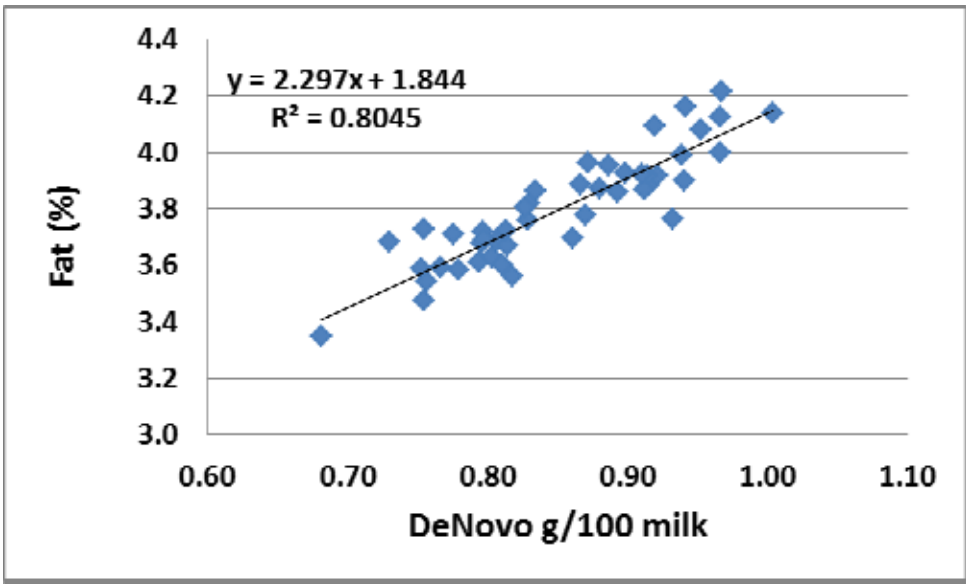

Figure 1. Relationship of bulk tank milk fat test to concentration (g/100 g milk) of de novo fatty acids in milk. In general, a farm needs to have a concentration of de novo fatty acids higher than 0.85 g/100 g milk to achieve a bulk tank fat test higher than 3.75%.

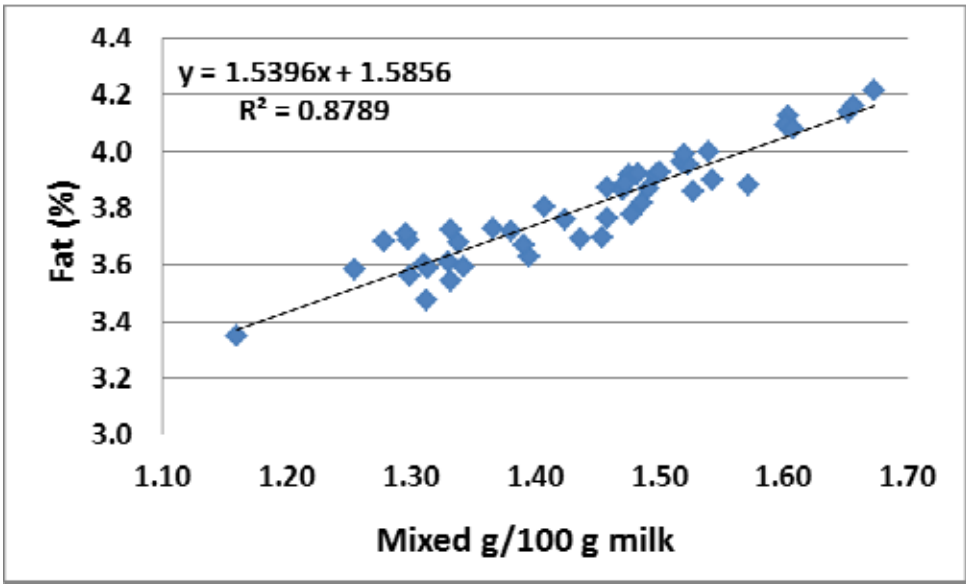

Figure 2. Relationship of bulk tank milk fat test to concentration (g/100 g milk) of mixed origin fatty acids in milk. In general, a farm needs to have a concentration of de novo fatty acids higher than 1.40 g/100 g milk to achieve a bulk tank fat test higher than 3.75%.

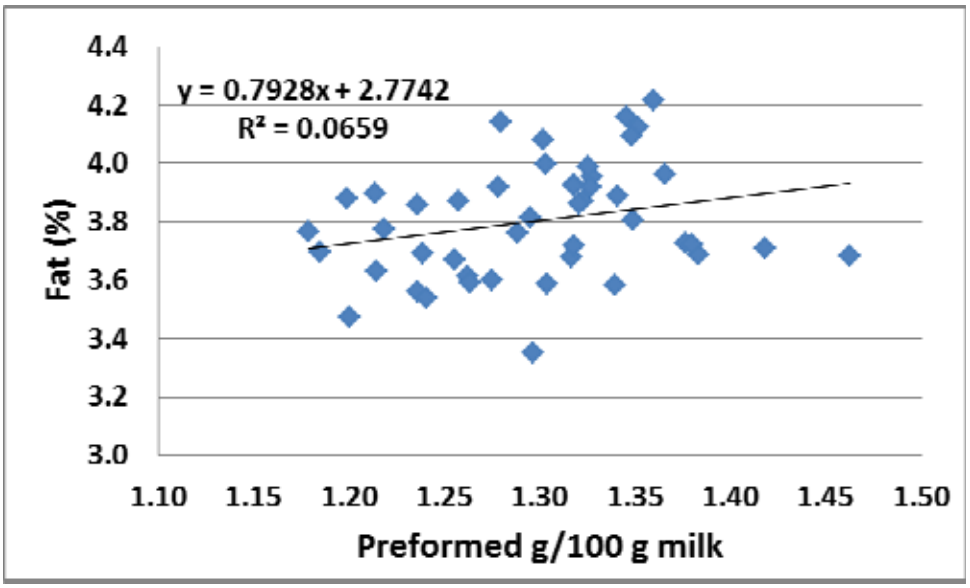

Figure 3. Relationship of bulk tank milk fat test to concentration (g/100 g milk) of preformed fatty acids in milk. In general, the variation in preformed fatty acid concentration in Holstein herds is less than de novo and mixed origin fatty acids and is not well correlated with bulk tank milk fat test.

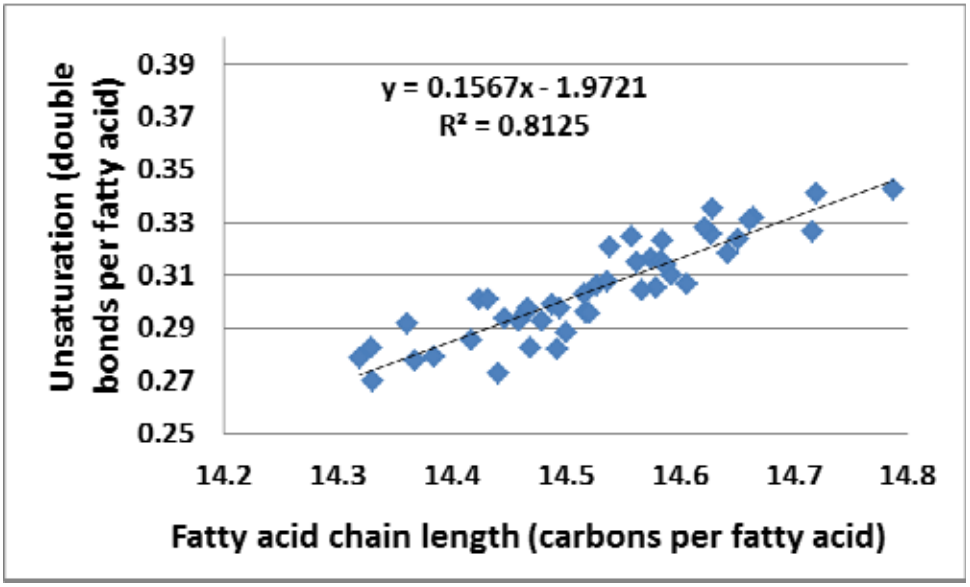

Figure 4. Relationship of bulk tank milk fat fatty acid unsaturation to fatty acid chain length. As fatty acid chain length fatty acid unsaturation increases and this appears to be due mostly to an increase in oleic acid (C18:1 cis 9).

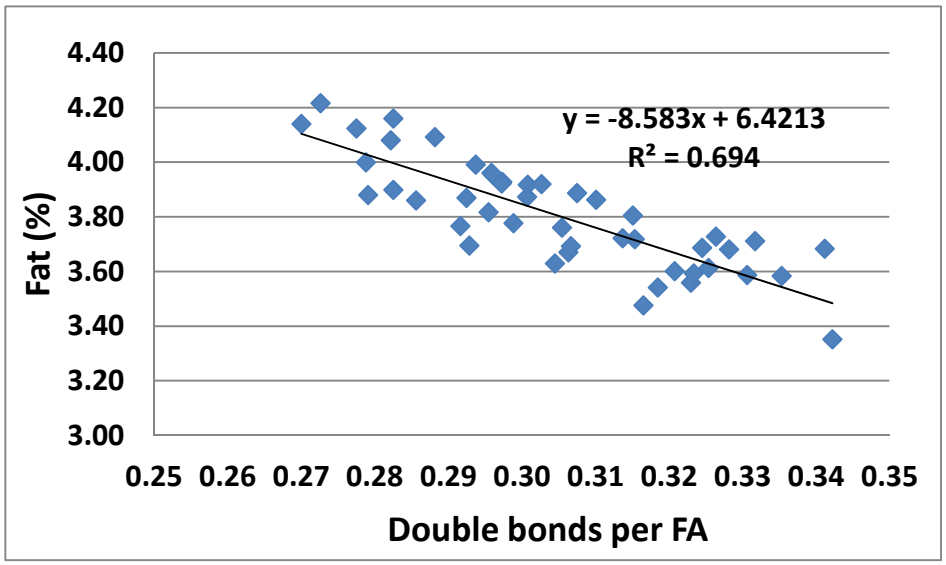

Figure 5. Relationship of bulk tank milk fat fatty acid unsaturation with bulk tank milk fat test. As double bonds per fatty acid increases the bulk tank milk fat test decreases. To achieve a 3.75% fat test a farm needs to have a double bond per fatty acid of less than 0.31. The double bonds per fatty acid may be an indirection of the rumen unsaturated fatty acid load (RUFAL) and the rate of unsaturated fat release from forage sources (e.g., corn silage, distiller grains, and oil seeds) in the rumen. The double bonds per fatty acid may be an index of the level of milk fat depression in a dairy herd.

The relationship between de novo milk fatty acid concentration across 40 farms and bulk tank milk protein test is shown in the figure below.

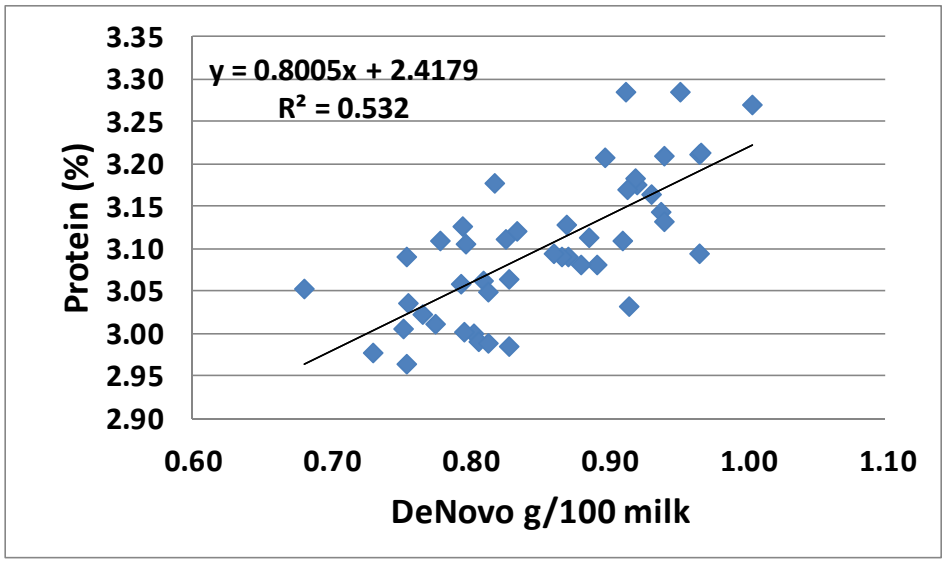

Figure 6. Relationship of bulk tank milk protein test to concentration (g/100 g milk) of de novo fatty acids in milk. In general, a farm needs to a concentration of de novo fatty acids > 0.85 g/100 g milk to achieve a bulk tank fat test higher than 3.10% true protein.

It is hypothesized that when de novo fatty acid production is high, the the biomass of rumen microflora is high and this provides a higher level of essential amino acids produced in the rumen. When double bonds per fatty acid increase bulk tank milk protein test decreases (data not shown).

The difference in income per cow between HDN and LDN herds would depend on the actual milk price at any point in time. However, 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, respectively. Therefore, at 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 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 30 kg of milk would result in a gross income difference of $9,125 for fat and $6,935 for true protein per 100 milking cows per year. High de novo farms tended to be more likely to deliver fresh feed twice versus once per day, have a freestall stocking density less than or equal to 110%, and provide greater than or equal to 46 cm of feed bunk space per cow. There were no detectable differences in forage quality or ration dry matter, crude protein, or starch content. However, ether extract was lower and physically effective neutral detergent fiber was higher for HDN compared with LDN farms. The results of this study indicate that feeding management, stocking density, dietary ether extract content, and the physical characteristics of the diet are related to de novo fatty acid, fat, and protein concentration in bulk tank milk from high-producing Holstein dairy farms.

SUMMARY OF BULK TANK MILK TESTING

The key FA parameter that was positively correlated with bulk tank milk fat and true protein concentration was *DeNovo* FA (g/100 g milk). Structural parameters of FA chain length (carbon number) and total unsaturation (double bonds /FA) were negatively correlated with fat and protein (g/100 g milk). This was true for both Jersey and Holstein. In general, a Holstein farm needs to have a concentration of de novo fatty acids higher than 0.85 g/100 g milk and a concentration of mixed origin fatty acids higher than 1.35 g/100 g milk to achieve a bulk tank fat test higher than 3.75%. As double bonds per fatty acid increase both fat and protein will decrease. Double bonds per fatty acid may be an index of effective RUFAL level in diet. Keeping the milk double bonds per fatty acid at 0.3 or lower produce higher milk and protein. Over crowding of cows in pens was correlated with lower de novo and mixed origin fatty acids and lower milk fat and protein test. Generally, when de novo fatty acid production is higher milk production per cow will be equal to or higher than when de novo is lower, but both milk fat and protein test (g/100 g of milk) will be higher. This will increase the income per unit of milk produced.

Milk Testing for Individual Cows (Barbano et al., 2015)

As the milk production per cow has increased, there is more demand placed on the physical and metabolic system of each individual dairy cow. More attention through automated information collections systems to the metabolic and physical condition of each cow is needed to keep each cow healthy and productive. Because each cow makes an individual contribution to both farm costs and income, it becomes a management challenge particularly in large dairy herds, to make each cow a "cow-of-interest" and make correct decision about health and reproduction to achieve improved overall performance of the dairy herd.

To achieve a focus on individual cow status, measurement of de novo, mixed origin, and preformed fatty acids in milk is also useful for individual cow milk testing, particularly during the transition period. The changes in de novo fatty acids are a relative percentage of total fatty acids reflects the energy balance status of the cow. Recently, we have developed a new milk mid-IR test that produces an estimate of blood NEFA level by testing the milk. This testing would be done on the same milk sample at the same time as the fat, protein, lactose, solids, MUN and fatty acid analysis using the mid-IR milk analyzer.

High blood NEFA indicates that a cow is mobilizing body fat and increases the risk of metabolic disorders. Milk and blood samples were collected from 60 lactating Holsteins once per week for the first 3 weeks of lactation. Cows were milked 3 times per day. Within + or – one milking of the time of blood collection, a milk sample was analyzed using a Delta Instruments (model FTA) mid-IR milk analyzer. A Wako NEFA HR test kit was used as an *in vitro* enzymatic colorimetric method for the quantitation of NEFA in blood serum and these values were used as reference values for development PLS regression model to predict blood NEFA from the mid-IR milk spectra. There are no NEFA in milk, so a model to predict blood NEFA from a milk sample uses differences in the milk spectra from sample to sample that are correlated with changes in blood NEFA. The final PLS model had 9 factors, used wavelengths in the following ranges (3000 to 2800, 1800 to 1700, 1585 to 1000 cm^{-1}) with a standard error of cross validation of 172 µEq/L. Validation milk and blood sample pairs (n = 53) were collected from Holstein cows from a different herd. The mean value for the blood reference test was 713 µEq/L of serum and the mean value for the milk based blood NEFA prediction was 703 µEq/L of serum with a standard deviation of the difference (SDD) of 218 µEq/L for the 53 validation samples. Blood NEFA measured on blood is a snapshot of the NEFA concentration at an instant in time, while blood NEFA predicted from milk analysis represents a time average for the total time between milkings. The FTIR milk analysis to estimate blood NEFA is rapid (about 10 seconds), done simultaneously with all other milk component and fatty acid measures, and uses no reagents. This approach could be useful for rapid evaluation of risks of ketosis, displaced abomasum and possibly reproductive disorders. The relationship between the milk estimated blood NEFA level and the change in de novo milk fatty acids may have predictive to power to provide an advanced warning that a cow is going to have a displaced abomasum.

Concepts for integration of mid-IR milk analysis directly into the milking systems on large farms are being considered. The combination of milk weight and the component concentrations (i.e., fat, protein, lactose, and milk NPN/Urea content) will allow calculation of energy output in the milk and in combination with feed input data will allow an estimate of energy and protein balance of individuals or groups of cows within the herd.

Some other measures that we have developed for use in individual cow milk testing are predicted blood NEFA for ketosis prediction, in addition to milk BHB and acetone concentrations. We are developing a milk estimated blood BHB method currently. The measurement and rate of change of blood NEFA estimated by milk analysis during the early transition period will provide a view of the metabolic status combined with energy balance estimates. Indirect measurement of rumen pH through milk analysis is in development and might provide insight into how a cow is interacting the complex mixture of nutrients in the rumen, as that impacts the chemistry of the milk.

Combinations of individual parameters that provide more predictive indices of feed efficency, ketosis, and probability of successful breeding may be derived from the current PLS models for milk analysis. In the future, development of models to determine pregnancy status and loss of pregnancy will bring further benefit in the applications of mid-IR milk testing for real-time farm management milk testing.

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