Prediction of Blood Non-Esterified Fatty Acid and Fatty Acid Analysis of Individual Cow Milk Samples

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Abstract

The overall objective of our research program on mid-infrared (**IR**) milk analysis is to develop a suite of milk analysis measurements that can be used as precision farm management tools. Currently, we have developed and tested rapid milk fatty acid analysis tools that can be used to determine the outcome of feeding and farm management changes by measuring de novo, mixed origin, and preformed milk fatty acids. These measures have been applied to a large number of dairy herds over a 4 year period, and a positive relationship between milk de novo fatty acid content and bulk tank milk fat and protein concentration were observed. Two 40 farm field studies in 2 different years (2014 and 2015) were conducted and farm management and feeding practices were identified that were related to higher milk de novo fatty acid content and higher bulk tank fat and protein tests. In both years, the high de novo farms had higher fat and protein tests. The high de novo farms tended to have more feed bunk space per cow, lower free stall stocking density, and had lower fat content in the ration. In 2014, at 25 kg/cow/day of milk, the average high de novo (**HDN**) farm earned a gross of $5.50 and $7.72/cow for fat and protein, respectively. The average low de novo (**LDN**) farm at 25 kg/cow/day milk earned a gross of $5.26 and $7.29/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. In 2015, at 30 kg/cow/day of milk, the average HDN farm earned a gross of $5.00 and $5.49/cow for fat and protein, respectively. The average LDN farm at 30 kg/cow/day milk earned a gross of $4.01 and $5.30/cow for fat and protein, respectively. These differences for fat and 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 protein per 100 milking cows per year. When the fatty acid analysis method and the newly developed blood nonesterified fatty acid test based on an mid-Fourier transform infrared (**FTIR**) analysis of milk are applied to milks from individual cows on a weekly basis, the metabolic status with respect to fat mobilization, ketosis, and displaced abomasum in transition cows can be rapidly determined. Work is on-going to determine how to best use mid-FTIR milk testing for real-time farm management decision making.

Introduction

Mid-IR transmittance milk analysis has been used routinely for about 40 years to measure fat, protein, and lactose contents of milk for both payment of dairy farmers and analysis of individual cow milk for dairy herd improvement record keeping. The sample preparation technology is simple (i.e., does not

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use any chemical reagents), the milk is only warmed to about 40oC, mixed, and pumped directly into the instrument. The instruments can be purchased in various configurations that will test between 50 and 600 samples per hour for all measured components. The basis of the measurement of fat, protein, and lactose is the vibration of specific chemical bonds that are characteristic in the structure of fat, protein, and lactose in milk. The first generation of mid-IR milk analyzers used individual optical filters in pairs, that selected 2 bands (sample and reference) of wavelengths to measure each milk component (Kaylegian et al., 2009). Each filter was mounted in a filter wheel within the instrument and each filter was rotated into the light path, and an absorbance reading at each band of wavelengths was recorded. In the late 1990's, with the routine use of lasers and affordable computing power, there was a significant change in the internal optical system of IR milk analyzers from a physical optical filter system to an interferometer based Fourier transform (**FT**) optical system. With this change, a complete mid-FTIR spectra of every sample was produced within the instrument. At that time, neither the equipment manufacturers nor the users of the instruments knew what to do with the additional information at other wavelengths within the full spectra. Therefore, the first versions of FTIR instrument software reduced the spectra to a copy of what the optical filters would have produced (i.e., created virtual filters within the software) and then used information to predict fat, protein, and lactose contents of milk by the traditional filter approach (Kaylegian et al., 2009). There were 2 immediate advantages of the change in hardware: 1) the analysis speed could be increased and 2) consistency of virtual filters from one instrument to another was much better and would enable achievement of better agreement of results among instruments.

With time, both researchers within the instrument manufacturing companies and other groups of researchers started to explore the rest of the mid-IR spectra to determine if there was other information that could be used to predict other characteristics of milk. Partial least square (**PLS**) regression analysis was used to analyze the absorbance data from the full spectra. One of the first new parameters to be measured by this approach was milk urea nitrogen (**MUN**), with the goal of using the new information as a dairy herd management tool to evaluate how effectively dairy cows were using protein in the dairy ration. The MUN is closely related to blood urea nitrogen and thus the milk analysis becomes a proxy for collecting and analyzing a blood sample for urea. Over the years, this measurement has become routine in dairy herd improvement (**DHI**) milk testing and has also been included in most bulk milk testing for herd management informational purposes, along with the milk payment testing for milk fat, protein, and solids. With time, researchers developed other measures of milk characteristics based on information in the mid-FTIR spectra of milk. The beauty of this approach is that it only takes computer analysis of the spectra to do this. The analysis time and procedure for milk analysis by the instrument remains the same. So additional value is derived from the same milk spectra, while the per sample operational cost is virtually the same. The cost of adding new measures is the cost of development of the new PLS models.

As a result, PLS models have been developed to measure milk beta-hydroxyl butyrate (**BHB**) and milk acetone (Duffield et al., 1997; de Roos et al., 2006; Rutten et al., 2009; van Knegsel et al., 2010). Soyeurt et al. (2006) developed PLS models to measure the fatty acid composition of the milk fat portion of milk directly from the milk spectra. These new PLS prediction models were targeted mainly for producing data to be used for genetic selection of

cows, but more recently, their value as potential herd management tools has been evolving. The PLS approach continues to be applied to develop of metrics that may be useful in the dairy industry.

Milk Fatty Acid Composition

Bulk tank milk

Soyeurt, et al. (2006) quantified milk fatty acids by mid-FTIR, but they also measured some groups of fatty acids (e.g., saturated, monounsaturated, and polyunsaturated). The information on groups of fatty acids was of primary interest to dairy product manufacturers because these groups of fatty acids need to be listed on the nutritional label of dairy foods, but can also be applied to milk from individual cows. It was thought that if a rapid measurement of these groups of fatty acids was available, then it might be feasible to use genetic selection or feeding approaches to produce less saturated fat and more unsaturated fat. Some progress can be made in this area of modification of milk fatty acid composition with bypass fat feeding. However, in practice, it is very difficult to make large enough changes in milk fatty acid composition for the Food and Drug Administration to allow a food label claim.

Another potential application of mid-IR fatty acid measurement would be to obtain milk fatty acid data in a form that would be useful for more tactical feeding and farm management decision making. To address this application, Barbano et al. (2014a,b) were the first to develop PLS fatty acid prediction models to measure groups of fatty acids as they relate to the biosynthetic origin of the milk fatty acids (i.e., de novo – C4 thru C14, mixed origin – C16, and preformed > C18) from mid-FTIR spectra of milk. Once these fatty acid prediction models were developed and operational in the software of a commercial infrared milk analyzer (Delta Instruments, Model FTA, Drachten, The Netherlands), a survey of bulk tank milk fatty composition was initiated with the St Albans Cooperative Creamery (St Albans, VT). Because the fatty acid results are derived from the same sample and spectra used to obtain the milk payment test for fat and protein, we were able to start collecting data on milk fatty acid composition of bulk tank milk from 430 farms in the cooperative at a frequency of 3 to 20 times per month. At the present time, we have 4 years of data for these farms. Barbano et al. (2014a,b) reported a positive relationship between increasing levels of de novo fatty acids as a percentage of milk total fatty acids, grams of de novo fatty acids per 100 grams of milk, and the fat and protein concentration in bulk tank milk. In general, as de novo fatty acids increased, fat (Figures 1a,b) and protein (Figure 2a,b) concentration in the bulk tank increased for both Holstein and Jersey farms. Given this relationship that we observed in data from the 430 farms, we selected a subpopulation of 40 farms (20 low de novo and 20 high de novo) in 2014 and then another 40 farms again in 2015 to determine differences in feeding and management practices between high and low de novo herds, the relationship to bulk tank milk composition, and differences in milk payment.

The 2014 field study identified management practices (Woolpert et al., 2015), such as higher stall stocking density and lower feeding frequency that were related to lower de novo fatty acid (**FA**) content in bulk tank milk. Farms with lower de novo FA, on average, produced less milk fat and protein per cow per day. Milk yield tended to be higher for HDN farms (P = 0.06). Milk fat yield, protein yield, and protein content were higher (P = 0.01) on HDN farms, while milk fat content tended to be higher (P = 0.10). The higher milk fat and protein yields per cow per day for HDN farms

would indicate that gross milk income per cow was higher on HDN farms during the period of the study. The difference in income per cow would depend on the actual milk price at any point in time. However, the average fat and protein prices 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/cow/day of milk, the average HDN farm earned a gross of $5.50 and $7.72/cow for fat and protein, respectively. The average LDN farm at 25 kg/cow/day milk earned a gross of $5.26 and $7.29/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.

In the 2015 study (Woolpert, 2016), cow comfort indicators and dietary physically effective neutral detergent fiber (**peNDF**) were related to de novo FA concentration in bulk tank milk on high-producing, Holstein dairy farms. Again, both management (i.e., frequent feed delivery and increased feed bunk space per cow) and dietary factors (i.e., adequate peNDF and lower ether extract) that differed between HDN and LDN farms have been shown to affect rumen function; therefore, de novo FA concentration may be an important tool to monitor cows' rumen function on commercial dairy farms. However, the average fat and protein price for the Federal Milk Order No. 1 for February through April, 2015 was $4.19 and $5.74 per kg, respectively. Therefore, at 30 kg of milk/cow/day, the average HDN farm earned a gross of $5.00 and $5.49/cow for fat and protein, respectively. The average LDN farm at 30 kg/cow/day milk earned a gross of $4.01 and $5.30/cow for fat and protein, respectively. These differences for fat and 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 protein per 100 milking cows per year.

Individual cow milk

Recently, we have applied the mid-IR milk fatty acid analysis models to individual cow milks. Lynch et al. (1992) reported that milk fatty acid composition (based on gas chromatography analysis) for individual cows changed systematically with days in milk, particularly during the transition period. Generally, the relative percentage of total fatty acids that are de novo fatty acids increases with days in milk and becomes relatively stable for the remainder of lactation after cows have reached positive energy balance. We have begun monitoring individual cow milks from the dairy herd at Miner Institute using a mid-FTIR (Delta Instruments, Model FTA, Drachten, The Netherlands) at the farm at 3 consecutive milkings, one day per week, and we have observed the same milk fatty composition behavior as was reported by Lynch et al., 1992. However, there is considerable cow-to-cow variation in level and the temporal patterns of change in the relative proportions of the de novo, mixed, and preformed milk fatty acids that seem to reflect real-time cow-to-cow differences in energy balance and metabolic health status of individual cows. Generally, healthy cows at day 7 in lactation that do not have excessively high blood NEFA will have a relatively high percentage of total fatty acids that are de novo fatty acids (20% or higher), and with increasing days in milk, the de novo value as a proportion of total fatty acids should be in the range of 27 to 30% of total fatty acids when the cow reaches positive energy balance. Generally, the mixed origin fatty acids as a percentage of total fatty acids will increase with days in milk and preformed fatty acids will decrease.

Blood NEFA

The concentration of nonesterfied fatty acids (**NEFA**) in the blood of lactating dairy

cows is used as an index of how much fat is being mobilized by a dairy cow from adipose tissue at the beginning of lactation. When blood NEFA and blood BHB are too high, cows are susceptible to a range of metabolic health issues, such as displaced abomasum, ketosis, retained placenta, and others (Ospina et al., 2010; McArt et al., 2012). Barbano et al. (2015) were the first to report and validate a blood NEFA prediction model based on the analysis of milk samples from individual cows. Milk and blood samples were collected from 60 lactating Holstein cows 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 mid-IR milk analyzer (Delta Instruments, model FTA, Drachten, The Netherlands). A Wako NEFA HR test kit (WAKO Chemicals USA, Inc., Richmond, VA) 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 of a 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 uEq/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 uEq/L of serum and the mean value for the milk based blood NEFA prediction was 703 uEq/L of serum with a standard deviation of the difference (**SDD**) of 218 uEq/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 measures, and uses no reagents. This approach could be useful for rapid evaluation of risk for ketosis, displaced abomasum, and possibly reproductive disorders. In the same test on the same milk, the fatty acid composition of the milk fat is also determined. We have observed that there is a relationship between the milk estimated blood NEFA concentration and the change in de novo fatty acids as a percentage of total fatty acids. The combination of many measured parameters in milk as a group and their inter-relationships may have predictive power to provide an advanced warning that a cow is going to have a displaced abomasum.

Conclusions

The application of mid-IR analysis of both bulk tank and individual cow milk samples for parameters that may be useful in support of farm management decision making has potential to enable farm managers to improve the economic performance and sustainability of milk production by improving feed efficiency. Farm management and feeding practices that increase de novo fatty acids as a percentage of total milk fatty acids is correlated with achievement of higher fat and protein tests in the bulk tank. In studies on individual farms where data on milk produced per cow was collected, the production per cow was the same or higher for high de novo fatty herds, so there was higher output per day of fat and protein when de novo fatty acid was higher. More individual cow diagnostic tests using mid-FTIR milk analysis are being developed. In larger herds, the possibility of an economically feasible approach to on-farm, real-time milk analysis by mid-FTIR should be explored as a management tool.

Acknowledgments

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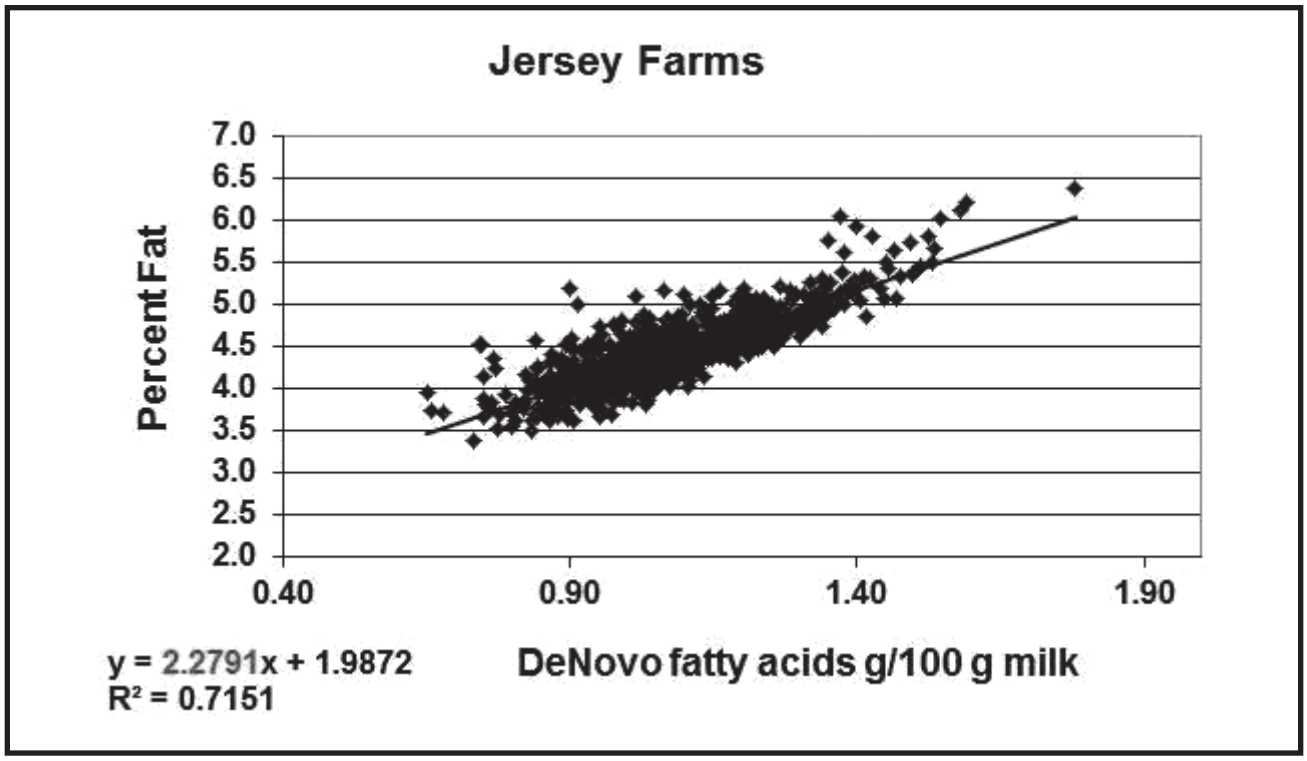

Figure 1a. Percent fat in the bulk tank milk plotted as a function of de novo fatty acids (grams per 100 grams of milk) for Jersey farms.

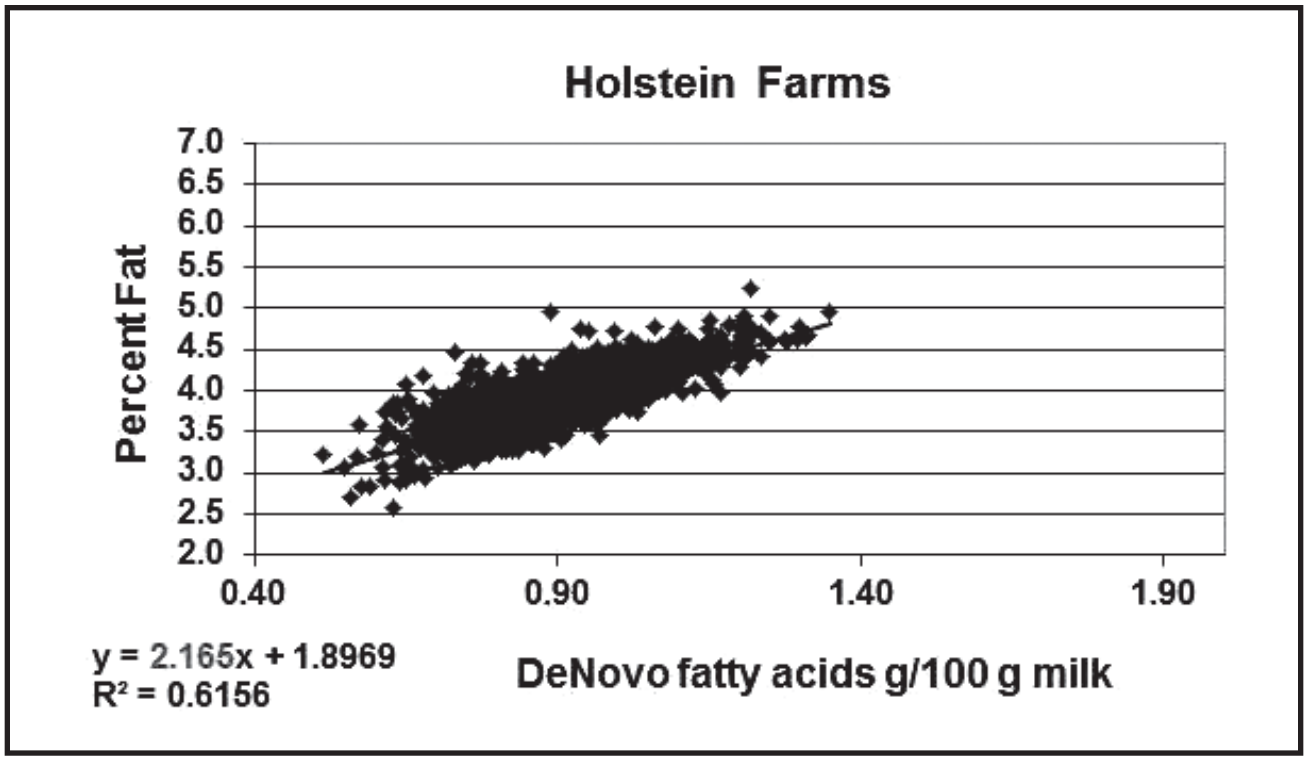

Figure 1b. Percent fat in the bulk tank milk plotted as a function of de novo fatty acids (grams per 100 grams of milk) for Holstein farms.

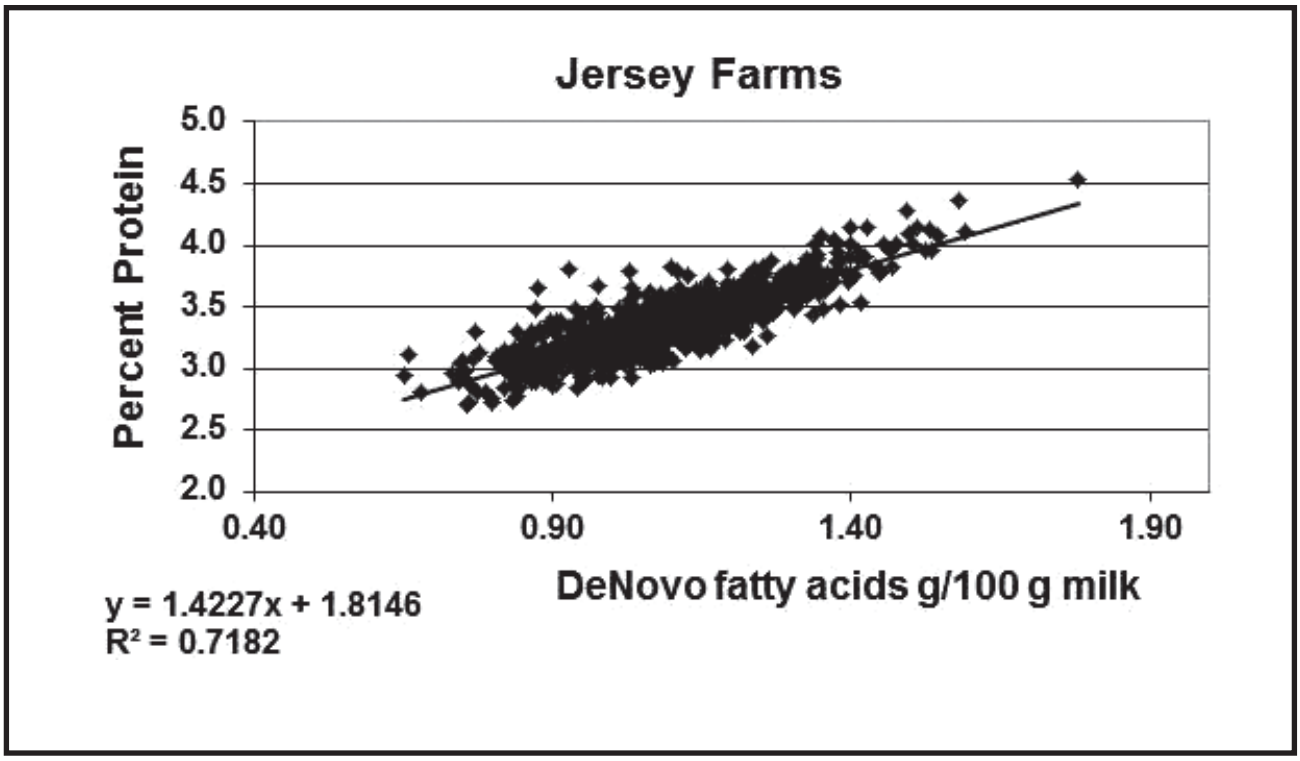

Figure 2a. Percent true protein in the bulk tank milk plotted as a function of de novo fatty acids (grams per 100 grams of milk) for Jersey farms.

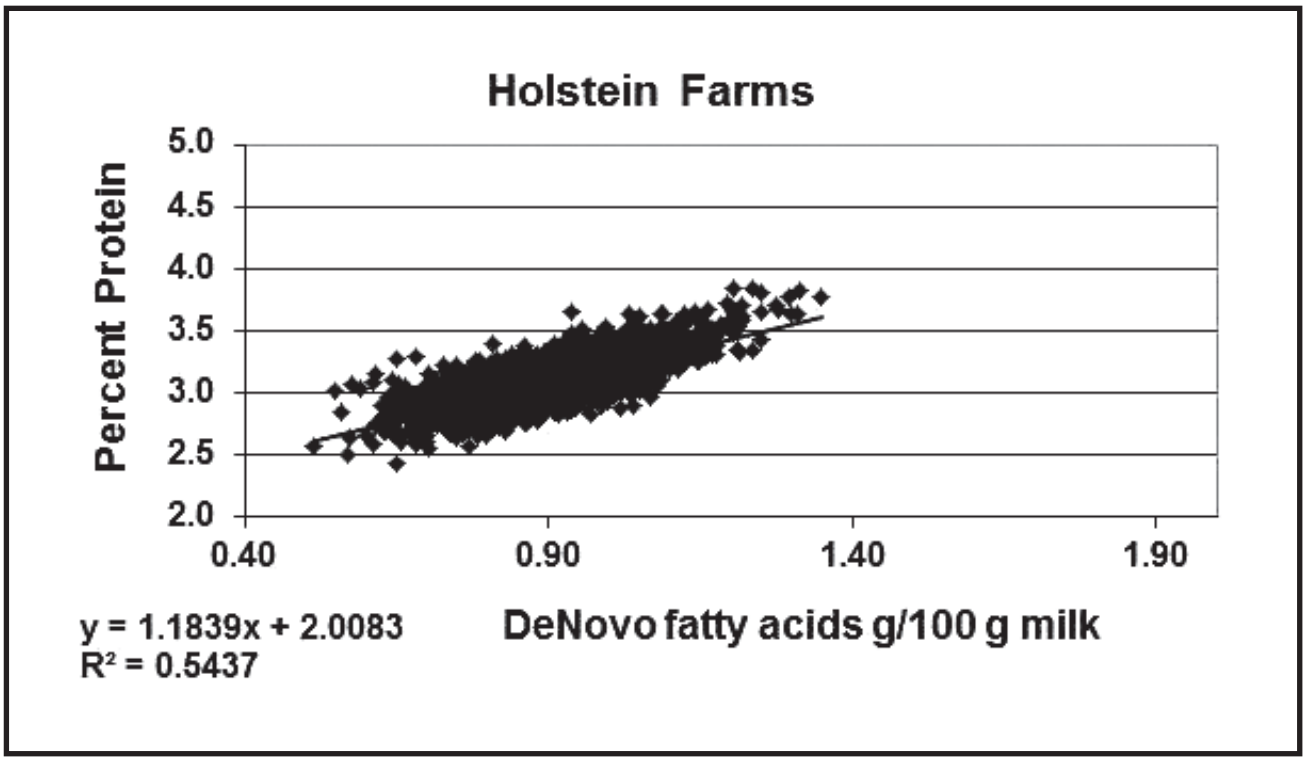

Figure 2b. Percent true protein in the bulk tank milk plotted as a function of de novo fatty acids (grams per 100 grams of milk) for Holstein farms.