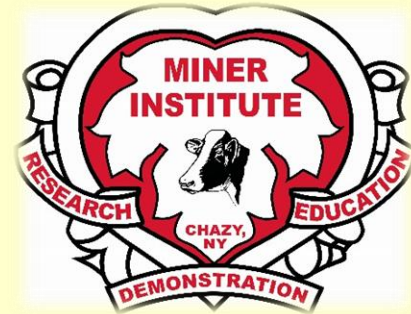
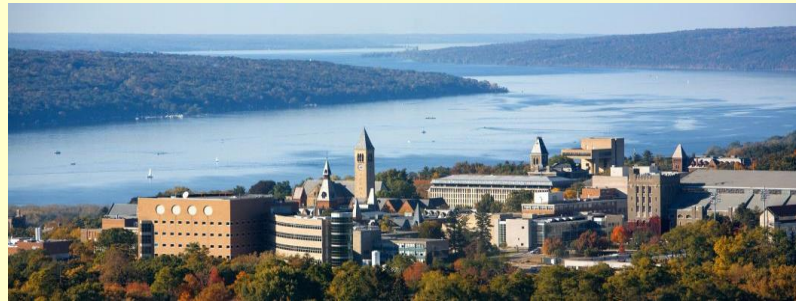


New milk mid-FTIR metrics for dairy cattle management

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„Milch bietet mehr!“, Cologne, Germany

March 25, 2019

Milk Analysis for Dairy Herd Management

- **Past:** Where we have been?
- **Present:** Where we are?
- **Future:** Where we are going?

Milk Analysis for Dairy Herd Management

- **Past: Where we have been?**
 - **2014:** CNC meeting first presentation on IR milk fatty acid analysis for dairy herd management. Introduction of a rapid method to measure de novo, mixed origin, and preformed fatty acids and fatty chain length and unsaturation.
 - The first herd management data was reported from the St Albans Cooperative (430 farms) showing a strong positive correlation between bulk tank milk fat and protein test and de novo fatty acid concentration using the models developed at Cornell.

PLS Model Development

- **Herd Management – fatty acid and protein models**
 - **Eskildsen et al. 2014 (JDS 97:7940-7951) correctly indicated the prediction of milk fatty acid can be challenging given the natural interrelation (collinearity) between total fat and milk fatty acid composition.**

PLS Model Development

- **Herd Management – fatty acid models**

Therefore, the approach used in our work to develop the new herd management models was designed to eliminate the collinearity issue in the modeling itself.

This was done by using about 20% of the PLS modeling set as milks that had a wide range of concentration of fat, protein, and lactose with no correlation among these components and no correlation with the change in milk fatty acid. The remainder of the samples in the modeling set were bulk tank milks and individual cow milks designed to provide a wide range in milk fatty acid composition. The output of the models is g/100 g milk for all concentration based models. The models are calibrated (slope and intercept adjustment) with an orthogonal sample set with a wide range in fatty acid concentration.

Milk Analysis

- **PLS (partial least squares) Models**
 - **PLS statistical performance metrics from modeling**
 - **Modeling statistics – RPD**
 - RPD is the standard deviation of the reference chemistry values of the population of samples used for the modeling divided by the standard error of cross validation (SECV) in a one out CV validation PLS modeling
 - How do you interpret an RPD value.
 - Models with RPD's less than 3 are generally very weak analytically. They might be useful for sorting sample into those with high and low predicted values
 - Models with RPD's between 3 and 5 can be good for qualitative screening.
 - Models with RPD's > 6 are good for quantitative analysis.
 - **External Validation of Model Performance**

Herd Management Fatty Acid Models

Woolpert et al. 2016.
J. Dairy Science.
99:8486–8497. First
generation herd
management models and
field study results.

Current Mid-FTIR PLS prediction models used on Delta Instruments

	Total FA	De novo FA	Mixed FA	Preformed FA
Number of samples	268	268	268	268
Mean	3.36	0.83	1.03	1.51
SD	0.9	0.26	0.29	0.41
Minimum	0.19	0.05	0.06	0.08
Maximum	6.15	1.82	2.02	2.51
Number of factors	8	10	9	11
SECV	0.019	0.025	0.047	0.056
R-square	0.999	0.991	0.975	0.981
F-Ratio (PRESS)	1.1623	1.272	1.165	1.105
F-Test (FPRESS)	0.8894	0.975	0.893	0.792
RPD	47.6	10.4	6.2	7.3

Herd Management Fatty Acid Models

Wojciechowski and
Barbano. 2016. J. Dairy
Science. 99:8561–8570.

Parameter	MIR PLS prediction models	
	Chain length	Unsaturation
Number of samples	268	268
Mean	14.55	0.33
SD	0.24	0.04
Minimum	13.95	0.22
Maximum	15.43	0.47
Number of factors	9	10
SECV	0.112	0.012
F-Ratio (PRESS)	1.07	1.10
F-Test (FPRESS)	0.70	0.78
RPD	2.1	3.3

Fatty Acid Reference Chemistry: Publications

Gas chromatography method. A detailed description of the methylation, gas chromatography conditions, recovery of denovo fatty acids. **Wojciechowski and Barbano. 2016. J. Dairy Science. 99:8561–8570.**

Standardization of Calculation of denovo, mixed origin, preformed, fatty acid chain length, and double bonds per fatty acid **reference values** with the specific group of fatty acids included in the calculation. If other fatty acids are included in the routine calibration reference the results will not be comparable.
Kaylegian et al. 2009. J. Dairy Science. 92:2502–2513.

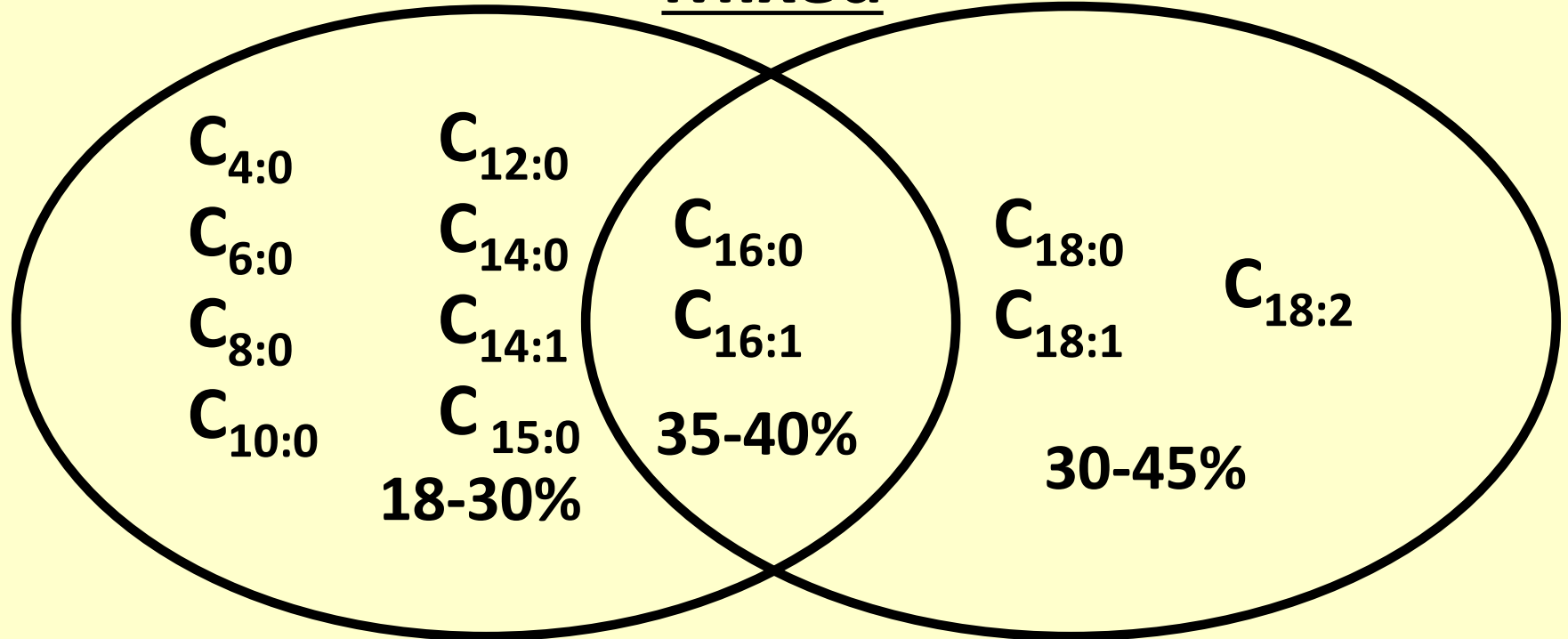
Calibration sample production. The method of production of the orthogonal calibration sample set is described. In the future, there will be modifications (addition of an orthogonal MUN and a fatty chain length and double bond taper).
Kaylegian et al. 2006. J. Dairy Science. 89:2817–2832

Milk Fatty Acid Origin

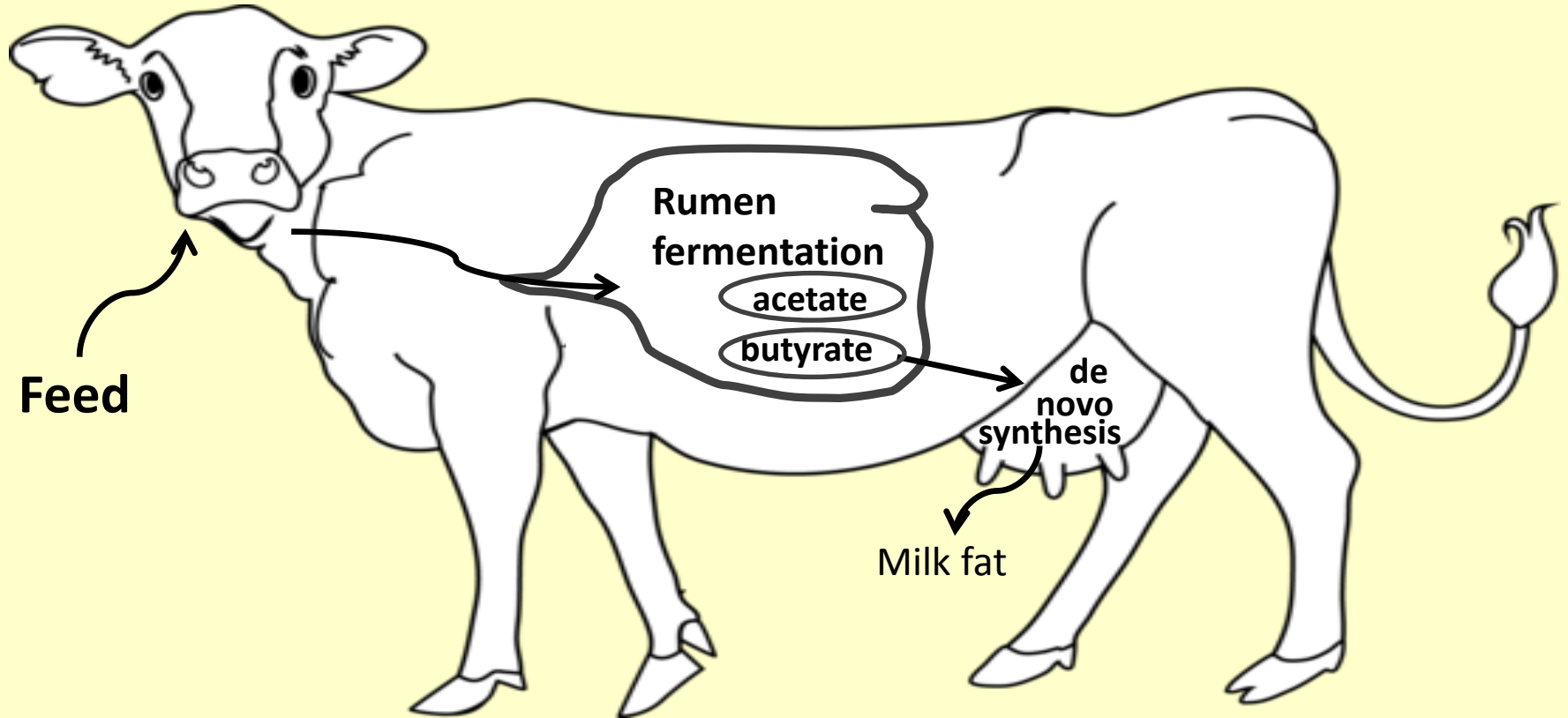
De novo

Mixed

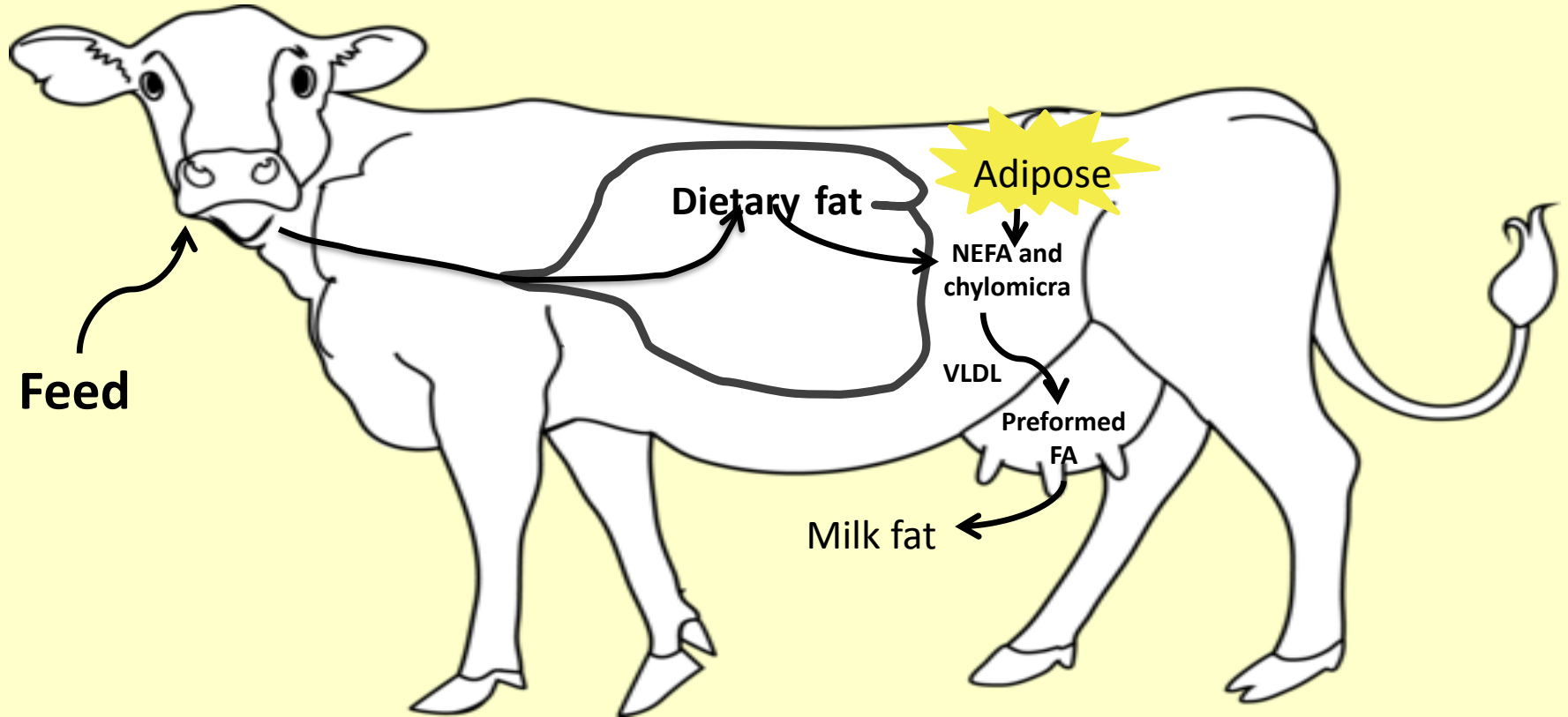
Preformed



De novo Fatty Acid Synthesis



Preformed Fatty Acids



Milk Analysis for Dairy Herd Management

- **Past: Where we have been?**
 - **2016: CNC meeting: Introduction to the “Cow of Interest” and the quest for real-time dairy herd management detailed milk analysis began.**
 - **Results were presented for two 40 herd field studies showing herd management factors that influence de novo fatty acids and fat and protein tests.**
 - **Graphs of relationship between milk fatty acids and bulk tank fat and protein tests for Holsteins.**
 - **First introduction of milk estimated blood NEFA by mid-infrared milk analysis.**

What Do **Dairy Farmers** Want?

In the end, milk production is all about the sum of the performance of all the individual cows. The farmer needs **information** upon which to make decisions, not data.

So how can today's new technology be better harnessed to manage each individual cow?

Each cow needs to be a “**Cow of Interest**”

Main Conclusions from Bulk Tank Milks

The **strongest correlation** between milk fatty acid composition and the concentration of fat and protein in milk **was with *de novo* fatty acid production.**

De novo fatty acid level seems to be barometer of rumen health and proper rumen function.

Thus, feeding and farm management strategies that produce an increase in synthesis of *de novo* fatty acids may produce an increase milk fat and milk protein percentage and possibly output of fat and protein per cow per day.

In the field studies, over crowding showed up clearly as a factor causing low de novo fatty acids, lower bulk tank fat and protein tests.

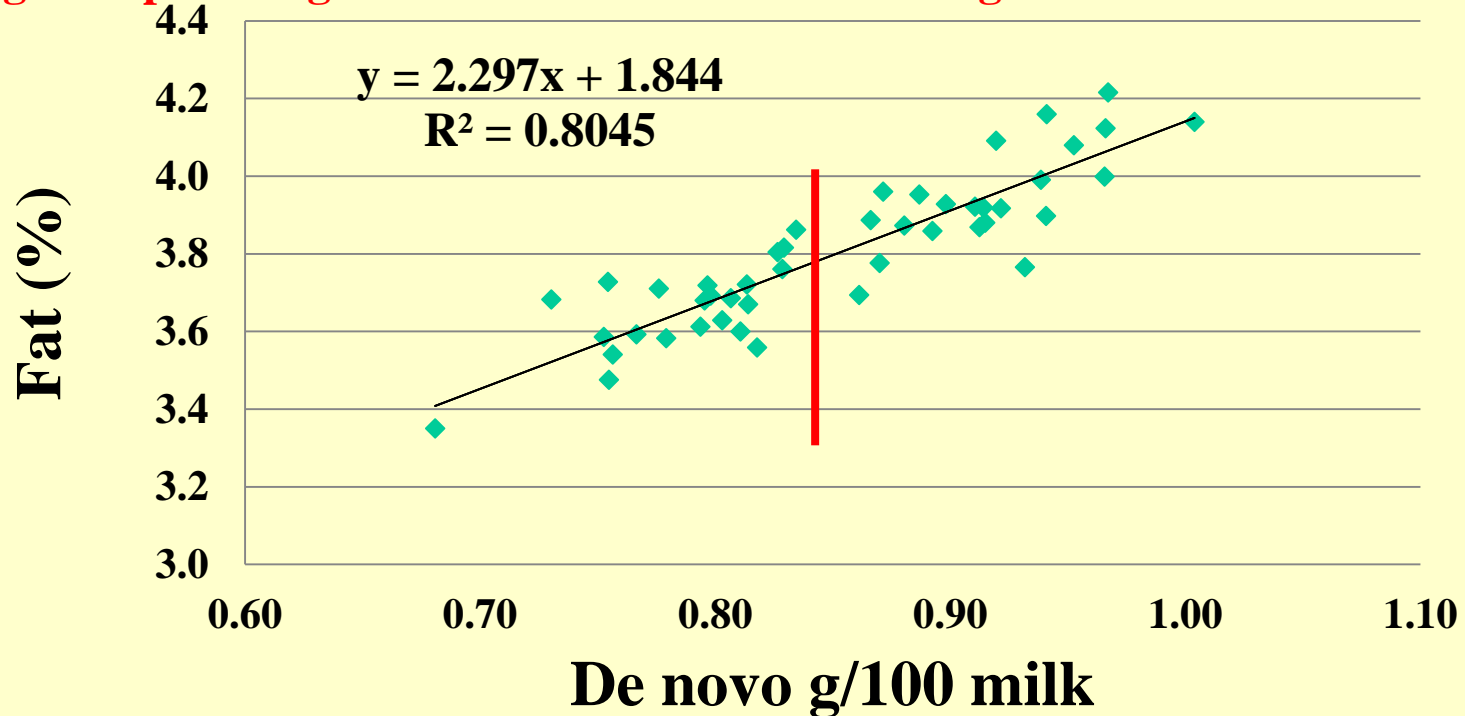
Milk Analysis for Dairy Herd Management

- **Past: Where we have been.**
 - **2016: CNC meeting: Introduction to the “Cow of Interest” and the beginning of quest for real-time dairy herd management milk analysis.**
 - **Results presented for two 40 herd field studies showing herd management factors that influence de novo fatty acids and fat and protein tests. Herds with higher de novo fatty acids had higher production of kg of fat and protein per cow per day.**
 - **Graphs of relationship between milk fatty acids and bulk tank fat and protein tests for Holsteins.**

40 Holstein Farms 2015

St Albans - Fat

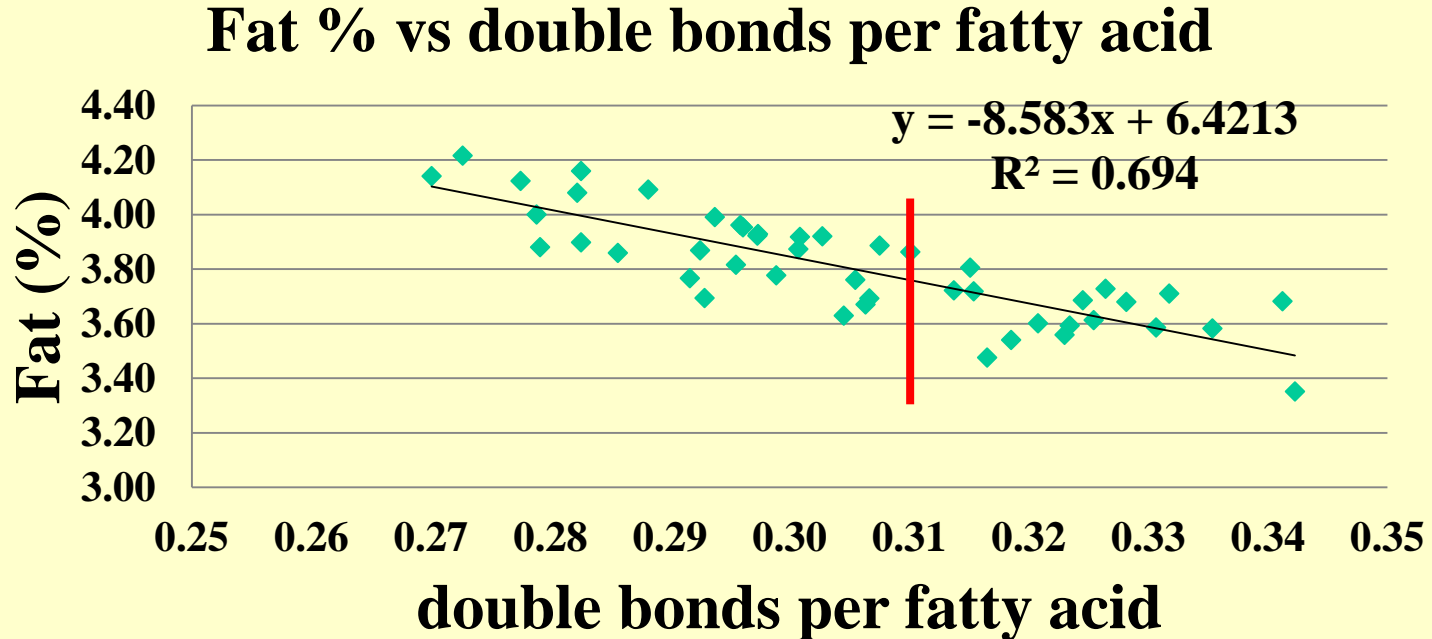
If you want a fat test > 3.75% fat in bulk tank with Holsteins, then the de novo fatty acids in grams per 100 grams of milk needs to be > 0.85 g/100 milk



40 Holstein Farms 2015

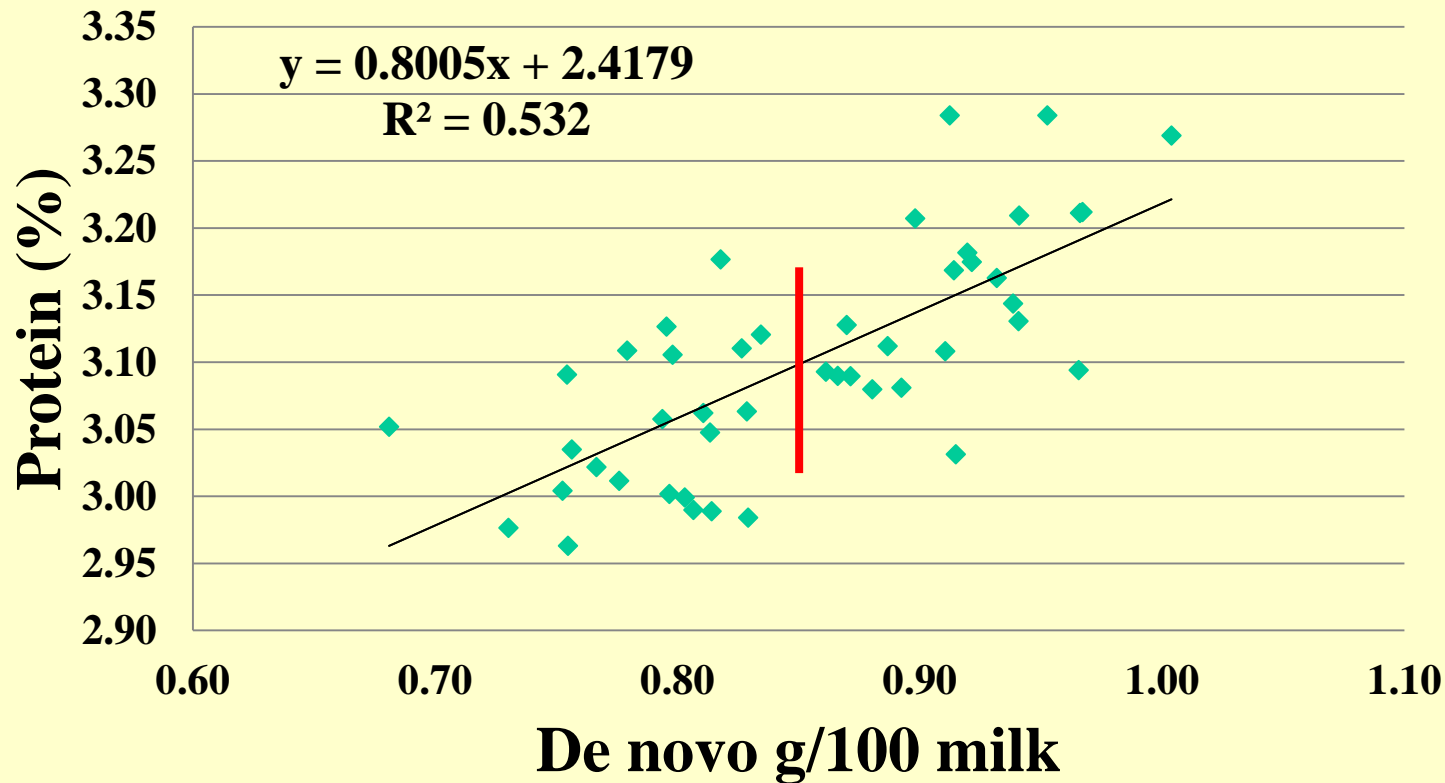
St Albans - Fat

If you want a fat test > 3.75% fat in bulk tank with Holsteins, then the double bonds per fatty acid in milk fat needs to < 0.31.



40 Holstein Farms 2015

St Albans - Protein

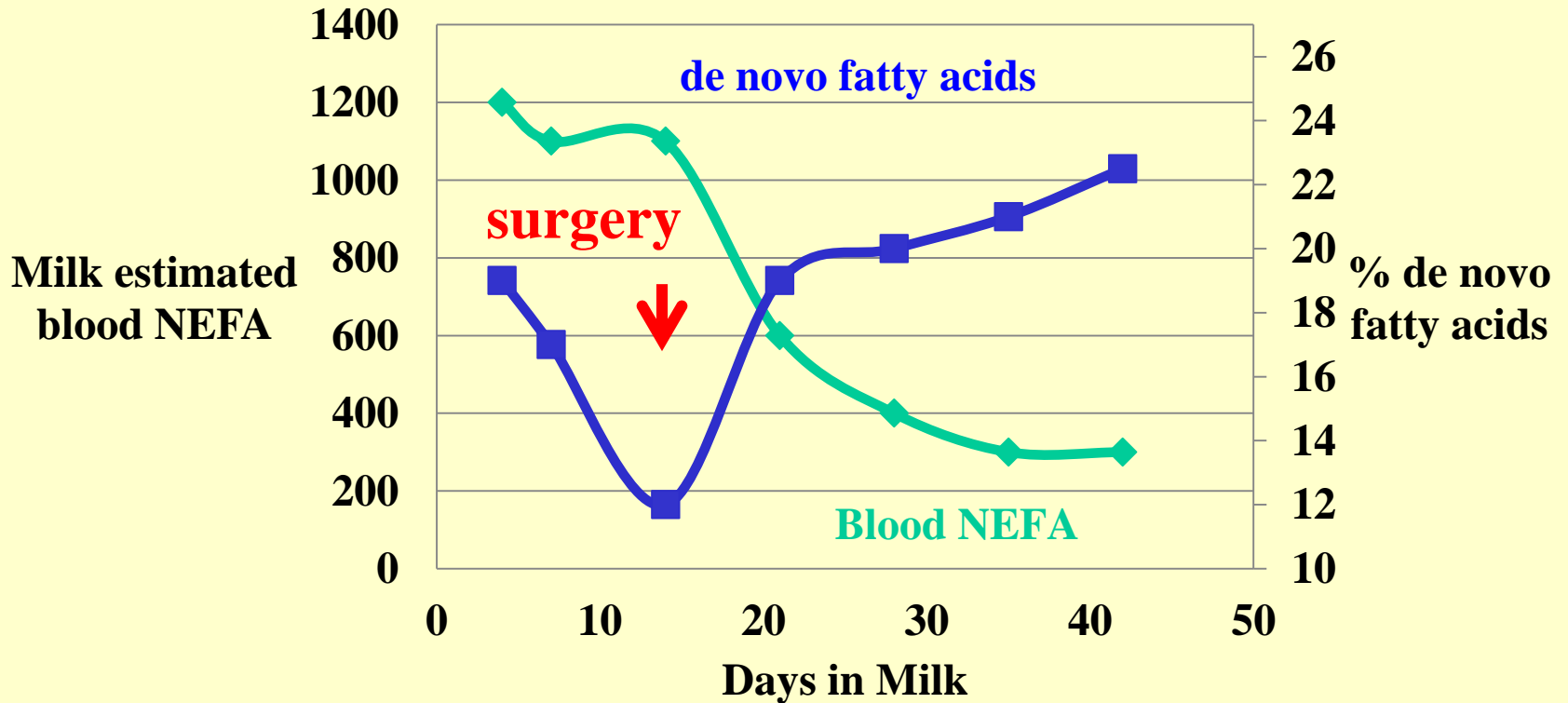


Milk Analysis for Dairy Herd Management

- **Past: Where we have been.**
 - **2016: CNC meeting: Introduction to the “Cow of Interest” and the beginning of quest for real-time dairy herd management milk analysis.**
 - Results presented for two 40 herd field studies showing herd management factors that influence de novo fatty acids and fat and protein tests.
 - Graphs of relationship between milk fatty acids and bulk tank fat and protein tests for Holsteins.
 - First introduction of milk estimated blood NEFA testing by mid-infrared milk analysis.

Sample Individual Cow Health Data

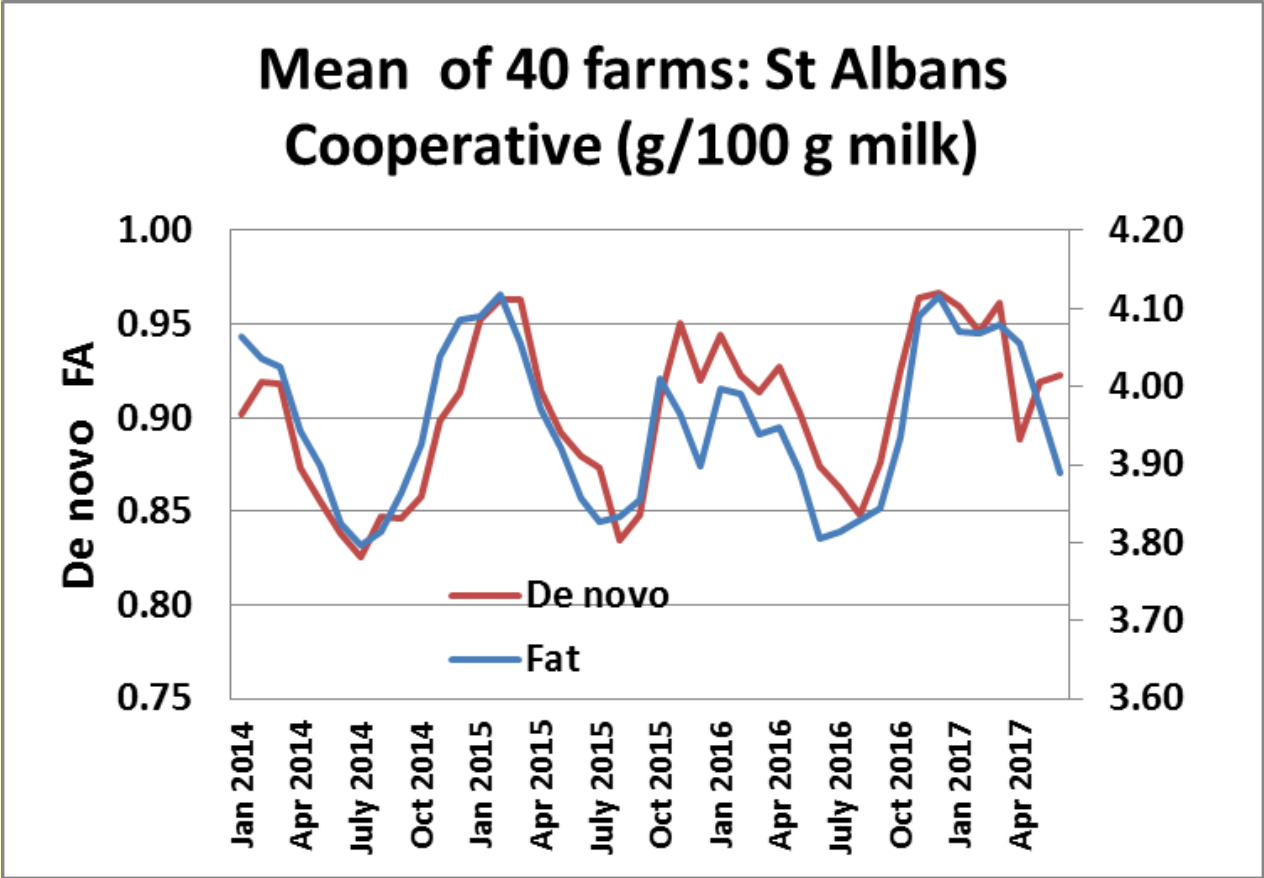
displaced abomasum



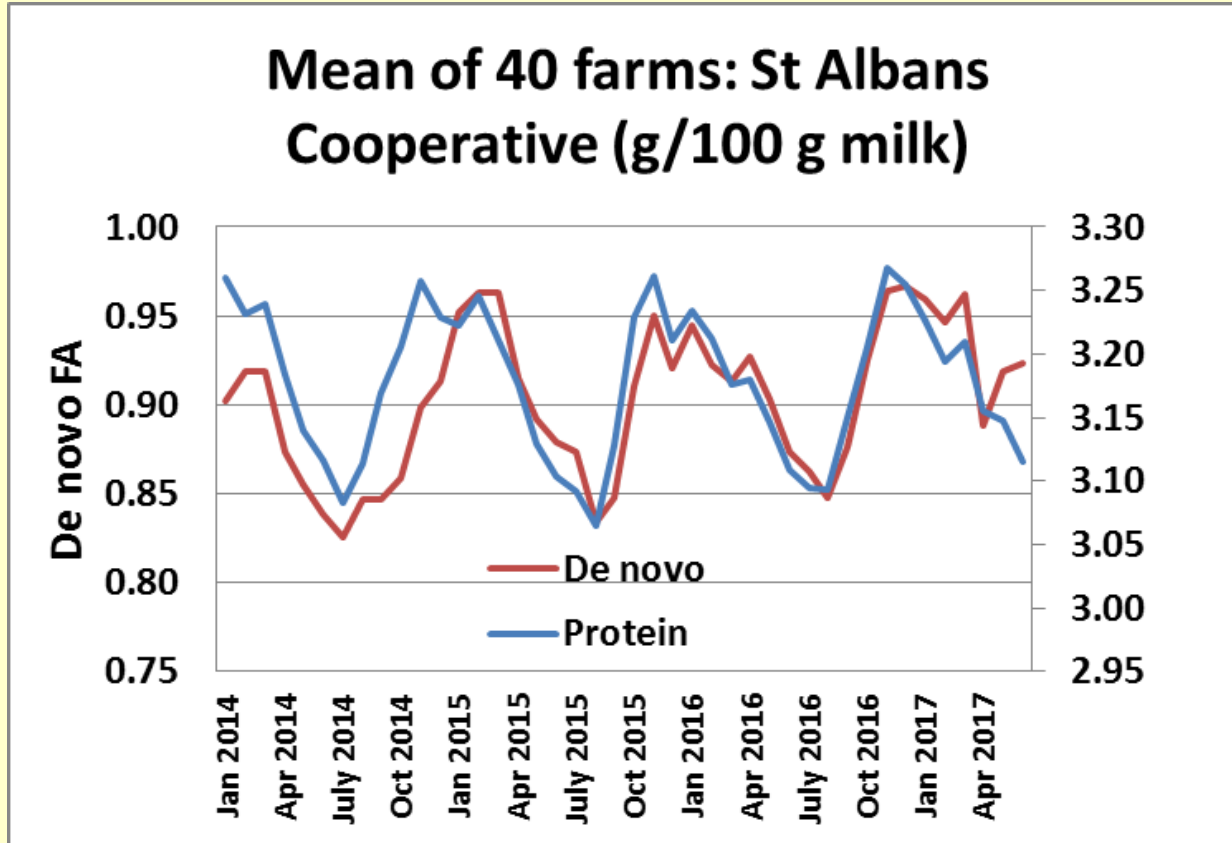
Milk Analysis for Dairy Herd Management

- **Past: Where we have been?**
 - **2017: CNC meeting: new data (herd level and individual cow level)**
 - **milk fatty acids: relation to seasonality of fat and protein**

Seasonality of Bulk Tank Milk - Fat



Seasonality of Bulk Tank Milk – Protein



Milk Analysis for Dairy Herd Management

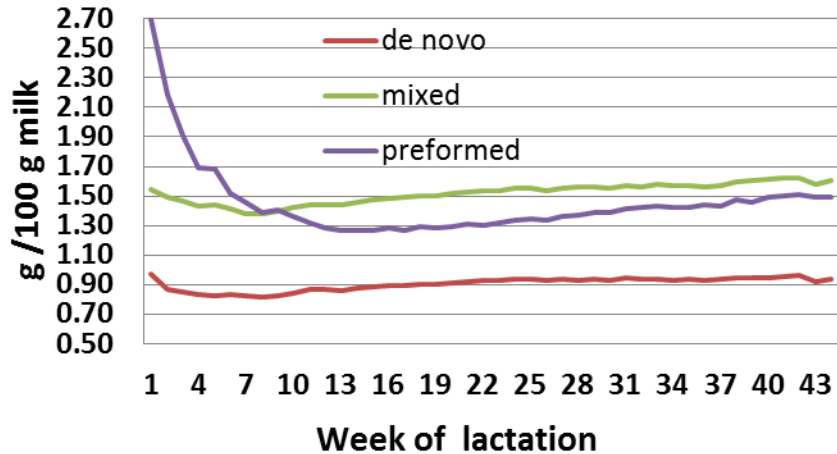
- **Past: Where we have been?**
 - **2017: CNC meeting: new data (herd level and individual cow level)**
 - **milk fatty acids: relation to seasonality of fat and protein**
 - **167 farm study of milk fatty acid from herds distributed all over the US – basically the same relationships between de novo, mixed, and preformed fatty acid with fat and protein test that we had seen in the Northeast**

Milk Analysis for Dairy Herd Management

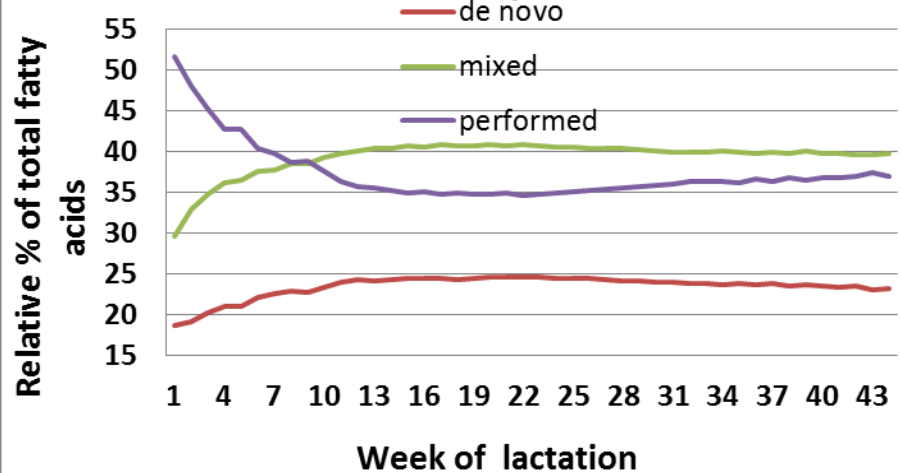
- **Past: Where we have been?**
 - **2017: CNC meeting: new data (herd level and individual cow level)**
 - **milk fatty acids: relation to seasonality of fat and protein**
 - **167 farm study of milk fatty acid from herds**
 - **Stage of lactation effect on milk fatty composition**

Stage of Lactation – Holstein

De novo, mixed and preformed FA



De novo, mixed, preformed FA

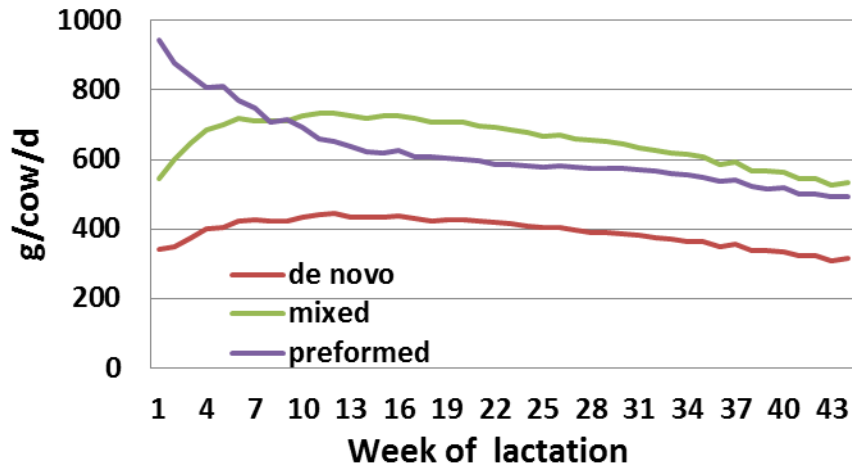


Herd producing an average of about 92 lb (41.77 kg) per cow per day on TMR feeding system.

Stage of Lactation – Holstein

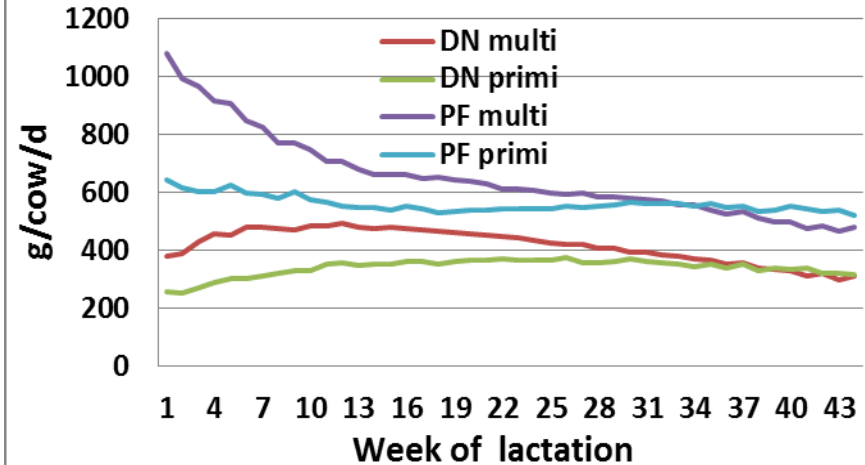
All Cows

Output of milk fatty acids



DN & PF by Parity

Multi & Primi Parous Cow Fatty Acids



Herd producing an average of about 92 lb (41.77 kg) per cow per day on TMR feeding system.

Milk Analysis for Dairy Herd Management

- **Current 2019: Where are we?**
 - **Instruments** testing milk for de novo, mixed performed, chain length, and unsaturation (**total of 14 Delta Instruments**)
 - St Albans Cooperative, AgriMark Cooperative, and Cayuga Marketing Cooperative (1 instrument each), Merieux-Siliker, Salida CA (2 instruments March 2019)
 - Sterns County and Zumbrota DHIA Labs, Minnesota (2 each), ADM DHIA Lab, Clovis, New Mexico
 - Cornell University (2), North Carolina State, Miner Institute, and Texas Federal Milk Market Laboratory

Milk Analysis for Dairy Herd Management

- **Current 2019: Where are we?**
 - **What factors influence PLS model calibration across time and from instrument to instrument?**
 - Accuracy of wavelength calibration
 - Cuvette pathlength
 - Change in power out put of the light source
 - Change in homogenizer efficiency across time and differences from instrument to instrument.
 - **A non milk method can be used to control the instrument variation for the first three parameters listed above, but not the homogenizer, therefore calibration samples are needed.**

Milk Analysis for Dairy Herd Management

- **Current 2019: Where are we?**
 - **Calibration Samples**
 - A 14 sample sets of milk samples for calibration of mid-infrared milk analyzers are being produced 13 times a year at Cornell.
 - All farm management parameters on Delta instruments are calibrated with these samples
 - These samples are available to calibrate other brands of infrared milk analyzers when those instruments have herd management milk fatty acid models available.

Milk Analysis for Dairy Herd Management

Current 2019: Where are we?

- **How well do results agree among instruments on fatty acids?**

If the milk fatty acid parameters on each infrared milk analyzer are **not** calibrated with reference samples, then instruments will not agree very well. Calibration is needed if you want accurate results. Calibration should be done on a g/100 g milk basis and values per 100 g fatty acids should be a calculated parameter.

In 2018, we did the first two multi-lab comparisons of instrument results for milk fatty acid testing. There another one scheduled for next week.

Multi-lab Validation of Results (Delta Instruments)

Instruments: A mixture of 9 Delta FTA's and Delta Combi's
Calibration:

1. De novo, mixed, and preformed calibrated every 4 weeks with Cornell calibration samples.
2. Chain length and double bonds/fatty acid calibrated once per year. That frequency will increase in the future.

Validation: Individual farm milks (8) from 4 different regions of the US. None of these milks were part of the PLS model development samples or calibration adjustment.

Multi-lab Comparison of Results (Delta Instruments)

de novo		Lab	Lab	Lab	Lab	Lab	Lab	Lab	Lab	lab
Reference		1	2	3	4	5	6	7	8	9
1	0.8991	0.860	0.862	0.874	0.860	0.870	0.894	0.920	0.890	0.890
2	0.8484	0.820	0.810	0.838	0.820	0.822	0.828	0.840	0.820	0.830
3	0.7209	0.720	0.732	0.743	0.730	0.715	0.748	0.750	0.720	0.720
4	0.8179	0.810	0.811	0.819	0.800	0.789	0.804	0.840	0.800	0.830
5	0.7540	0.720	0.729	0.754	0.750	0.731	0.740	0.740	0.730	0.740
6	0.9635	0.930	0.937	0.964	0.940	0.933	0.953	0.950	0.930	0.950
7	0.7910	0.810	0.798	0.803	0.820	0.796	0.804	0.840	0.810	0.810
8	1.3033	1.220	1.224	1.252	1.240	1.234	1.220	1.240	1.230	1.250
0.887 Mean		0.861	0.863	0.881	0.870	0.861	0.874	0.890	0.866	0.878
MD		-0.026	-0.024	-0.006	-0.017	-0.026	-0.013	0.003	-0.021	-0.010
SDD		0.031	0.029	0.023	0.029	0.022	0.032	0.035	0.027	0.022

Multi-lab Comparison of Results (Delta Instruments)

	Mixed Reference	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	lab 9
1	1.3295	1.480	1.445	1.438	1.420	1.419	1.471	1.480	1.490	1.460
2	1.1070	1.220	1.170	1.162	1.180	1.163	1.168	1.170	1.220	1.200
3	0.9481	1.050	1.042	1.041	1.010	0.996	1.035	1.030	1.060	1.040
4	1.1063	1.240	1.232	1.208	1.210	1.158	1.186	1.260	1.260	1.230
5	1.0260	1.100	1.098	1.103	1.100	1.049	1.078	1.070	1.100	1.080
6	1.3599	1.490	1.455	1.472	1.440	1.414	1.482	1.440	1.450	1.460
7	1.3105	1.330	1.261	1.267	1.300	1.227	1.225	1.290	1.300	1.280
8	1.5220	1.660	1.625	1.648	1.640	1.580	1.630	1.650	1.680	1.620
Mean	1.2136	1.321	1.291	1.292	1.288	1.251	1.285	1.299	1.320	1.296
	MD	0.108	0.077	0.079	0.074	0.037	0.071	0.085	0.106	0.083
	SDD	0.043	0.055	0.054	0.039	0.052	0.070	0.059	0.057	0.051

Multi-lab Comparison of Results (Delta Instruments)

	Preformed	Lab	Lab	Lab	Lab	Lab	Lab	Lab	Lab	lab
	Reference	1	2	3	4	5	6	7	8	9
1	1.4988	1.370	1.419	1.426	1.480	1.451	1.405	1.410	1.380	1.390
2	1.4982	1.390	1.479	1.492	1.450	1.468	1.484	1.470	1.400	1.440
3	1.5371	1.410	1.438	1.427	1.460	1.480	1.458	1.470	1.390	1.490
4	1.5798	1.440	1.471	1.544	1.510	1.561	1.563	1.430	1.400	1.490
5	1.4224	1.370	1.371	1.370	1.380	1.438	1.429	1.440	1.350	1.460
6	1.7128	1.560	1.635	1.606	1.690	1.677	1.622	1.660	1.620	1.660
7	1.3716	1.310	1.414	1.434	1.370	1.442	1.477	1.410	1.340	1.400
8	1.7819	1.690	1.739	1.695	1.750	1.784	1.774	1.730	1.650	1.760
Mean	1.5503	1.443	1.496	1.499	1.511	1.538	1.526	1.503	1.441	1.511
	MD	-0.108	-0.055	-0.051	-0.039	-0.013	-0.024	-0.048	-0.109	-0.039
	SDD	0.036	0.049	0.058	0.026	0.041	0.066	0.059	0.046	0.052

Multi-lab Comparison of Results (Delta Instruments)

	CL	Lab	Lab	Lab	Lab	Lab	Lab	Lab	Lab	Lab
	Reference	1	2	3	4	5	6	7	8	9
1	14.7434	14.63	14.76	14.80	14.72	14.65	14.65	14.67	14.76	14.76
2	14.7429	14.64	14.78	14.79	14.69	14.61	14.69	14.71	14.77	14.78
3	14.8803	14.75	14.85	14.91	14.83	14.76	14.73	14.82	14.88	14.88
4	14.7634	14.64	14.72	14.76	14.68	14.64	14.65	14.64	14.77	14.73
5	14.7897	14.67	14.75	14.78	14.71	14.66	14.67	14.73	14.76	14.78
6	14.8062	14.61	14.74	14.77	14.69	14.63	14.61	14.70	14.77	14.77
7	14.7861	14.67	14.79	14.83	14.73	14.68	14.69	14.73	14.76	14.82
8	14.4498	14.32	14.38	14.46	14.37	14.25	14.32	14.32	14.43	14.47
Mean	14.7452	14.616	14.721	14.763	14.678	14.610	14.626	14.665	14.738	14.749
	MD	-0.129	-0.024	0.017	-0.068	-0.135	-0.119	-0.080	-0.008	0.004
	SDD	0.029	0.039	0.032	0.028	0.037	0.043	0.035	0.023	0.028

Multi-lab Comparison of Results (Delta Instruments)

	DB/FA	Lab	Lab	Lab	Lab	Lab	Lab	Lab	Lab	Lab
	Reference	1	2	3	4	5	6	7	8	9
1	0.2651	0.260	0.275	0.289	0.270	0.281	0.277	0.260	0.290	0.270
2	0.2974	0.290	0.308	0.318	0.288	0.301	0.310	0.300	0.310	0.300
3	0.3405	0.320	0.329	0.344	0.326	0.334	0.328	0.330	0.340	0.340
4	0.2987	0.290	0.299	0.311	0.291	0.307	0.309	0.290	0.310	0.300
5	0.3237	0.310	0.316	0.325	0.305	0.319	0.321	0.310	0.320	0.320
6	0.3065	0.290	0.299	0.310	0.286	0.301	0.293	0.300	0.310	0.300
7	0.2841	0.280	0.302	0.311	0.282	0.302	0.306	0.290	0.300	0.300
8	0.2649	0.250	0.255	0.273	0.245	0.259	0.268	0.250	0.260	0.250
Mean	0.2976	0.286	0.298	0.310	0.287	0.301	0.302	0.291	0.305	0.298
	MD	-0.011	0.000	0.013	-0.011	0.003	0.004	-0.006	0.007	0.000
	SDD	0.006	0.011	0.010	0.009	0.010	0.013	0.007	0.010	0.009

Milk Analysis for Dairy Herd Management

Current **2019**: Where are we?

- **Increased experience for interpretation of fatty acid results.**
 - **Examples**
 - **Trans fatty acid induced milk fat depression**
 - **Immune system activation **not** due to mastitis**
 - **Immune system activation **due** to mastitis**
 - **Error in ration sampling, testing, and formulation that lowers ration energy density**

Interpretation (Milk Fat Depression Example)

Table 1. Example of expected changes in milk production and composition for bulk tank milk on corn based total mixed ration fed Holstein cows that are progressively moving into rumen produced trans fatty acid induced milk fat depression.

A Holstein Farm Transitioning into Trans Fatty Acid Induced Milk Fat Depression												
		X1000	fatty acids per 100 g milk					fatty acids per 100 g milk			carbon #	DB/FA
week	lbs	SCC	Fat	Lactose	Protein	MUN	Denovo	Mixed	Preformed	FA CL	FA Unsat	
1	92.0	147	3.88	4.61	3.25	9.7	0.91	1.41	1.34	14.54	0.28	
2	91.8	155	3.80	4.63	3.25	9.9	0.90	1.35	1.34	14.60	0.30	
3	91.6	162	3.71	4.62	3.17	10.3	0.85	1.30	1.36	14.68	0.31	
4	91.4	170	3.63	4.61	3.14	10.7	0.80	1.25	1.38	14.78	0.33	
5	91.3	158	3.42	4.61	3.10	11.2	0.72	1.15	1.36	14.90	0.34	
fatty acids												
	Milk kg	Denovo	Mixed	Preformed	Lactose	Fat	Protein	fatty acids per 100 g fatty acids				
week	per day	g/day	g/day	g/day	g/day	g/day	g/day	Denovo	Mixed	Preformed		
1	41.8	380	589	560	1927	1621	1358	24.86	38.50	36.64		
2	41.7	375	563	559	1930	1584	1355	25.06	37.59	37.36		
3	41.6	353	541	566	1921	1543	1318	24.22	37.04	38.75		
4	41.5	332	519	573	1915	1506	1303	23.32	36.44	40.23		
5	41.5	300	477	564	1911	1418	1284	22.40	35.55	42.05		

Interpretation (Immune System Activation – High SCC)

Table 3. Expected changes in milk production and composition for bulk tank milk for Holstein cows that are progressively experiencing a mammary infection immune system challenge that is characterized by an increase in milk SCC. The key change is the progressive decrease in grams of lactose per cow per day, while concentration of lactose in milk decreases slightly and milk fat and protein stay the same, milk component output per cow per day decreases, and the milk fatty acid composition remains relatively stable.

A Holstein Farm an immune system challenge due to increasing milk SCC.												
		X1000	fatty acids per 100 g milk				fatty acids per 100 g milk			carbon #	DB/FA	
week	lbs	SCC	Fat	Lactose	Protein	MUN	Denovo	Mixed	Preformed	FA CL	FA Unsat	
1	92.0	150	3.89	4.65	3.25	9.7	0.91	1.40	1.36	14.54	0.29	
2	90.0	237	3.88	4.61	3.24	9.9	0.90	1.38	1.38	14.60	0.30	
3	88.0	324	3.88	4.57	3.23	10.3	0.90	1.39	1.38	14.61	0.31	
4	86.0	411	3.89	4.54	3.25	10.7	0.90	1.38	1.40	14.58	0.30	
5	84.0	500	3.90	4.52	3.26	11.2	0.90	1.39	1.39	14.60	0.31	
fatty acids												
	Milk kg	Denovo	Mixed	Preformed	Lactose	Fat	Protein	fatty acids per 100 g fatty acids				
week	per day	g/day	g/day	g/day	g/day	g/day	g/day	Denovo	Mixed	Preformed		
1	41.8	380	587	568	1942	1626	1358	24.78	38.22	37.00		
2	40.9	368	564	564	1884	1585	1324	24.59	37.70	37.70		
3	40.0	360	555	551	1826	1550	1290	24.52	37.87	37.60		
4	39.0	351	539	547	1773	1519	1269	24.46	37.50	38.04		
5	38.1	343	530	530	1724	1487	1243	24.46	37.77	37.77		

Interpretation (TMR reformulation error)

Table 4. Example of expected changes in milk production and composition for bulk tank milk for Holstein cows due to a TMR reformulation where an error in sampling or feed analysis caused the energy density of the new TMR to be lower than the old TMR. **The time line in this table is DAYS instead of weeks that was in previous tables.**

A Holstein Farm with a ration formulation error that unintentionally decreased the energy density of the ration.

		X1000	fatty acids per 100 g milk				fatty acids per 100 g milk			carbon #	DB/FA
Day	lbs	SCC	Fat	Lactose	Protein	MUN	Denovo	Mixed	Preformed	FA CL	FA Unsat
1	92.0	147	3.89	4.61	3.25	9.7	0.91	1.40	1.36	14.54	0.29
2	92.0	155	3.88	4.64	3.24	9.9	0.90	1.38	1.38	14.60	0.30
3	91.8	162	3.85	4.61	3.20	9.0	0.88	1.34	1.42	14.68	0.31
4	91.4	170	3.79	4.62	3.18	8.7	0.85	1.32	1.42	14.72	0.30
5	90.1	158	3.70	4.61	3.17	7.9	0.80	1.26	1.44	14.75	0.31

fatty acids											
	Milk kg	Denovo	Mixed	Preformed	Lactose	Fat	Protein	fatty acids per 100 g fatty acids			
Day	per day	g/day	g/day	g/day	g/day	g/day	g/day	Denovo	Mixed	Preformed	
1	41.8	380	587	568	1927	1626	1358	24.78	38.22	37.00	
2	41.8	376	576	576	1938	1621	1353	24.59	37.70	37.70	
3	41.7	367	558	592	1923	1605	1334	24.18	36.81	39.01	
4	41.5	353	548	589	1917	1573	1320	23.68	36.77	39.55	
5	40.9	327	515	589	1886	1513	1297	22.86	36.00	41.14	

Milk Analysis for Dairy Herd Management

- **Future:** Where we are going?
 - De novo, mixed, preformed, chain length and double bonds per fatty acid graphs for Jersey cattle.
 - Improved current milk analysis metrics (2nd generation).
 - More milk estimated blood metrics.

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Questions??

