Mid-Infrared Analyzers: Herd management milk fatty acid calibration and validation of multiple instruments

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Abstract

Mid-infrared (MIR) analyzers require calibration with samples that have known reference chemistry values. Traditional milk calibration samples are individual farm, or cow milks, with reference chemistry for each sample. A more advanced procedure for making an orthogonal design (fat, protein, lactose, urea) sample calibration set (14 samples) with all-lab mean (n=8 to 10) reference chemistry was published and updated in 2020. Recently, this same sample set has been used for milk fatty acid calibration. The fatty acid reference chemistry is from gas chromatography run on the extracted fat from the ether extraction used for the fat payment test. Reference values for major individual fatty acids and those used for the most useful dairy herd management decision making (i.e., de novo, mixed origin, preformed fatty acids, and double bonds per fatty acid) are produced for this orthogonal design sample set. Reference chemistry for groups of milk fatty acids utilizes the values for only the major fatty acids (C4, 6, 8, 10, 12, 14, 16:0, C16:1, C18:0, C18:1, C18:2, C18:3) normalized to 100% and expressed as g/100 g of milk. Using only the major fatty acids will achieve better between laboratory agreement and consistency for GLC fatty acid methods. Glycerol is approximately 5.5% of the weight of milk fat. A useful quality control metric for MIR data is the sum of the de novo, mixed origin, and preformed fatty acids (g/100 g milk) divided by the fat test, and should be between 93 and 96% of the fat test in g/100 g milk. If outside this range, there is a problem with ether the MIR fat test or one or more of the MIR values for fatty acid groups. Nine MIR milk analyzers located in different regions of the US were calibrated with samples described above and then validated with a group of 8 individual farm milks from collected from different regions of the US by comparison to GLC reference chemistry on the same milks. The best agreement (g/100 g milk) for the mean of all instruments with reference chemistry was for de novo (MD -0.016 and SDD 0.028) and double bonds per fatty acid (MD 0.00 and SDD 0.01). Mixed and preformed had MD of 0.08 and -0.054 and SDD of 0.053 and 0.048, respectively. Mixed and preformed fatty acid models are more sensitive to variation in homogenizer performance than *de novo*.

Introduction

History and first field application herd management fatty acid models

The development of PLS models for mid-infrared (MIR) milk analysis of dairy herd management parameters was initiated in 2011 in a collaborative program among researchers from Cornell University (Ithaca, NY), the laboratory of St Albans Cooperative (St Albans, Vermont), and Delta Instruments, Drachten, The Netherlands. The new herd management milk analysis parameters de novo, mixed origin, and preformed fatty acid (FA) models, fatty chain length, double bonds per fatty acid (i.e., milk fat depression index), milk estimated blood NEFA were initiated and were first applied in 2012 for routine testing of producer bulk tank milks from the St Albans Cooperative

(about 400 farms). Application of these same herd management models (plus models to measure milk urea, milk BHB, and milk acetone for individual cow milk testing was initiated in 2014 in a collaboration between Cornell researchers and researchers at the W. H. Miner Institute, Chazy, NY. Our first work on bulk tank milks from farms at the St Albans Cooperative found a positive relationship between higher de novo and de novo + mixed origin fatty acids with bulk tank fat and protein tests. The relationship between de novo , mixed origin, and preformed milk fatty acids with fat and protein test and fat and protein production per cow per day was the focus of joint field research study by Cornell University and W. H. Miner Institute.

The results of the field studies of the herd management milk analysis models were published in two research papers (Woolpert *et al.,* 2016 and Woolpert *et al.,* 2017). Woolpert *et al.,* 2016 and 2017 identified management practices, such as higher stall stocking density and lower feeding frequency, which were related to lower de novo FA content in bulk tank milk. Farms with lower de novo FA, on average, produced less milk fat and protein per cow per day. In addition, higher dietary EE was related to lower de novo FA content of milk. High de novo farms also had higher milk yield and fat and true protein content and yield. 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 high de novo and low de novo farms have been shown in earlier studies to affect ruminal function. At a constant level of milk production, the gross income per unit of milk produced was higher on the high de novo farms because of higher milk fat and true protein concentrations. Therefore, the de novo FA concentration of bulk tank milk may be an important tool for monitoring ruminal function on commercial dairy farms.

Dairy herd management milk analysis model development

Our strategy for development of herd management milk analysis PLS models was to develop milk analysis metrics that could be used more strategically and tactically to identify the causes of increases and decreases in milk fat and protein production per cow per day. With respect to herd management milk testing for diagnostic determination of why milk fat has increased or decreased, the strategy was to develop milk analysis models that provide information on milk fat production by two different milk fatty acid sources:

1. De novo synthesis of fatty acid in the mammary cells and
2. Transfer of performed fatty acid from the blood stream into mammary secretory cells.

The development and modeling performance metrics (i.e., RSD values) of the PLS models for measurement of milk de novo, mixed origin, preformed, and total milk fatty acids were described by Woolpert *et al*. (2016). The development and modeling performance metrics for the fatty chain length (mean carbon number) and the milk double bonds per fatty acid models (i.e., milk fat depression index) were described by Wojciechowski and Barbano (2016). The development and modeling performance metrics for the milk estimated blood NEFA model were described by Bach *et al*. (2021).

Materials and methods

Calibration of MIR milk fatty acid models

Like all other MIR milk analysis metrics (e.g., fat, protein, solids, urea, etc.), all milk fatty prediction models need to calibrated with reference samples with known reference values. The reference values for milk fatty acids are determined by gas liquid chromatography (GLC). The sample extraction, methylation and GLC method used in our studies was described by Wojciechowski and Barbano (2016). Briefly milk fat was extracted from each sample by an ether extraction milk that is the reference for milk fat payment (AOACI, 2021, method 989.05), the formation of methyl esters was catalyzed by methanolic KOH and boron triflouide, and methyl esters of fatty acids were determined GLC as described by Wojciechowski and Barbano (2016). The calculation of milk fatty acids, fatty acid chain length and double bonds per fatty acid was described by Kaylegian *et al*. (2009). The steps of accounting for recovery of short chain fatty acids, normalization of fatty acids to 100% and removal of the impact the added methyl group on the relative proportion on each fatty acid chain length are important steps described by Kaylegian *et al*. (2009). These methods and the approach of measuring fatty acid groups (de novo, mixed origin, and performed) are applied to an orthogonal design set of MIR calibration milks (Kaylegian *et al*. 2006) that was modified to include milk urea by Portnoy *et al*. (2021). The reference values for each of the 14 milks in the calibration set for de novo, mixed origin, and preformed fatty acids are expressed as grams of fatty acid per 100 g milk, as shown in Figure 1.

Three hundred and forty sets of the calibration samples shown in Figure 1 are produced once every 4 weeks and distributed laboratories for calibration of components and milk fatty acids.

Cornell University Fatty Acid Calibration Standards

Reference Values for Fatty Acid Calibration and milk components

Sample	total grams de novo fatty acid (g/100g milk)	total grams mixed origin fatty acid (g/100g milk)	total grams preformed fatty acid (g/100g milk)	Fat	Protein	Solids	Lactose (anhydrous)	MUN	SNF	OS
1	0.0506	0.0752	0.0813	0.2192	4.2354	9.5217	3.9957		9.30	5.07
2	0.1480	0.2201	0.2379	0.6412	2.2222	8.4280	4.5419		7.79	5.56
3	0.2473	0.3679	0.3977	1.0716	3.8950	11.2754	5.0897		10.20	6.31
4	0.3453	0.5137	0.5552	1.4962	2.5608	10.1190	4.9484		8.62	6.06
5	0.4437	0.6601	0.7135	1.9227	3.5669	10.8796	4.2728		8.96	5.39
6	0.5419	0.8062	0.8713	2.3480	2.8979	10.8918	4.5492		8.54	5.65
7	0.6422	0.9554	1.0326	2.7827	3.2329	11.6934	4.5429		8.91	5.68
8	0.7384	1.0985	1.1872	3.1995	3.0759	11.7890	4.4157		8.59	5.51
9	0.8375	1.2459	1.3466	3.6289	3.4041	12.8757	4.6757		9.25	5.84
10	0.9344	1.3901	1.5024	4.0488	2.7419	11.9600	4.1412		7.91	5.17
11	1.0337	1.5378	1.6621	4.4791	3.7500	14.2347	4.8220		9.76	6.01
12	1.1310	1.6825	1.8185	4.9006	2.4144	12.2930	4.0055		7.39	4.98
13	1.2313	1.8317	1.9797	5.3350	4.0805	15.2020	4.5430		9.87	5.79
14	1.3290	1.9770	2.1368	5.7583	2.0770	13.9981	5.0924		8.24	6.16
Mean	0.6896	1.0259	1.1088	2.9880	3.1539	11.7972	4.5454		8.8093	5.6553
min	0.0506	0.0752	0.0813	0.2192	2.0770	8.4280	3.9957		7.3925	4.9781
max	1.3290	1.9770	2.1368	5.7583	4.2354	15.2020	5.0924		10.2037	6.3087
Range	1.2784	1.9018	2.0554	5.5391	2.1585	6.7740	1.0967		2.8113	1.3307

Figure 1. Milk calibration samples and reference chemistry (components and fatty acids).

Results and discussion

Between laboratory agreement: milk fatty acid validation testing

Four times per year, a set of 8 unknown individual farm milk are sent to all laboratories for performance evaluation. The farms selected for this testing are 2 farms from each of 4 regions of the US. These 8 farm milks are sent to one laboratory and they split and shipped to all laboratories for testing by MIR milk analysis and for reference testing. Tables 1 through 5 below contain an example of the results from multi-laboratory testing of the same milks. The results in the Tables below are from a group of instruments that are a mixture of Delta FTA and Delta Combi milk analyzers. Recently, additional instruments by Bentley and Foss have been using our calibration samples and in general their performance on the milk fatty acids is similar to what we have observed for the Delta instruments.

On average for this set of unknown farm milks, most MIR laboratories produced a mean estimate of the de novo fatty acids was lower than the reference chemistry. However, on an absolute basis all labs had a mean difference that was ≤ 2.93% relative of the reference chemistry mean for the sample set.

On average for this set of unknown farm milks, most MIR laboratories produced a mean estimate of mixed origin fatty acids that was higher than the reference chemistry. However, on an absolute basis all labs had a mean difference that was ≤ 8.9% relative of the reference chemistry mean for the sample set.

On average for this set of unknown farm milks, most MIR laboratories produced a mean estimate of performed fatty acids that was lower than the reference chemistry. However, on an absolute basis all labs had a mean difference that was ≤ 7.0% relative of the reference chemistry mean for the sample set.

Table 1. Between laboratory comparison of de novo fatty acid analysis (g/100 of milk) and calculated mean difference (MD) and standard deviation of the differences from reference chemistry for the 8 samples.

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
0.8873	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

Table 2. Between laboratory comparison of mixed origin fatty acid analysis (g/100 of milk) and calculated mean difference (MD) and standard deviation of the differences from reference chemistry for the 8 samples.

	Mixed Origin		Lab	Lab	Lab	Lab	Lab	Lab	Lab	Lab	lab
	Reference		1	2	3	4	5	6	7	8	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

Table 3. Between laboratory comparison of preformed fatty acid analysis (g/100 of milk) and calculated mean difference (MD) and standard deviation of the differences from reference chemistry for the 8 samples.

	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

We have observed that the mixed and preformed milk fatty acids tend to deviate in opposite direction relative reference chemistry values. The mixed origin and preformed fatty acid models are more sensitive to variation in homogenizer performance than de novo fatty predictions or prediction of the main milk components.

On average for this set of unknown farm milks, most MIR laboratories produced a mean estimate of fatty acid chain length that was lower than the reference chemistry. However, on an absolute basis all labs had a mean difference that was ≤ 0.92% relative of the reference chemistry mean for the sample set.

On average for this set of unknown farm milks, most MIR laboratories produced a mean estimate of mean fatty acid unsaturation that was very close to the reference chemistry. On an absolute basis all labs had a mean difference that was ≤ 4.4% relative of the reference chemistry mean for the sample set. The between lab agreement on this parameter

Recognizing when there is laboratory problem with MIR fatty acid data

- **Bulk tank producer milks**: The sum of de novo, mixed, and preformed fatty acids (g/100 g milk) should be about 94.5% of the fat test (g/100 g milk). This will vary from farm-to-farm, but all values should be between 93 and 96%. If sum of de novo, mixed and preformed as a % of fat test increases or decreases with change in fat concentration for a population of farms, the error is in the slope setting for the prediction of total fat, not the fatty acid testing.
- If all tests are lower than 93 or higher than 96% then it could be bias error in fat test, or the wrong reference chemistry has been used to adjust the slope and intercept on one of the 3 fatty acid measures.

Table 4.. Between laboratory comparison of fatty acid chain length analysis (carbons per fatty acid) and calculated mean difference (MD) and standard deviation of the differences from reference chemistry for the 8 samples.

	CL Reference		Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 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

Table 5. Between laboratory comparison of mean fatty acid unsaturation (double bonds per fatty acid) and calculated mean difference (MD) and standard deviation of the differences from reference chemistry for the 8 samples.

	DB/FA Reference		Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 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

- **Individual cow milks**: The sum of de novo, mixed, and preformed fatty acids should be about 94.5% of the fat test. This will vary from cow-to-cow. The errors discussed on the previous slide for bulk tank milk can have the same impact on individual cow milk tests.
- The PLS fatty acid herd management models were developed for bulk tank milks and for milks from individual cows in positive energy balance. Thus, these fatty acid models will not work well on milks from cows at less than 5 days in lactation. Very early lactation milks will give values for the sum of the fatty acid (g/100 g milk) that exceeds the fat test in g/100 g milk. These samples are outside the scope of the ability of the current models.
- **Herd management application of milk fatty data.** This is a discussion that is beyond the scope of this presentation, but we have presented data from field studies and provided examples of how to interpret the fatty acid data from bulk tank milks. Those examples are provided in a series of papers presented at the annual Cornell Dairy Conference (Barbano *et al* 2014,2017, 2018, 2019 ; Barbano and Mellili 2016).

Conclusion

Milk fatty acid analysis is a useful tool for dairy herd management. Most dairy herd management milk fatty acid analysis in the USA has been applied to bulk tank and tanker load samples from individual farms in milk payment testing laboratories. Today bulk tank milk is tested on virtually every pick up and for large farms every tanker load of milk is tested daily. Results from this testing are usually posted within 36 h of sample collection. This has provided a valuable resource for nutrition management on dairy farms in the USA and dairy nutritionists have rapidly improved their skills for interpretation of the data. Monitoring milk fatty acid composition across time on the same farm (g/100 g milk) has proved very useful in management of dairy rations.

Individual cow milk testing is still a challenge. In general, the frequency of milk sampling and testing of milks from individual cows is too low to provide useful information for farm management. The interpretation of the data is more complex because there are normal and systematic changes in milk fatty acid composition with stage of lactation and these factors need to be considered when interpreting milk fatty acid data from individual cows. In the long- term future, the dairy industry needs to strive to achieve milk testing hardware innovations that allow individual cow milk analysis to occur in real-time on the farm during milking. If that is achieved, monitoring and management of individual cow health will advance rapidly.

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