Effect of Supplementing Diets with Fat on Infrared Analysis of Milk Fat[1]

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ABSTRACT

Individual a.m. and p.m. milk samples (n = 1340) were collected in two feeding trials to investigate the effects of amount and source of dietary fat on the determination of milk fat by different analytical methods. Percentage of milk fat was determined by the modified Babcock procedure and infrared instrumentation using either the A filter (5.73 μm) or a combination of the A and B (3.48 μm) filters. In Trial 1, 24 cows were fed either 0 or .45 kg/d of calcium soap. Mean percentages of milk fat measured by the Babcock, A, and AB methods were, respectively: control, 3.88, 3.88, 3.88; calcium soap, 3.92, 3.93, 3.93. Differences among methods were not significant for either the control or fat-supplemented treatments. In Trial 2, 20 cows were fed 0 or .63 kg/d of either tallow or yellow grease. Milk fat percentages were, respectively: control, 3.43, 3.44, 3.43; tallow, 3.59, 3.61, 3.56; yellow grease, 3.36, 3.36, 3.33. Percentage of milk fat was not affected by analytical method for the three diets. Using data from Trial 1, week of lactation had a greater effect on mean molecular weight of fatty acids in milk than did the feeding of fat. Additional research is warranted to analyze fat in milk from cows under more widely differing dietary conditions.

INTRODUCTION

Milk fat remains an influential component of milk pricing, breeding decisions, and feeding practices. Therefore, the accuracy of milk fat measurements is imperative to the dairy industry. In addition to accuracy, milk marketing organizations and DHI laboratories depend upon speed and simplicity for rapid turnaround of information.

The Babcock method (1) for determining milk fat was developed in 1890. However, with the advancement of infrared (IR) spectroscopy technology for milk fat determination (12), analyses can be automated, the use of corrosive acids is not necessary, and time required for analyses is reduced. However, IR instruments must be calibrated with results from direct chemical analysis. The Babcock procedure has been the chemical method most commonly used, but accurate ether extraction methods also exist (1, 2).

Different functional groups in organic molecules absorb light at different wavelengths. The carbonyl group (C=O) is most commonly measured at a wavelength of 5.73 μm (referred to as the "A" filter). Therefore, the A filter quantitates the number of ester linkages present in a fat molecule but will not determine changes in molecular weight of the fatty acids or presence of nonesterified fatty acids. Recently, the B filter (3.48 μm) has been introduced with absorbance based on carbon-hydrogen groups. The B filter reflects changes in molecular weight of fatty acids, but degree of unsaturation may provide a source of error. Lactose contains several carbon-hydrogen groups; thus, lactose must be determined when the B filter is used, reducing the speed of analyses in comparison with the A filter. Equipment fitted with both filters (AB) and having the flexibility of varying the proportion of each filter is presently available. Present IR instruments have been developed to handle the accuracy and dependability needed by today's standards (14).

Most research on the use of different wavelengths for analysis of milk fat is recent. Lipolysis reduces the estimate of milk fat content by the A filter (8, 13, 16) but not by the B filter

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(16). Accuracies of measuring milk fat content by the A and B filters are similar, whereas that of AB is superior (4). Different forage types appear to affect the analysis of milk fat by the filters differently (4). Feeding fat to dairy cows almost invariably increases the long-chain fatty acid content of milk fat (10); an increase in mean molecular weight of milk fatty acids may influence quantification of milk fat by IR (5). The objective of this research was to determine whether different quantities and sources of dietary fat cause significant variation in milk fat content as determined by different analytical methods.

MATERIALS AND METHODS

Individual a.m. and p.m. milk samples (n = 1340) were collected from cows fed different diets and split into two portions. One portion was sent to the DHI Central Laboratory (DHI Cooperative, Inc., Powell, OH) and the other to the Milk Market Administration Laboratory (Milk Market Administration, Cleveland, OH). The samples did not contain a preservative but were kept cool by refrigeration and ice packs. Analyses with the A filter were conducted at the DHI Central Laboratory using a Multispec Model M (Foss Food Technology Corp., Eden Prairie, MN). The Babcock procedure (1), modified to achieve a specific milk-acid reaction temperature (2), and AB analyses (50% A and 50% B), also conducted on a Multispec Model M, were carried out by the Milk Market Administration Laboratory. The Multispec Model M was calibrated to correct for lactose interference with the AB analyses. The DHI Central Laboratory received standard samples for calibration of their IR instrument from the Milk Market Administration. Milk protein content was determined at both locations by IR instruments calibrated from samples analyzed by Kjeldahl methods (1, 3).

In Trial 1, 24 cows (12 Holstein, 6 Ayrshire, 3 Brown Swiss, 3 Guernsey) at The Ohio State University, Columbus, were fed either 0 or .45 kg/d of calcium soap (Church and Dwight Co., Inc., Princeton, NJ) beginning immediately after parturition. The diets consisted of alfalfa silage, corn silage and a concentrate mixture in a 1:1:2 ratio (DM basis). Milk samples were collected weekly for the analysis of fat and every 4 wk for determination of fatty acid composition.

Corn silage, either alfalfa hay or silage, and a concentrate mixture were fed in a 1:2:2 ratio (DM basis) during Trial 2 to 20 Holstein cows at The Ohio Agricultural Research and Development Center, Wooster. Supplemental fat (tallow or yellow grease) was fed at a rate of 0 or .63 kg/d. Milk samples were analyzed weekly for fat and fatty acid composition was deter-

TABLE 1. Fatty acid composition (weight percentage of methyl esters) of milk fat from cows fed the experimental diets.

Fatty acid	Trial 1		Trial 2		
	Control	Ca Soap[1]	Control	Tallow	Yellow grease
4:0	1.71	1.61	1.75	1.84	1.82
6:0	1.52	1.52	1.56	1.39	1.36
8:0	1.07	1.05	1.17	.99	.94
10:0	2.50	2.43	3.04	2.43	2.24
12:0	2.93	2.86	3.76	2.93	2.72
14:0	9.77	9.82	12.23	10.90	10.83
14:1	1.02	1.08	1.53	1.38	1.37
15:0	.93	.85	1.26	1.13	1.00
16:0	28.70	32.20	32.17	29.86	29.13
16:1	1.72	1.45	1.98	1.81	1.82
17:0	.72	.54	.63	.84	.66
18:0	13.62	11.96	9.65	11.56	11.38
18:1	26.91	25.82	21.80	24.82	26.73
18:2	2.58	2.51	2.16	1.95	2.11
18:3	.43	.41	.62	.49	.52

[1]Calcium salt of palm fatty acid distillate. Provided by Church and Dwight Co., Inc., Princeton, NJ.

TABLE 2. Characteristics of milk fat from cows fed the experimental diets.

Characteristic	Trial 1		Trial 2		
	Control	Ca Soap	Control	Tallow	Yellow grease
Molecular weight of fatty acid	253.8	253.3	249.0	252.1	253.2
Double bonds/fatty acid	.37	.36	.33	.35	.39
Molecular weight/mole triacylglycerol	802	801	788	797	801
Double bonds/mole triacylglycerol	1.11	1.08	.99	1.05	1.17

mined three times for each cow over the course of the trial.

Fatty acid composition (9) of milk samples is in Table 1. Mean molecular weight and unsaturation of fatty acids in milk fat were calculated for each diet from the fatty acid composition data (Table 2). After characterizing a typical triacylglycerol molecule for each diet, the models discussed by McKenna (7) were used to predict the expected variation in percentage of milk fat when using the different filters. For this illustration, it was assumed that the IR instrument was calibrated with milk containing 3.6% fat. The typical mole of triacylglycerol in the standard milk was considered to have a molecular weight of 800 with one double bond. Molecular weight was the only adjustment used for the A filter: (800/x)3.6, where x is the molecular weight of the mole of triacylglycerol for a specific dietary treatment. The B filter predictions accounted for both molecular weight and degree of unsaturation. Glycerol, carbonyl groups, three-fourths of the methylene groups adjacent to the carbonyls, one-half of the methylene groups adjacent to double bonds, and the CH=CH groups were considered unabsorbing units for the B filter ($204.5 + 42y = z$; y = number of double bonds and z = molecular weight of fat molecule unaccounted for by the B filter). Then, the number of absorbing units (CH_2) per unit of molecular weight was determined and compared to the base mole of triacylglycerol. The AB prediction was made using 27.27% of A and 72.73% of B.

Fatty acid composition of milk samples from individual cows in Trial 1 were used to determine molecular weight of fatty acids during wk 1 to 17 of lactation. Effects of fat supplementation and week of lactation on differences in milk fat concentration between analytical methods were compared using paired milk samples.

Data for the effect of analytical methods on milk fat concentration as influenced by the different dietary treatments were analyzed by the mixed model least squares and maximum likelihood computer program (6). Analysis of data for effects of fat supplementation and week of lactation on molecular weight of fatty acids and differences between analytical methods was conducted by using the General Linear Model for analysis of variance (11). Means were compared using Duncan's new multiple range test (15) with $\alpha = .05$.

RESULTS AND DISCUSSION

Milk protein was not different ($P>.05$) between the DHI and Milk Market Administration laboratories (3.07 and 3.08%, respectively). Thus, a location effect on measurements for milk fat does not appear warranted. Milk fat concentrations determined by the Babcock, A, and AB procedures are in Table 3. No differences were found ($P>.05$) among analytical methods across diets in both trials and no diets by treatment interaction occurred. Milk fat in Trial 1 was essentially identical among analytical methods for the control and fat supplemented cows. Concentrations in Trial 2 were slightly more variable, particularly from cows fed tallow.

The predicted milk fat concentrations, based on the model presented by McKenna (7), in Table 4 demonstrated the expected variation among the filters when characteristics of triacylglycerols changed according to diets in Table 2. It was assumed the IR instrument was

TABLE 3. Milk fat percentages as determined by the Babcock, A, and AB procedures.

Diet	n	Method of analysis					
		Babcock		A		AB	
		$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
Trial 1							
Control	392	3.88	.05	3.88	.05	3.88	.05
Ca Soap	480	3.92	.04	3.93	.04	3.93	.04
Trial 2							
Control	153	3.43	.08	3.44	.08	3.43	.08
Tallow	157	3.59	.08	3.61	.08	3.56	.08
Yellow grease	158	3.36	.07	3.36	.07	3.33	.07

calibrated with milk containing 3.6% fat with one double bond and a molecular weight of 800. Differences between the base 3.6% and predicted percentage of milk fat for the different diets and filters were not compared statistically and although these differences were small, the expected trends occurred. The A filter overestimated milk fat concentration in Trial 2 for the control and the tallow diets because their molecular weights were less than 800. The B filter underestimated percentage of fat for all diets. Milk fat percentage for the control diet in Trial 1 and the yellow grease diet in Trial 2 were slightly more underestimated; these two diets resulted in the greatest number of double bonds per mole of triacylglycerol. The AB predictions were between the A and B predictions.

Feeding the calcium soap in Trial 1 increased ($P<.05$) the mean molecular weight of fatty acids in milk (Table 5). Mean molecular weight of fatty acids decreased ($P<.05$) with advancing weeks of lactation, paralleling the reduction that occurs in incorporation of long-chain fatty acids from adipose tissue into milk fat with advancing days in milk (10). No interaction occurred between diet and weeks of lactation ($P>.05$). Although both fat supplementation and week of lactation affected mean molecular weight of fatty acids, week of lactation had a greater effect ($P=.0001$ versus $P=.0026$). Week of lactation accounted for a significant ($P<.05$; $R^2 = .44$) portion of the variation in molecular weight of fatty acids with the following regression equation: $Y = 259.6 - 2.03x$, where Y = molecular weight of fatty acids and x = week of lactation.

Comparable with the other data presented, no variation ($P>.05$) was found in the differences between analytical methods. However, the greatest difference between the infrared

TABLE 4. Prediction of percentage of milk fat for the A, B, and AB filters, assuming milk used for calibration contains 3.6% fat with a molecular weight of 800 and one double bond.[1]

Diet	Filter					
	A		B		AB	
	(%)	Diff.[2]	(%)	Diff.	(%)	Diff.
Trial 1						
Control	3.59	−.01	3.57	−.03	3.58	−.02
Ca Soap	3.60	0	3.58	−.02	3.59	−.01
Trial 2						
Control	3.65	+.05	3.58	−.02	3.60	0
Tallow	3.61	+.01	3.58	−.02	3.59	−.01
Yellow grease	3.60	0	3.56	−.04	3.57	−.03

[1]Calculations based on models presented by McKenna (7).

[2]Diff. = Difference.

TABLE 5. Effects of fat supplementation and week of lactation in Trial 1 on molecular weight of fatty acids and differences between analytical methods.

Item	Mean molecular weight of fatty acid	A–AB	A–Babcock	AB–Babcock
Fat				
Control	252.5[a]	.012	.001	–.011
Ca Soap	254.9[b]	.041	.038	–.004
Week of lactation				
1	260.5[a]	.165	.084	–.081
5	254.7[b]	–.023	.009	.032
9	252.6[b,c]	–.003	–.003	–.001
13	250.7[c,d]	.003	.004	0.000
17	250.1[d]	–.010	.003	.013

[a,b,c,d]Means in the same column within measurements with different superscripts differ ($P<.05$).

methods occurred when mean molecular weight was the highest. This observation agrees with the calculations in Table 4 where the AB filters would be expected to result in lower milk fat than the A filter, given the characteristics of the fatty acids in this study.

Theoretically, milk fat concentration can vary with analytical procedure used. The A and B filters are not without sources of error. The A filter is independent of changes in amount of unsaturation, and the B filter is less sensitive to changes in molecular weight. Therefore, the AB method has distinct advantages in that it is independent of changes in molecular weight and less sensitive to changes in degree of unsaturation (5). The more fluctuations occur in the characteristics (such as molecular weight, degree of unsaturation, and degree of lipolysis) of fat molecules in milk, the more potential exists for differences among analytical methods. Although the degree of change in the nature of the fat molecule necessary to change results using different filters is not clearly known from a practical approach, consistency among laboratories needs to be enhanced. Even if the AB procedure was accepted to yield the best results, laboratories must still decide what ratio of A to B should be used. In addition, variations between milk fat analyses among laboratories will likely continue due to differences in testing intervals and sample handling procedures.

Perhaps milk fat content did not differ among methods in this study with the feeding of fat because the nature of the fat molecule was not altered enough to cause such changes to be detectable. Nevertheless, these data document that fat content in milk from cows fed high fat diets can be determined by IR with confidence. The data also demonstrate that stage of lactation can have a greater effect on mean molecular weight of fatty acids in milk than dietary fat. Changes in mean molecular weight of fatty acids may affect milk fat concentrations when determined by different IR instruments. Therefore, typical changes in the characteristics of milk fat during different stages of lactation could have as much, if not more, of an effect on milk fat concentration when analyzed by IR instruments than other factors such as dietary changes and milk handling procedures. Further research is warranted to analyze fat in milk from cows under more widely differing dietary conditions and to compare the different filters on a single instrument.

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