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Estimating Fatty Acid Content in Cow Milk Using Mid-Infrared Spectrometry

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ABSTRACT

Interest in the fatty acid composition of dairy products is increasing; however, the measurement of fatty acids requires using gas-liquid chromatography. Although this method is suitable, it involves a time-consuming procedure, expensive reagents, and qualified staff. By comparison, the mid-infrared (MIR) spectrometry method could be a good alternative for assessing the fatty acid profile of dairy products. The objective of this study was to explore the calibration of MIR spectrometry for estimating fatty acid concentrations in milk and milk fat. Estimated concentrations in milk fat were less reliable than those for the same fatty acids in milk. Results also showed that when the fatty acid concentrations in milk increased, the efficiency of the infrared analysis method in predicting these values simultaneously increased. Selected prediction equations must have a high cross-validation coefficient of determination, a high ratio of standard error of cross-validation to standard deviation, and good repeatability of chromatographic data. Results from this study showed that the calibration equations predicting 12:0, 14:0, 16:0, 16:1*cis*-9, 18:1, and saturated and monounsaturated fatty acids in milk could be used. Thus, with its potential for use in regular milk recording, this infrared analysis method offers the possibility of assessing and improving the quality of milk produced. Indeed, it enables the fatty acid composition in milk to be estimated for each cow and the estimates to be used as indicator traits to determine the genetic values of underlying fatty acid concentrations. The knowledge of these genetic values would open up opportunities for animal selection aimed at improving the nutritional quality of cow milk.
Key words: milk, fatty acid, mid-infrared, quality

INTRODUCTION

Cow milk fat typically contains 70% saturated fatty acids (**SAT**), 25% monounsaturated fatty acids (**MONO**), and 5% polyunsaturated fatty acids (**POLY**; Grummer, 1991). A milk lipid composition more favorable to human health would be about 30% SAT (Pascal, 1996), 60% MONO, and 10% POLY (Hayes and Kosla, 1992). The fatty acid profile of cow milk is therefore far from optimal. However, the observed variations in SAT, MONO, and POLY suggest that the milk fat composition could be modified by various means, such as through feeding and genetics (Palmquist et al., 1993), and could come closer to the optimal profile. Several researchers have been focusing on ways to improve the nutritional quality of bovine milk fat by feed supplementation (e.g., Demeyer and Doreau, 1999; Chilliard et al., 2000). However, all the fatty acids in a specific class (SAT, MONO, or POLY) do not have the same effects on human health. In the case of SAT, although myristic acid is known for its negative effects on cardiovascular diseases, stearic acid does not seem to have this effect (Hu et al., 1999). Similarly, in POLY, the n-6 fatty acids appear to have negative effects on human health because of their prevalence in Western nutrition. Indeed, the current ratio n-6:n-3 is estimated to be 15:1 to 20:1 (Simopoulos, 2003). It is therefore important to check the global fatty acid profile in milk if one wants to assess the nutritional quality of bovine milk fat. As stated earlier, influencing the nutritional quality of milk fat has been the topic of several recent research papers (e.g., Demeyer and Doreau, 1999; Chilliard et al., 2000), some of which have concentrated on milk fat enriched in n-3, in line with the current interest in dairy products that are enriched in n-3. However, there has been less focus on assessing the fatty acid content,

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because this requires chromatographic analysis. Although this method is suitable (e.g., Dorey et al., 1988; Collomb and Bühler, 2000), it is time-consuming and requires skilled staff.

Mid-infrared (**MIR**) spectrometry is an alternative to gas chromatography, with advantages such as a very high throughput (up to 500 samples/h; FOSS, 2005), ease of use, and availability. The infrared spectrum is caused by the absorptions of electromagnetic radiation at frequencies that are correlated to the vibrations of specific chemical bonds within a molecule (Coates, 2000). The spectrum therefore illustrates these absorptions at different wavenumbers (cm^{-1}) for a specific chemical composition (Smith, 1996). Mid-infrared spectrometry (400 to 4,000 cm^{-1}) is particularly interesting because it is very highly sensitive to the chemical environment, as the fundamental absorptions of molecular vibrations occur in this region (Belton, 1997). Mid-infrared spectrometry can be used to estimate various traits quantitatively based on calibration equations. The purpose of our research was to develop the predicted equations necessary for measuring the fatty acid content in milk and milk fat using MIR spectrometry.

MATERIALS AND METHODS

Sampling and Recording Spectra Files

Milk samples were taken from cows in 7 herds selected according to the following criteria: their participation in the milk recording system in Wallonia, the observed variation in the percentage of milk fat and the number of breeds in the herds. An 80-mL sample of milk taken during routine milk recording was divided into 2 parts (60 + 20 mL). The samples were collected for all the cows milked in the herds on a given test day. Following standard procedures (International Committee for Animal Recording, 2004), the samples represented 50% morning milk and 50% evening milk. The 20-mL sample was then analyzed using MIR spectrometry (MilkoScan FT6000; FOSS, 2005) following the normal milk recording procedure (International Committee for Animal Recording, 2004). The MilkoScan FT6000 works within the MIR region from 1,000 to 5,000 cm^{-1} and uses an interferometer. From the resulting interferogram, MIR spectra are generated by means of fast Fourier transformations (FOSS, 2005). The spectra files generated were recorded in a database. The second sample (60 mL) was frozen at –26 ± 2°C. In this procedure, 600 samples were taken between April and June 2005 from 275 cows from 6 breeds (Dual Purpose Belgian Blue, Holstein-Friesian, Jersey, Normande, Montbeliarde, and Red and White). Not all the farms were tested 3 times, and some cows were dried off or had calved during the study. The milk fat percentage of the collected samples ranged between 2.97 and 7.73 g/dL of milk. This large variation indicated that a good calibration could be made.

Reference Values

Using principal component analysis based on the spectral variability, 49 samples were chosen and used from the 600 samples collected. The milk fat was extracted according to ISO Standard 14156:2001 (International Organization for Standardization, 2001). These milk fat samples were analyzed using gas chromatography, based on a method derived from Collomb and Bühler (2000). The gas chromatograph (model 6890; Agilent Technologies, Inc., Palo Alto, CA) was equipped with a CPSil-57 CB capillary column (Varian, Inc., Palo Alto, CA), with a length of 50 m, an internal diameter of 0.25 mm, and a film thickness of 0.20 μm. The retention gap was a "methyl deactivated nonpolar" (Varian, Inc.) with a length of 20 cm and an internal diameter of 0.53 mm. The conditions for the chromatographic analyses were as follows: carrier gas, helium; average velocity, 35 cm/s; cold on-column injector; flame ionization detector at 265°C; and a temperature program from 40°C (2 min) to 150°C (at 30°C/min), then 150 to 250°C (at 2°C/min). The volume injected was 0.5 μL. To measure the fatty acid concentrations, the response factors used were the same as those described by Collomb and Bühler (2000) because the experimental conditions were similar.

For each sample, 2 groups of reference values were generated (g of fatty acid/100 g of fat, and g of fatty acid/dL of milk) and were recorded in the database. The repeatability and accuracy of this method were estimated from several reference butter samples produced by BIPEA (Bureau InterProfessionnel d'Etude Analytique, http://www.bipea.org).

Calibration Equations

From the chromatographic and spectral data, a specific program for multivariate calibration (WINISI III; http://www.winisi.com/) was used to compute the calibration equations using partial least squares regression (**PLS**). Partial least squares regression has 2 important advantages over multiple linear regression or regression on principal components. First, like principal components regression, it uses all the spectral data for the calibration (Frank et al., 1984). Second, in one step, the PLS method compresses the data (Martens and Jensen, 1982) and maximizes the variability of the dependent variable (Martens and Naes, 1987). The PLS method is therefore considered more efficient for calibration than the regression on principal components or multi-

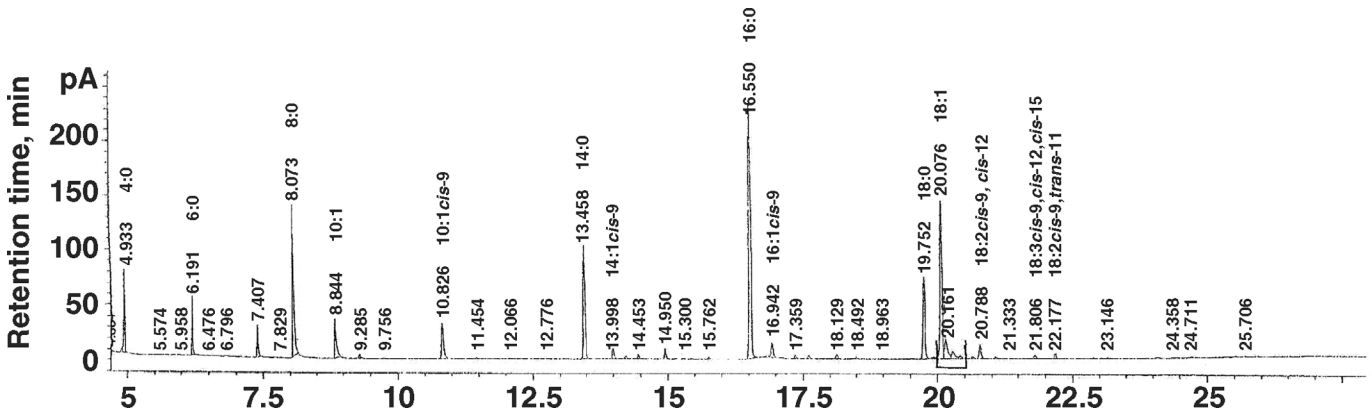

Figure 1. Studied peaks in the chromatogram.

ple linear regression (Prévot, 2004). The number of factors used in the equation was determined by cross-validation, which was also needed to estimate its robustness. Although various pretreatments were studied, none was used on the spectral data before the calibration. Finally, 41 prediction equations were elaborated to estimate the fatty acid profile in milk and milk fat. To assess the efficiency of the calibration equations, various statistical parameters were estimated and analyzed: mean, standard deviation, standard error of calibration (**SEC**), calibration coefficient of determination, standard error of cross-validation (**SECV**), and cross-validation coefficient of determination ($\mathbf{R^2_{CV}}$). The ratio of SECV to standard deviation (**RPD**) was also calculated (Williams and Norris, 2001) to assess the efficiency of the calibration.

RESULTS AND DISCUSSION

Reference Values

Figure 1 presents a typical gas chromatogram recorded under the conditions described earlier. Because of the poor resolution between 18:1 isomers, they were not studied individually. Thus, the concentration indicated for 18:1 in this study is the result of the sum of the different 18:1 isomer contents.

The accuracy of the chromatographic method was assessed by comparing the mean values obtained with the reference values set by BIPEA (Table 1). For all fatty acids, with the exception of 4:0 and 18:1*cis*-9, the mean results obtained were in the variation limits of the reference values. The divergence for 4:0 with the values set by BIPEA might be explained by the more cautious procedure for volatile fatty acids followed during the experiment: The Sovirel tubes (VWR International, Leuven, Belgium) were closed by a hermetic cap between all manipulations. The divergence for 18:1*cis*-9 might be explained by the poor chromatographic resolution between this fatty acid and other 18:1 isomers.

Table 1 shows the coefficients of variation for the butter samples analyzed by BIPEA. In this study, repeatability for a fatty acid was considered acceptable if its coefficient of variation was less than or equal to 5%. With good repeatability obtained for most of the fatty acids studied, 2 decimal places were used in Table 1. In this study, 6:0 and 8:0 showed poor repeatability, which was attributed to some losses owing to their relative volatility. Nevertheless, when pooled in the group

Table 1. Mean concentrations of fatty acids (g/100 g of fat) obtained for reference butter and the reference concentrations of fatty acids in the same butter, set by BIPEA (http://www.bipea.org/)[1]

Fatty acid	Mean	CV	Reference value
4:0	4.43 ± 0.21	4.74	3.3 ± 0.4
6:0	2.31 ± 0.19	8.22	2.0 ± 0.4
8:0	1.72 ± 0.09	5.23	1.3 ± 0.4
10:0	3.42 ± 0.05	1.46	3.1 ± 0.4
10:1*cis*-9	0.34 ± 0.01	2.94	0.3 ± 0.4
12:0	3.83 ± 0.11	2.87	3.8 ± 0.4
14:0	12.13 ± 0.30	2.47	11.8 ± 0.9
14:1	1.08 ± 0.03	2.77	1.1 ± 0.4
15:0	1.22 ± 0.04	3.28	1.2 ± 0.4
16:0	33.35 ± 1.05	3.15	32.0 ± 2.5
16:1	1.43 ± 0.07	4.89	1.8 ± 0.4
18:0	9.97 ± 0.25	2.51	9.8 ± 0.8
18:1*cis*-9	19.41 ± 1.71	8.81	22.3 ± 1.8
18:2*cis*-9,*cis*-12	1.54 ± 0.05	3.25	NA
18:3*cis*-9,*cis*-12,*cis*-15	0.42 ± 0.01	2.38	0.5 ± 0.4
18:2*cis*-9,*trans*-11	0.64 ± 0.01	1.56	NA
SAT	69.38 ± 0.94	1.35	NA
UNSAT	30.62 ± 0.94	3.07	NA
MONO	27.73 ± 0.93	3.35	NA
POLY	2.89 ± 0.12	4.15	NA

[1]Mean = Mean ± standard deviation estimated from 7 samples of the same BIPEA butter; reference value = mean ± tolerance estimated by 26 laboratories; NA = value not set by BIPEA; SAT = saturated fatty acids; UNSAT = unsaturated fatty acids; MONO = monounsaturated fatty acids; POLY = polyunsaturated fatty acids.

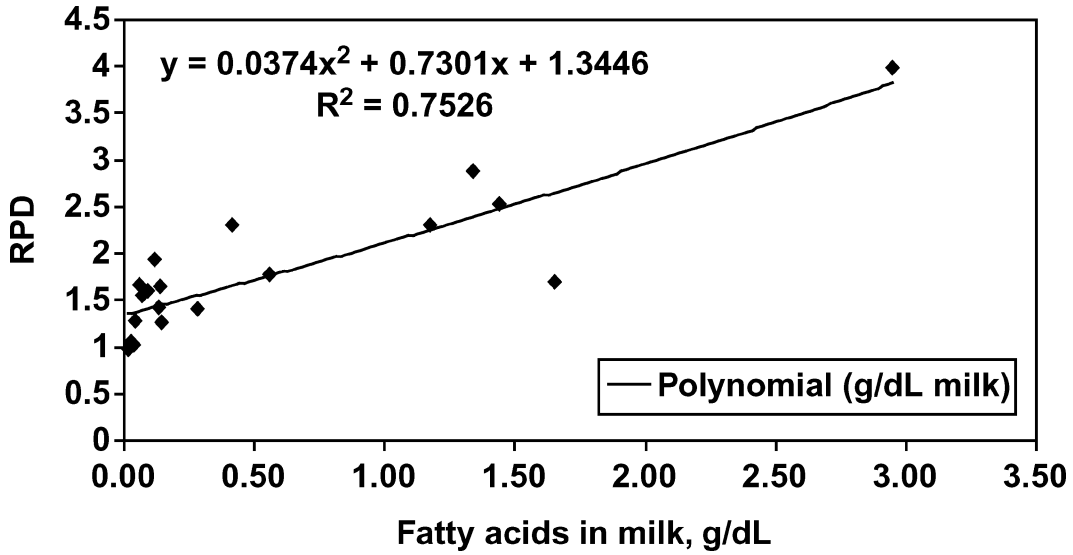

Figure 2. Variation trend of RPD, the ratio of the standard error of cross-validation (SECV) to the standard deviation (SD) as a function of the concentration of fatty acids in milk.

of SAT, the repeatability was acceptable. Generally, good repeatability was observed for fatty acids with longer carbon chains. Overall, except for short-chain fatty acids, the accuracy of the chromatographic method used was acceptable.

Calibration Equations

The applied PLS analysis resulted in equations with approximately 10 factors combining more than 500 values in each equation.

The potential for estimating the fatty acid composition in milk using MIR spectrometry might be explained by the absorptions of electromagnetic radiation at frequencies that are correlated to the vibrations of specific chemical bonds within molecules (Coates, 2000). This explanation is easy with a simple matrix as a mix of 2 different components, but the milk matrix is very complex. The spectrum is therefore the result of successive interactions due to the chemical bonds from all the constituents (fatty acids, proteins, lactose, etc.). By comparing the milk spectrum and specific fatty acid spectrum, the principal MIR regions that were implicated in estimating the fatty acid profile were located between 1,736 and 1,805 cm^{-1} and between 2,823 and 3,016 cm^{-1}. The implication of the first region is logical because Coates (2000) indicated that 1,745 cm^{-1} is the frequency correlated with the vibration of the fatty acid carbonyl group.

Table 2 shows the estimated statistical parameters for each calibration equation. These show that the correlations for predicting fatty acid concentrations in milk were better than those for predicting the same fatty acid concentrations in milk fat. This might be explained by a different dispersion of values obtained for concentrations of fatty acids in milk or milk fat. Indeed, 2 milk samples can have the same fat profile but different percentages of fat in the milk. The profile values expressed in grams per deciliter of milk were autocorrelated more closely than those expressed in grams per 100 grams of fat. The reference values used to establish the predicted equation for fat in milk came from the predicted values obtained using the Milkoscan FT6000. This explains the high result of R^2_{CV} obtained for this calibration equation.

The SEC parameter underestimates the mean square error of the model because the residual variance is not taken into account. Therefore, SEC neglects the variance of regression coefficients and, in the context of calibration model validation, SECV is preferred (Prévot, 2004). To estimate the efficiency of the calibration, the RPD was calculated (Table 2, Figure 2). For potential use, high R^2_{CV} and high RPD parameters would be required. Generally, if the value of R^2_{CV} was high, the value of RPD was high (Table 2).

Based on the results in Table 2, our study also showed that there was a second-order polynomial relationship ($y = 0.0374x^2 + 0.7301x + 1.3446$) between the fatty acid concentrations in milk and the value of RPD ($r^2 = 0.75$). Generally, therefore, if the concentration of fatty acid is high, the potential for predicting the concentration using MIR spectrometry analysis is also high.

To verify whether the predicted concentrations of fatty acids obtained by the calibration equations were

Table 2. Estimated statistical parameters for each calibration equation that estimated the concentrations of fatty acid in milk (g/dL of milk) and in milk fat (g/100 g of fat)[1]

	g/dL of milk							g/100 g of fat						
Fatty acid	Mean	SD	SEC	R^2_C	SECV	R^2_{CV}	RPD	Mean	SD	SEC	R^2_C	SECV	R^2_{CV}	RPD
FAT	4.55	1.18	0.05	1.00	0.06	1.00	20.90	NA	NA	NA	NA	NA	NA	NA
4:0	0.28	0.11	0.07	0.59	0.08	0.51	1.41	6.26	2.02	1.42	0.50	1.60	0.39	1.27
6:0	0.13	0.06	0.04	0.69	0.04	0.52	1.43	2.90	1.26	0.97	0.41	0.98	0.41	1.28
8:0	0.07	0.03	0.02	0.75	0.02	0.59	1.55	1.54	0.68	0.43	0.60	0.50	0.46	1.35
10:0	0.14	0.06	0.03	0.77	0.04	0.64	1.65	3.06	1.31	0.69	0.72	0.90	0.53	1.45
10:1*cis*-9	0.01	0.01	0.01	0.05	0.01	0.01	0.98	0.27	0.16	0.10	0.64	0.12	0.45	1.33
12:0	0.12	0.04	0.02	0.82	0.02	0.74	1.93	2.71	0.87	0.38	0.81	0.53	0.64	1.65
14:0	0.41	0.12	0.04	0.90	0.05	0.82	2.30	9.28	1.95	0.87	0.80	1.14	0.67	1.71
14:1*cis*-9	0.03	0.01	0.01	0.12	0.01	0.07	1.02	0.71	0.32	0.26	0.34	0.28	0.23	1.13
15:0	0.04	0.01	0.01	0.58	0.01	0.40	1.28	0.98	0.29	0.18	0.61	0.20	0.53	1.44
16:0	1.17	0.39	0.11	0.91	0.17	0.82	2.30	25.67	4.89	1.63	0.89	3.50	0.50	1.40
16:1*cis*-9	0.06	0.03	0.02	0.75	0.02	0.65	1.66	1.32	0.46	0.18	0.86	0.37	0.37	1.24
18:0	0.56	0.24	0.12	0.73	0.13	0.69	1.77	11.97	2.87	2.66	0.14	2.77	0.09	1.04
18:1	1.34	0.51	0.12	0.95	0.18	0.88	2.88	29.19	5.74	3.14	0.70	3.99	0.53	1.44
18:2*cis*-9,*cis*-12	0.09	0.03	0.02	0.76	0.02	0.62	1.61	1.96	0.46	0.35	0.41	0.44	0.11	1.05
18:3*cis*-9,*cis*-12,*cis*-15	0.03	0.01	0.01	0.20	0.01	0.14	1.06	0.58	0.22	0.19	0.27	0.20	0.20	1.10
18:2*cis*-9,*trans*-11	0.04	0.02	0.02	0.12	0.02	0.07	1.02	0.82	0.45	0.20	0.80	0.37	0.34	1.21
SAT	2.95	0.78	0.12	0.98	0.20	0.94	3.99	64.87	6.13	2.94	0.77	3.75	0.63	1.64
UNSAT	1.65	0.57	0.29	0.74	0.34	0.66	1.69	35.13	6.13	2.94	0.77	3.75	0.63	1.64
MONO	1.44	0.55	0.18	0.89	0.22	0.85	2.54	31.74	5.87	3.26	0.69	4.10	0.52	1.43
POLY	0.14	0.05	0.03	0.43	0.04	0.39	1.27	3.39	0.77	0.68	0.22	0.74	0.10	1.05

[1]SEC = Standard error of calibration; R^2_C = calibration coefficient of determination; SECV = standard error of cross-validation; R^2_{CV} = cross-validation coefficient of determination; RPD = ratio of standard error of cross-validation to standard deviation; FAT = percentage of milk fat; NA = data not available; SAT = saturated fatty acids; UNSAT = unsaturated fatty acids; MONO = monounsaturated fatty acids; POLY = polyunsaturated fatty acids.

due to real absorbance of these fatty acids or only to the correlations between the total fat content and fatty acids, the correlations between total fat and the studied fatty acids were calculated (Table 3). If the calibration correlations (R_{CV}) were not due to real absorbances specific to fatty acids, these correlations would not be higher than the correlations between total fat and fatty acids. Thus, the predicted concentrations for these fatty acids resulted more from a real infrared prediction than the correlation with the fat. It is interesting to observe that the differences between the correlation with fat and R_{CV} for short-chain fatty acids (4:0, 6:0, 8:0) were higher than the others. Therefore, specific spectral information can be extracted by the PLS model independently from the correlation with total fat.

Table 3. Correlations between the percentage of milk fat and different concentrations of studied fatty acids in milk[1]

	Correlation	
Fatty acid	FAT	R_{CV}
4:0	0.38	0.71
6:0	0.24	0.72
8:0	0.21	0.77
10:0	0.14	0.80
10:1*cis*-9	–0.04	0.09
12:0	0.21	0.86
14:0	0.36	0.90
14:1*cis*-9	0.08	0.26
15:0	0.09	0.63
16:0	0.59	0.90
16:1*cis*-9	0.48	0.80
18:0	0.68	0.83
18:1	0.62	0.94
18:2*cis*-9,*cis*-12	0.51	0.79
18:3*cis*-9,*cis*-12,*cis*-15	0.20	0.37
18:2*cis*-9,*trans*-11	0.05	0.26

[1]Correlation FAT = Value for the correlation between the percentage of milk fat (FAT) and different concentrations of fatty acid; R_{CV} = square root of the R^2_{CV} value (where R^2_{CV} is the cross-validation coefficient of determination).

CONCLUSIONS

The estimation of fatty acid concentrations in milk and in milk fat using MIR spectrometry seems feasible. Results from this study showed that the calibration equations predicting 10:0, 12:0, 14:0, 16:0, 16:1*cis*-9, 18:1, 18:2*cis*-9,*cis*-12, SAT, and MONO in milk could be used. Although some fatty acids present in low concentrations in milk could not be predicted accurately (e.g., n-3 and 14:1*cis*-9), MIR spectrometry could predict most fatty acids (e.g., 14:0, 16:0, 18:0, 18:1, SAT, and MONO). Indeed, most associated coefficients of determination were clearly lower than 1, but they were significantly different from zero. Therefore, and because of the speed of analysis and the present application of the methodology in routine milk testing, MIR spectrometry is an important alternative in the dairy sector for providing indications of the fatty acid profiles in cow

milk. This option seems particularly useful for application in milk recording schemes. The composition of milk fat for each milk sample from an animal could be estimated using the calibration equations established in this study. The estimates based on MIR could be used as indicator traits for real underlying fatty acid concentrations, potentially in a multitrait setting using the appropriate selection index theory. Knowledge of these genetic values would open up opportunities for animal selection aimed at improving the nutritional quality of cow milk.

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