Variation in fat globule size in bovine milk and its prediction using mid-infrared spectroscopy

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ABSTRACT

The objectives of this study were to investigate the sources of variation in milk fat globule (MFG) size in bovine milk and its prediction using mid-infrared (MIR) spectroscopy. Mean MFG size was measured in 2,076 milk samples from 399 Ayrshire, Brown Swiss, Holstein, and Jersey cows, and expressed as volume moment mean (D[4,3]) and surface moment mean (D[3,2]). The mid-infrared spectra of the samples and milk performance data were also recorded during routine milk recording and testing. The effects of breed, herd nested within breed, days in milk, season, milking period, age at calving, parity, and individual animal on the variation observed in MFG size were investigated. Breed, herd nested within breed, days in milk, season, and milking period significantly affected mean MFG size. Milk fat globule size was the largest at the beginning of lactation and subsequently decreased. Milk samples with the smallest MFG on average came from Holstein cows, and those with the largest were from Jersey and Brown Swiss cows. Partial least squares regression was used to predict MFG size from MIR spectra of samples with a calibration data set containing 2,034 and 2,032 samples for D[4,3] and D[3,2], respectively. Coefficients of determination of cross validation for D[4,3] and D[3,2] prediction models were 0.51 and 0.54, respectively. The associated ratio of performance deviation values were 1.43 and 1.48 for D[4,3] and D[3,2], respectively. With these models, individual mean MFG size could not be accurately predicted, but results may be sufficient to screen samples for having either small or large MFG on average. Significant but low correlations of D[4,3] and D[3,2] with milk fat yield were estimated (0.16 and

0.21, respectively). Significant and moderate Pearson correlation coefficients for fat percent with D[4,3] and D[3,2] were assessed (0.34 and 0.36, respectively). This correlation was greater between milk fat percentage and predicted MFG size than with measured MFG size with coefficients of 0.47 and 0.49 for D[4,3] and D[3,2], respectively. The MIR prediction equations are potentially overusing the correlation between fat and MFG size and exploiting the strong relationship between the MIR spectra and total milk fat. However, the predictions of MFG size are able to determine variation in mean globule size beyond what would be achieved just by looking at the correlation with fat production.

Key words: milk fat globule, mid-infrared spectroscopy

INTRODUCTION

Over 95% of the total fat in milk is present in the form of milk fat globules (MFG; Keenan and Dylewski, 1995), a triglyceride globule surrounded by a tripartite cellular membrane. The MFG are secreted in a wide variety of sizes ranging in diameter from about 0.1 to 15 μm . The average bovine MFG is approximately 2.5 to 4.6 μm in diameter although globule size shows considerable variation (Huppertz and Kelly, 2006). The size distribution and average diameter of MFG may depend on a variety of factors, including breed, the individual animal, stage of lactation, diet, and season (Mulder and Walstra, 1974; Wiking et al., 2003; Carroll et al., 2006; Logan et al., 2014; Martini et al., 2016). Most studies examining variation in MFG size have used a small sample size to explore these effects (<80 cows, and cows from a single farm).

The main compositional differences in lipids between small and large MFG relate to the ratio of the MFG core to the membrane. Small MFG have more membrane material per unit of fat than large MFG. The MFG membrane is valuable to the human diet as it is

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naturally rich in important minor lipids and glycoproteins. Bovine MFG membrane has been suggested as a prospective nutraceutical due to the many potential health-benefits of its components (Spitsberg, 2005; Hintze et al., 2011).

Milk fat globule size has critical implications for the technological and sensory properties of many dairy products. This trait is of particular interest in the manufacturing of cheeses, as the interaction between the surface of MFG and the casein matrix influences both the structure and texture of the finished product (Michalski et al., 2003).

Routine determination of MFG size is unfeasible due to the time, cost, and complexity of the requirement of fresh milk. Mid-infrared (**MIR**) technology provides an opportunity to obtain phenotypes on a considerable number of samples at a low cost, with technology already used in regular milk recording. Recently, researchers have been looking at using MIR spectroscopy to phenotype additional, more detailed milk composition, milk properties, and cow characteristics (De Marchi et al., 2014). Prediction is possible—with varying success—for some fatty acids (Soyeurt et al., 2011), protein composition (Rutten et al., 2011), lactoferrin (Soyeurt et al., 2012), minerals (Soyeurt et al., 2009), β -hydroxybutyrate, and acetone (de Roos et al., 2007). Mid-infrared spectroscopy could be used to predict mean MFG size by way of the compositional differences of samples with differing MFG sizes.

The determination of MFG size in milk samples and the factors influencing size may be of interest to the dairy industry in terms of both nutrition and manufacturing. The objectives of this study were (1) to investigate the observed variation in mean MFG size from a large number of individual milk samples; (2) to examine the effectiveness of MIR spectroscopy in predicting mean MFG size; and (3) to determine the relationship of MFG size with routinely recorded milk production traits.

MATERIALS AND METHODS

Data Collection

A total of 44 herds with Ayrshire, Brown Swiss, Holstein, or Jersey cows that were enrolled in Canadian DHI milk recording programs were selected from across the provinces of Alberta, Ontario, and Quebec. Approximately 10 cows from each herd, 5 at the beginning of lactation and 5 in mid lactation on the first test, were identified for milk collection. Individual milk samples (50 mL) were collected from cows multiple times throughout their lactation, and potentially their subsequent lactation, during routine milk testing by

Canadian DHI partners CanWest DHI (Guelph, ON, Canada) for Alberta and Ontario herds and Valacta (Ste-Anne-de-Bellevue, QC, Canada) for Quebec herds. Collection occurred between March 2013 and October 2014 for CanWest DHI and between December 2013 and May 2015 for Valacta.

Milk samples were transported to either a CanWest DHI or Valacta milk laboratory. A portion of the milk sample was removed and analyzed by a MIR spectrometer (MilkoScan FT6000; Foss, Hillerød, Denmark) using standard milk recording procedures. Spectra were collected from 2 machines, one at CanWest DHI and one at Valacta. The MIR data for each sample contained 1,060 data points in the infrared range of 5,000 to 900 cm^{-1} . At the same laboratories, fat and protein content were determined from the spectra. Standardization of the historical spectra between the 2 machines and across time was performed as described by Bonfatti et al. (2017) through the correction of shifts observed in the analyzed principal components of the collected spectra.

The remaining quantity of the milk sample was sent fresh (never frozen) from the DHI laboratory to the University of Guelph (Guelph, ON, Canada) for MFG measurement. Milk samples were analyzed between 1 and 21 d after collection (on average within 5.5 d from the collection). The size distribution of MFG was measured by integrated light scattering with a Malvern Mastersizer 2000 (Southborough, MA). Immediately before measurement, samples were diluted 1:1 in 80 mM EDTA/NaOH solution (pH 7) to minimize the signal from the casein micelles by adding 1 mL of EDTA buffer to 1 mL of milk and vortexing. The absorption coefficients of liquid milk fat used for measurement were 0.5×10^{-5} and 1.7×10^{-5} at 633 and 466 nm, respectively. The refractive indices of MFG in water were 1.458 and 1.460 at 633 and 466 nm, respectively. Whole milk was measured at a stirring speed of 2,800 rpm. The obscuration was 10 to 15%. The diameter of the MFG was recorded as volume moment mean (**D[4,3]**), and surface moment mean (**D[3,2]**) defined by the equation:

$$D[k, z] = \frac{\sum N_i d_i^k}{\sum N_i d_i^z},$$

where N_i is the number of globules in a size class of d_i , $k = 4$ and $z = 3$ for **D[4,3]**, and $k = 3$ and $z = 2$ for **D[3,2]**.

At the conclusion of collection, 2,083 milk samples had mean MFG size measured from 392 cows representing the 4 breeds with saved MIR spectra. The number of samples per cow ranged from 1 to 14 and averaged 5.31 samples per cow; 26 cows had only one MFG size

record. Sampling numbers varied greatly due to the number of times a herd was visited, sample spoiling or freezing during transport, cow health complications, cows entering their dry period, and cow removal from the herd. Two samples were removed because their D[3,2] measurement was greater than that for D[4,3] and a recording error was assumed. An additional 5 records with values greater than 4 standard deviations from the mean were removed, to eliminate measurement errors and milk samples where MFG aggregation likely occurred. This left 2,076 samples in the data set. The number of cows, samples, and herds with measured MFG size and their descriptive statistics are shown in Table 1. The distributions of the samples collected had skewness and kurtosis values of -0.17 and 0.01 , respectively, for D[3,2], and 0.80 and 1.19 , respectively, for D[4,3]. The selection of herds was geared toward obtaining as much variation in the fine milk components as possible, which included finding herds using a wide range of management practices. Many of these herds had Holsteins, the most prominent dairy breed in Canada, and thus the majority of the samples have come from Holstein cows.

Phenotypic Analysis

Complete information on the test day and the cow was available for 1,826 of the analyzed samples coming from 361 animals in 42 herds. Information included herd, breed, DIM, season, milking period (a.m. or p.m.), age at calving, and parity. Data were analyzed using the MIXED procedure of SAS (SAS Institute, 2013), with a repeated statement to allow for multiple records per cow using the following linear model:

$$y_{ijklmno} = \mu + H_i(B)_j + B_j + DIM_k + S_l + MP_m + a_n + e_{ijklmno},$$

where $y_{ijklmno}$ is the dependent variable D[4,3] or D[3,2]; μ is the overall mean; $H_i(B)_j$ is the fixed herd effect nested within breed ($i = 1$ to 42); B_j is the fixed effect

of breed ($j = 1$ to 4), DIM_k is the fixed effect of days in milk class ($k = 1$ to 11 ; class 1: 5–30 d, class 2: 31–60 d, class 3: 61–90 d, ..., class 10: 271–305 d, and class 11: >305 d after calving), S_l is the fixed effect of season ($l = 1$ to 4 ; class 1: January–March, class 2: April–June, class 3: July–September, class 4: October–December); MP_m is the fixed effect of milking period ($m = 1$ to 2 ; class 1: a.m., class 2: p.m.), a_n is the random effect of animal ($n = 1$ to 361); and $e_{ijklmno}$ is the random error.

No relationship matrix was used and animals were therefore assumed unrelated. Parity and age at calving did not have a significant effect on MFG size ($P = 0.87$ and $P = 0.60$, respectively) and were excluded from the final model. A significance level of $P < 0.05$ was used. Least squares means (LSM) were compared by use of Scheffé’s adjustment to determine significant differences between the factor levels. Orthogonal contrasts were estimated between LSM for the effects of DIM (linear, quadratic, cubic, and quartic components).

Predictive MIR Model

Prediction equations were obtained by partial least squares (PLS) regression using PLS procedures of SAS (SAS Institute, 2013). Regions of the MIR spectra from $3,105$ to $3,444\text{ cm}^{-1}$ and $1,628$ to $1,658\text{ cm}^{-1}$, characterized by low signal-to-noise ratio caused by the high absorption of water, were not used for the PLS analysis. Thus, 862 spectral variables were used. No further spectral pretreatments were used to develop the prediction equations. Milk sample spectral outliers were assessed and removed during the PLS procedure. Originally, all samples with both MFG size and spectral data available ($n = 2,076$) were included in the calibration set and models were created. The root mean sum of squares of residuals for standardized predictors were examined for each sample as a measure of the distance between the data point and the model plane in X-space. A maximum tolerable level was set to 3 standard deviations (SD) from the mean, with values over this limit considered outliers and removed. The PLS procedures

Table 1. Number of cows, records, and herds and the descriptive statistics of measured milk fat globule volume moment mean (D[4,3]) and surface moment mean (D[3,2]) for Ayrshire (AY), Brown Swiss (BS), Holstein (HO), and Jersey (JE) cows

Breed	No. of cows	No. of records	No. of herds ¹	Mean (μm)		SD (μm)	
				D[4,3]	D[3,2]	D[4,3]	D[3,2]
AY	55	194	7	4.37	3.69	0.39	0.28
BS	23	97	3	4.23	3.61	0.49	0.30
HO	254	1,584	29	4.14	3.47	0.50	0.34
JE	60	201	8	4.90	3.88	0.58	0.29
Total	392	2,076	44	4.24	3.54	0.55	0.35

¹Three herds had multiple breeds.

were then used on the remaining data ($n = 2,034$ and $2,032$ for D[4,3] and D[3,2], respectively) to produce the final calibration equation. Full cross-validation was used with a leave-one-out cross-validation (**LOOCV**) procedure, whereby one sample is removed and the remaining samples are used to produce the model and predict the discarded sample. The process is repeated until each sample has been predicted once, with the validation errors saved each time to produce the standard error of cross-validation (**SECV**). To assess the accuracy and utility of the calibration equations, the coefficient of determination of cross-validation (R^2_{cv}), the ratio of performance deviation (**RPD**), and the range error ratio (**RER**) were calculated. For RPD (the ratio of SD to SECV), a higher value is desired, with an RPD >2 enabling good predictions (De Marchi et al., 2011). The RER is calculated by dividing the range of the reference data by the SECV, and can be used to determine the practical application of the prediction equations. Calibration equations with an RER value between 7 and 20 are considered poor to fair and may be adequate for screening purposes; those with values between 21 and 40 are good to very good and more suitable for quality and process control applications (Williams, 2001). An independent data set was not available on which to perform an external validation of the predictive models. Therefore, to assess any possible overfitting with the LOOCV model, a 5-fold cross-validation was also performed. Samples were randomly allocated into 5 subsets and, one at a time, a single subset was retained as a validation set and the remaining subsets were used as the training set. This 5-fold cross-validation with random allocation was repeated 100 times with fitting statistics calculated and averaged.

Correlation with Production Traits

Milk production from milk recording and fat and protein yields calculated routinely from MIR spectra by DHI labs were available for 1,957 of the samples used in the MFG calibration data set. Correlation coefficients were estimated with SAS among the measured and predicted measures of MFG size along with milk yield, fat yield and percentage, and protein yield and percentage. Correlations with P -values < 0.05 were deemed significant.

RESULTS AND DISCUSSION

Phenotypic Analysis

Descriptive statistics of measured MFG size are summarized in Table 1. The average D[4,3] for all samples

analyzed was $4.24 \mu\text{m}$ (SD: $0.55 \mu\text{m}$) and the average D[3,2] was $3.53 \mu\text{m}$ (SD: $0.35 \mu\text{m}$). Herd nested within breed, breed, DIM, season, and milking period all had a significant effect on MFG size ($P < 0.05$). An effect of milking period was found, with evening milk having significantly larger MFG on average ($P < 0.05$) than morning milk. Least squares means for D[4,3] were $4.65 \mu\text{m}$ for morning and $4.68 \mu\text{m}$ for evening milk. For D[3,2], the LSM were $3.74 \mu\text{m}$ for morning milk and $3.78 \mu\text{m}$ for evening milk. In this study, information was only available on the time of day of milking and not milking frequency or interval between milkings. The categorization of this variable may be misguided if participating herds use unequal intervals between milkings, which is a factor likely to have an effect, as observed by Wiking et al. (2006).

We found a significant effect ($P < 0.001$) of season on MFG size. For D[4,3] and D[3,2], milk collected in the spring (April to June) had larger MFG on average than milk collected later in the year (Figure 1). Also, for D[4,3] only, MFG size was larger in the first 3 mo compared with the last 3 mo of the year. The large MFG in spring milk in this study is in contrast to Mulder and Walstra (1974), who noted smaller MFG in spring milk compared with winter milk. Couvreur et al. (2006) observed a decrease in mean MFG size when a greater amount of fresh grass was included in the diet, which could occur in spring and summer. Diet and management practices of the contributing herds were not known, and operations may have similar feeding through the entire year. In addition, the spring season experienced in the present study may not be comparable, because primarily pasture feeding may not commence until much later in the year in some of the participating regions of the country.

Milk from Jersey and Brown Swiss cows had, on average, larger MFG than Ayrshire and Holstein cows, with Holstein having the smallest (Figure 2). Carroll et al. (2006) did find that MFG from Jersey cows were larger in diameter on average than that for Brown Swiss or Holstein cows. Mulder and Walstra (1974) also noted that milk from Jersey and Guernsey cows contained larger MFG on average than milk from Holstein-Friesian type cows.

The herd nested within breed effects had LSM ranging between 3.3 and $4.1 \mu\text{m}$ for D[3,2] and between 3.9 and $5.9 \mu\text{m}$ for D[4,3]. For both traits, we observed variation in the solutions for herds with the same breed. The exception was herds with Jersey cows, which tended to all have high least squares means.

Milk fat globule size was greatest at the start of lactation and generally decreased thereafter (Figure 3). In the present study, the relationship between both D[4,3] and D[3,2] with DIM was determined to be cubic in na-

ture ($P < 0.001$). Maximum MFG size at the beginning of lactation followed by a decrease was also noted by Mulder and Walstra (1974) and Wiking et al. (2003). The association between the effects of breed and DIM with MFG size may be connected to relationship of breed and DIM with fat yield.

MFG Calibration Equations

Descriptive statistics of the samples used to create the calibration equations, the predicted values, and the studied milk quality traits are summarized in Table 2. The fitting statistics of the predictive models for D[4,3] and D[3,2] using LOOCV are shown in Table 3. The model for D[3,2] predicted slightly better with an R^2_{cv} of 0.54 compared with 0.51 for D[4,3]. The corresponding RPD values of D[4,3] and D[3,2] prediction models

Figure 1. Least squares means of milk fat globule size measured as (a) volume moment mean (D[4,3]), and (b) surface moment mean (D[3,2]) for season. Means with different letters differ at $P < 0.05$. Error bars represent SEM.

Figure 2. Least squares means of milk fat globule size measured as (a) volume moment mean (D[4,3]), and (b) surface moment mean (D[3,2]) for Holstein (HO; $n = 1,406$ samples), Ayrshire (AY; $n = 171$), Brown Swiss (BS; $n = 60$), and Jersey (JE; $n = 189$). Means with different letters differ at $P < 0.05$. Error bars represent SEM.

were 1.43 and 1.48, respectively, showing that the models were doing a relatively poor job of accurately predicting mean MFG size. The RER values, which were both greater than the 7 stated by Williams (2001) as a minimum for screening purposes, also indicated that the models did explain enough of the variation in MFG size to be some gauge of the average MFG size. When averaged, the fitting statistics from the 5-fold cross-validation were almost identical to those found with LOOCV. However, a range did exist with the different random allocations into the subsets. The R^2_{cv} values ranged from 0.47 to 0.55 for D[4,3] and 0.50 to 0.57 for D[3,2]. The models performed poorly and the generated results were comparable to the LOOCV results.

Figure 4 displays the measured versus predicted values of D[4,3] and D[3,2]. The current MIR prediction equations do not quantify the size adequately but are

Table 2. Descriptive statistics of measured milk fat globule volume moment mean (D[4,3]) and surface moment mean (D[3,2]) used in prediction models, predicted milk fat globules volume moment mean (pD[4,3]) and surface moment mean (pD[3,2]), and milk production traits

Trait	No. of samples	Mean	SD	Minimum	Maximum
D[4,3] (μm)	2,034	4.24	0.55	2.78	6.35
D[3,2] (μm)	2,032	3.53	0.35	2.21	4.73
pD[4,3] (μm)	2,034	4.24	0.40	3.17	6.34
pD[3,2] (μm)	2,032	3.53	0.26	2.75	4.67
Fat (kg)	1,957	1.21	0.38	0.20	3.53
Fat (%)	1,957	3.98	0.75	1.34	8.03
Milk (kg)	1,957	31.46	10.78	5.00	72.60
Protein (kg)	1,957	1.03	0.29	0.20	2.06
Protein (%)	1,957	3.35	0.40	2.42	4.97

sufficient to screen milk samples and identify milk with large or small MFG on average. A relationship between the measured and predicted values could be noted, and generally milk samples with smaller MFG on average predict in the lower part of the range and samples with the larger MFG on average predict in the high part of the range. The standard deviations calculated for the predicted values of D[4,3] and D[3,2] were less than found in the measured values (Table 2). The models tended to predict MFG size toward the means, with small MFG samples predicting higher and large MFG predicted lower than their true values. The slightly better predictive ability of the D[3,2] measure makes it more appealing because the 2 traits are very similar in nature. In addition, D[3,2] is more relevant where specific surface area is meaningful, such as with the MFG membrane in improving the nutritional value of milk and milk products.

Figure 3. Least squares means of milk fat globule (MFG) size measured as volume moment mean (D[4,3]), and surface moment mean (D[3,2]) for classes of DIM.

The ability to predict milk components and properties from their MIR spectra has shown variable results depending on the trait. Much success has been achieved in using MIR spectra to predict individual and groups of fatty acids. Soyeurt et al. (2011) reported R^2 values >0.95 for saturated, unsaturated, short-, medium-, and long-chain fatty acid groups. The predictive ability of individual fatty acids is generally better for major fatty acids with a relationship between R^2 and concentration being observed (De Marchi et al., 2011). Differences in fine fat composition are thus detectable in milk samples using MIR spectroscopy, and MFG size prediction equations may draw on this information.

The intent in predicting MFG size from MIR spectra was to exploit the compositional differences between milk with small and large MFG, not to directly detect particle size. In the process of procuring the MIR spectra of a milk sample, a homogenizer is added to disrupt the MFG and make them smaller. This is necessary because MIR radiation has limited penetration depth, and therefore, the spectra are sensitive to the presence of MFG or fat biofilms and scattering could occur. The major compositional difference between differently sized MFG is the relative concentration of phospholipid, as well as other membrane materials, to triacylglycerol. The ratio of surface area to volume of a sphere is 3 divided by the radius, and consequently, compositional changes related to the ratio of membrane to core material with increasing size is a more complex relationship to predict from MIR spectra.

Other compositional differences have been noted between small and large MFG, primarily fatty acid concentrations, although many disparities exist, likely due to large differences in methodology. Average MFG diameter is positively correlated with C16, C16:1, C18, and C18:1 but not with C4 to C14, C18:2, or C18:3 according to Wiking et al. (2004). Conversely, Briard et al. (2003) discovered higher amounts of C18:1 and C18:2 in small MFG than in large MFG. Lopez et al. (2011)

Table 3. Fitting statistics of prediction models for milk fat globule volume moment mean (D[4,3]) and surface moment mean (D[3,2])¹ from leave-one-out cross-validation procedures

Trait	No. of samples	SECV	R ² _{cv}	RPD	RER
D[4,3]	2,034	0.39	0.51	1.43	9.26
D[3,2]	2,032	0.24	0.54	1.48	10.55

¹SECV = standard error of cross validation; R²_{cv} = coefficient of determination of cross validation; RPD = ratio of performance deviation; and RER = range error ratio.

did not find differences in the amount of total saturated and unsaturated fatty acids or in the amounts of short-chain fatty acids (C:6 to C10:0) between different sized MFG. They did, however, find significantly more C12:0, C14:0 C16:0, C18:1 *trans*, and C18:2 *cis*-9,*trans*-11 and

less C18:0 and C18:1 *cis*-9 in small MFG than in large MFG. Conversely, Timmen and Patton (1988) found less short-chain fatty acids (C4:0 to C10:0) in small MFG compared with large MFG, but also found less C18:0 and greater C18:1. It is, therefore, important to note that the nature and magnitude of compositional changes observed between small and large MFG may not be consistent and can be influenced by diet (Wiking et al., 2004; Lopez et al., 2008), breed (Gallier et al., 2011), and season (Briard et al., 2003). This may limit the suitability of using MIR technology to predict MFG size through compositional differences and a reason for the low coefficient of determination. Furthermore, in this study, mean diameter was the only measure available and no other valuation of the distribution of MFG size for a milk sample was known. The overall fat composition of a sample is liable to change depending on the shape of the MFG size distribution.

Correlation with Production Traits

Pearson correlation coefficients among measured and predicted MFG size and production traits are shown in Table 4. A high correlation of 0.90 was found between the 2 measures of MFG size. This relationship was expected because the 2 parameters are describing much the same trait, but the assessment of D[4,3] is more sensitive to the presence of large particles and D[3,2] is more sensitive to small particles in the size distribution. A low but significant correlation was found between MFG size and protein percent ($P < 0.001$ and $P = 0.04$ for D[4,3] and D[3,2], respectively), but no significant correlation existed with protein yield ($P = 0.14$ and $P = 0.72$, respectively). The relationships between milk yield, protein yield, and protein percent with MFG size were largely unchanged between the measured and predicted MFG size measures. A significant correlation ($P = 0.04$) was observed between milk yield and predicted D[4,3] that was not present between milk yield and D[4,3]. However, this correlation was still very small (-0.05).

Significant correlations ($P < 0.001$) between fat yield and measured D[4,3] and D[3,2] of 0.16 and 0.21,

Figure 4. Predicted versus measured milk fat globule size measured as (a) volume moment mean (D[4,3]), and (b) surface moment mean (D[3,2]).

Table 4. Pearson correlation coefficients between measured milk fat globule volume moment mean (D[4,3]) and surface moment mean (D[3,2]), predicted milk fat globule volume moment mean (pD[4,3]) and surface moment mean (pD[3,2]), and milk production traits

	D[4,3]	D[3,2])	pD[4,3]	pD[3,2]	Fat yield	Fat percent	Milk yield	Protein yield	Protein percent
D[4,3]		0.90***	0.72***	0.68***	0.16***	0.34***	−0.03	−0.03	0.08***
D[3,2]			0.70***	0.74***	0.21***	0.36***	−0.01	−0.01	0.05*
pD[4,3]				0.96***	0.23***	0.47***	−0.05*	−0.03	0.10***
pD[3,2]					0.30***	0.49***	−0.01	0.01	0.05*

* $P < 0.05$; *** $P < 0.001$.

respectively, were found. The correlations between fat percentage and D[4,3] and D[3,2] were greater at 0.34 and 0.36, respectively. Wiking et al. (2004) also found that D[4,3] was explained partially by diurnal fat production in Danish Holstein cows ($R^2 = 0.54$). They theorized that MFG membrane material synthesis might not be able to increase as fat yield increases and, as a result, fat droplets grow larger before they are enveloped with plasma membrane in the secretory apical membrane. Larger MFG could be secreted to reduce the amount of membrane lost per unit volume of fat when membrane material is limited. However, there is currently little understanding of how the eventual size of the MFG is decided during their formation and secretion, and whether fat production has a causative association with MFG size. The positive connection between MFG size and fat production has also been investigated through animal diet. When cows were fed concentrate with a high quantity of saturated lipids, Wiking et al. (2003) found that the milk had a greater fat content and significantly larger MFG than milk from cows fed other diets. This was also demonstrated by Carroll et al. (2006) who observed, with 12 cows from 3 dairy breeds, that average MFG diameter tended to increase with increased fat production as a result of feeding diets with increased fat.

The correlation coefficients of the MFG size predictions with fat yield and fat percent were greater than those of their measured equivalents. The correlation coefficients for fat yield increased to 0.23 and 0.30 for predicted D[4,3] and D[3,2], respectively. Higher correlations of 0.47 for predicted D[4,3] and 0.49 for predicted D[3,2] with fat percent were found. The increase in the correlation coefficient suggested that the predictive model, for both measures of MFG size, likely draw on the correlation between MFG size and fat content and using absorption bands more associated with total fat. However, the correlation coefficients between the measured and predicted D[4,3] and D[3,2] were 0.72 and 0.74, respectively, which are greater than the correlation with fat and, thus, are better indicators of MFG size.

Prediction equations are only beneficial if the model is in fact using information directly related to the trait or component. The PLS methods used to create the predictions for milk traits from MIR spectroscopy depend on the correlations between MIR absorption and the trait, but models may also inadvertently be built on indirect correlations between the trait of interest and absorption bands not directly caused by that trait. If these indirect correlations are not preserved, the predictions will lose accuracy and become ineffective. Eskildsen et al. (2014) suggested that MIR predictions of individual fatty acids might not be valuable because the models are providing information related to total fat rather than the individual fatty acid, and future samples may predict poorly if these correlations are inconsistent. The compositional differences between milk with varying MFG size are complex and may range as discussed above. Accordingly, it is conceivable that the predictive models may be using information indirectly correlated with components such as total fat content.

The measured and predicted values of MFG size at a common fat content (4%; the mode of the data) were investigated more closely, and predicted MFG size still showed variation similar to the measured values (Figure 5). For a given fat content, milk samples with small MFG on average had a lower predicted size than samples with large measured MFG. Although the MIR prediction exploits the correlation with milk fat, the prediction does apply information beyond milk because small and large MFG size on average can still be differentiated to some extent for a given fat content.

CONCLUSIONS

The present study investigated the variation observed in mean MFG size and MIR prediction as a potential tool for the screening of MFG size. Mean MFG size varied between breeds, days in milk, season, milking period, and individual animal. The current MIR prediction equations are poor for precise quantification but may be useful to differentiate samples with small and large MFG on average. The stronger correlation

Figure 5. Predicted versus measured milk fat globule size measured as surface moment mean (D[3,2]) for samples with a common (4.0%) milk fat content.

between predicted MFG size and milk fat content demonstrates that the models are using correlations not truly indicative of MFG size. However, predicted MFG size still shows variation within milk fat categories. The further analysis of the milk samples for additional minor milk components will give further insight into potential compositional changes in milk with differing MFG size that MIR models may be employing.

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