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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:

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

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