Heritabilities of measured and mid-infrared predicted milk fat globule size, milk fat and protein percentages, and their genetic correlations

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ABSTRACT

The objective of this study was to estimate the heritability of milk fat globule (MFG) size and mid-infrared (MIR) predicted MFG size in Holstein cattle. The genetic correlations between measured and predicted MFG size with milk fat and protein percentage were also investigated. Average MFG size was measured in 1,583 milk samples taken from 254 Holstein cows from 29 herds across Canada. Size was expressed as volume moment mean (D[4,3]) and surface moment mean (D[3,2]). Analyzed milk samples also had average MFG size predicted from their MIR spectral records. Fat and protein percentages were obtained for all test-day milk samples in the cow's lactation. Univariate and bivariate repeatability animal models were used to estimate heritability and genetic correlations. Moderate heritabilities of 0.364 and 0.466 were found for D[4,3] and D[3,2], respectively, and a strong genetic correlation was found between the 2 traits (0.98). The heritabilities for the MIR-predicted MFG size were lower than those estimated for the measured MFG size at 0.300 for predicted D[4,3] and 0.239 for predicted D[3,2]. The genetic correlation between measured and predicted D[4,3] was 0.685; the correlation was slightly higher between measured and predicted D[3,2] at 0.764, likely due to the better prediction accuracy of D[3,2]. Milk fat percentage had moderate genetic correlations with both D[4,3] and D[3,2] (0.538 and 0.681, respectively). The genetic correlation between predicted MFG size and fat percentage was much stronger (greater than 0.97 for both predicted D[4,3] and D[3,2]). The stronger correlation suggests a limitation for the use of the predicted values of MFG size as indicator traits for true

average MFG size in milk in selection programs. Larger samples sizes are required to provide better evidence of the estimated genetic parameters. A genetic component appears to exist for the average MFG size in bovine milk, and the variation could be exploited in selection programs.

Key words: milk fat globule, mid-infrared prediction, genetic parameter

INTRODUCTION

Fat is an important component of milk because it has a role in the economic value, nutrition, and technological properties of milk and milk products. More than 95% of the total fat in milk is present in the form of milk fat globules (MFG; Keenan and Dylewski, 1995), a triple layer cellular membrane surrounding a globule of primarily triacylglycerol. Walstra (1969) reported that MFG with a diameter of less than 1 μm represented 80% of all MFG, but only 5% of milk fat volume, while MFG ranging from 1 to 8 μm comprised approximately 94% of fat. The remaining 1 to 2% of the fat volume comes from a small number of large MFG with a diameter greater than 8 μm . Milk fat globule triacylglycerols are synthesized at the rough endoplasmic reticulum, accumulated, and then released as microlipid droplets into the cytosol with a surface coat of polar lipids and proteins derived from the endoplasmic reticulum and the mammary cell (Mather and Keenan, 1998). The droplets grow in size through coalescence with each other as they migrate from their sites of origin to the apical region, where growth is most pronounced (Stemberger and Patton, 1981). The mechanisms involved in the fusion of lipid droplets and whether these processes are regulated or random remain speculative. The average size of MFG can be affected by multiple factors including breed (Carroll et al., 2006), stage of lactation (Walstra, 1995), and

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diet (Wiking et al., 2003). Variation in MFG diameter between individual animals within a herd was reported by Logan et al. (2014), which suggests potential genetic differences for MFG size and the possibility of selecting cows for this trait.

Milk fat globule size is a trait of interest because it affects the technological and sensory properties and nutritional quality of milk and milk products (Martini et al., 2016). Milk fat globules affect the stability of the emulsions, the creaming rate, and the crystallization of milk fat (Huppertz and Kelly, 2006). Michalski et al. (2003) showed that using milk with either small or large MFG could alter the moisture content and texture of cheese. Of interest to consumers are the health benefits of many of the MFG membrane components, which include important minor lipids and glycoproteins (Spitsberg, 2005). The addition of MFG membrane to the diet of infants and children has been shown to have possible positive benefits for neurodevelopment and defense against infections (Hernell et al., 2016). At a given amount of fat, cows producing small MFG will be producing higher amounts of the beneficial MFG membrane material.

Mid-infrared (MIR) spectroscopy has been used to quantify fat and protein content in milk samples in dairy herd improvement for decades. The use of this technology has more recently expanded to predict additional milk components and properties, including fatty acid content (Rutten et al., 2009; Soyeurt et al., 2011), protein composition (Bonfatti et al., 2011), mineral content (Soyeurt et al., 2009), and milk coagulation properties (Cecchinato et al., 2009). In the context of cow physiology, MIR spectroscopy has been used to assess energy intake and efficiency (McParland et al., 2014). The MIR-predicted values could act as indicator traits to allow for the routine genetic evaluation of many meaningful traits for which large-scale phenotyping would otherwise be unattainable. Most importantly, MIR spectroscopy is already used in milk recording programs, and this system could be used to predict additional, novel traits with minimal added infrastructure. The adequacy of MIR predicted traits for selection purposes depends on the performance of the prediction equation. Knowledge of the genetic correlations between the predicted values, their true values, and other milk components could give insight into their utility in breeding programs.

Few studies have investigated the genetic influence on MFG size and the corresponding estimated genetic parameters. The objective of this study was to estimate genetic parameters of measured and MIR-predicted average MFG size and their genetic correlations with each other and with milk fat and protein percentages.

MATERIALS AND METHODS

Data

Individual cow milk samples were obtained from 254 Holstein cows from 29 participating herds between March 2013 and April 2015. Approximately 10 cows per participating herd were preselected, with half being at the beginning of their lactation and half at mid lactation. Milk was collected as a part of routine milk recording programs by trained field staff from Canadian DHI partners, CanWest DHI (Guelph, ON, Canada) and Valacta (Ste-Anne-de-Bellevue, QC, Canada). Sampling occurred multiple times throughout the project time frame, with the time of day of milking alternating with each DHI milk collection visit. The average number of milk samples per cow was 6.3 and ranged from 1 to 14. In total, 246 cows had more than one milk sample, and 123 of these cows had milk samples coming from a second lactation. In total, 1,589 milk samples were analyzed for average MFG size.

Milk fat globule size distribution was measured on fresh (never frozen) milk samples by integrated light scattering with a Malvern Mastersizer 2000 (Southborough, MA) using a 2-wavelength protocol. 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 2,800-rpm speed stirring. The obscuration was 10 to 15%. The mean diameter of the MFG was calculated by the integrated software and expressed 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 .

If **D**[4,3] was lower than **D**[3,2], a recording error was assumed and the corresponding records were omitted. Records for which **D**[4,3] or **D**[3,2] were farther than 4 standard deviations away from the mean were also removed. After edits, 1,583 milk samples had **D**[4,3] and 1,586 had **D**[3,2] data.

Predicted **D**[4,3] (**pD**[4,3]) and predicted **D**[3,2] (**pD**[3,2]) were obtained for these data from the saved MIR spectra of the milk samples and the calibration

equations previously described by Fleming et al. (2017). The MIR prediction equations were developed to predict average MFG size in milk samples using a multi-breed calibration set that included the Holstein samples used in the present study. The coefficients of determination of cross validation (R_{cv}^2) of the prediction equations were 0.51 and 0.54 for D[4,3] and D[3,2], respectively (Fleming et al., 2017). As a result of some milk samples not having recorded MIR spectra or having spectra deemed to be outliers, 1,551 samples had pD[3,2] and 1,549 had pD[3,2].

Test-day fat and protein percentages determined during milk recording procedures were obtained from the Canadian Dairy Network (Guelph, ON, Canada). If one test-day in a cow's lactation had MFG size recorded, the fat and protein percentage for all test days in that lactation were kept. As a result, 3,643 fat and protein percentage records were kept for these traits (average of 14.5 samples per cow). A full pedigree going back as many generations as available containing 22,819 individuals was provided by the Canadian Dairy Network. The 254 cows with records were the progeny of 169 different sires.

Genetic Analysis

Univariate and bivariate repeatability animal models were used to analyze the data. The genetic correlation between the repeated measurements was assumed to be 1 across the lactation and parities. Variance components were estimated using the average information-REML (AI-REML) procedure in the DMU package (Madsen and Jensen, 2008). The following linear animal model was applied to all traits:

$$y_{ijklmn} = \mu + HS_i + P_j + DIM_k + pe_l + a_m + e_{ijklmn},$$

where y_{ijklmn} is the dependent D[4,3], D[3,2], pD[4,3], pD[3,2], fat percentage, or protein percentage; μ is the overall mean; HS_i is the fixed herd season effect ($i = 1-114$); P_j is the fixed effect of parity ($j = 1$ to 4 and more); DIM_k is the fixed effect of days in milk class ($k = 1-11$; 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); pe_l is the random permanent environment effect ($l = 1-254$); a_m is the random additive genetic effect of the animal ($m = 1-22,819$); and e_{ijklmn} is the random error term.

Heritability was calculated using variance components estimated from the univariate analysis of each trait as $\sigma_a^2 / (\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2)$, where σ_a^2 is the additive

genetic variance, σ_{pe}^2 is the permanent environmental variance, and σ_e^2 is the residual variance. Repeatability was defined as $(\sigma_a^2 + \sigma_{pe}^2) / (\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2)$. Genetic correlations between traits were estimated from the series of 14 bivariate analyses with D[4,3], D[3,2], pD[4,3], and pD[3,2] among themselves and with fat and protein percentages.

RESULTS AND DISCUSSION

Descriptive Statistics

Descriptive statistics for all examined traits in the edited data set are displayed in Table 1. The mean D[4,3] was $4.130 \pm 0.483 \mu\text{m}$, whereas D[3,2] had a lower mean of $3.468 \pm 0.339 \mu\text{m}$. These values are within the range reported by Huppertz and Kelly (2006) and Martini et al. (2016). The MIR predicted values for these traits had similar means to the measured MFG, but the variation observed in the predicted traits was lower. This phenomenon is a consequence of the low predictive power of the model whereby values in the extreme parts of the distribution (i.e., in the tails) are predicted to be closer to the mean than their corresponding true measured values.

Heritabilities and Repeatabilities

Heritability estimates calculated from the univariate analysis are shown in Table 2. The milk fat and protein percentages had heritabilities of 0.370 and 0.364, respectively. Miglior et al. (2007) reported for Canadian Holstein cattle heritabilities ranging from 0.533 to 0.555 for fat percentage and 0.561 to 0.586 for protein percentage. These heritabilities are greater than those found in the current study, but when the standard errors are considered, the heritabilities are only slightly lower than expected. The observed differences are attributable to different population and

Table 1. Descriptive statistics of milk fat globule volume moment mean (D[4,3]), surface moment mean (D[3,2]), predicted volume moment mean (pD[4,3]), predicted surface moment mean (pD[3,2]), and milk fat and protein percentages

Trait	N	Mean	SD
D[4,3] (μm)	1,583	4.130	0.483
D[3,2] (μm)	1,586	3.468	0.339
pD[4,3] (μm)	1,551	4.171	0.357
pD[3,2] (μm)	1,549	3.489	0.243
Fat (%)	3,643	3.951	0.704
Protein (%)	3,643	3.282	0.357

simple genetic models used in the present study. Moderate heritabilities of 0.364 and 0.466 were found for D[4,3] and D[3,2], respectively. This finding suggests that the genetics of the cow plays a significant role in determining the average size of the MFG that are formed and secreted in milk, and selection for this trait is possible. Limited studies have examined the heritability of average MFG size in milk. Hassaneyn (1965) reported a high heritability of 0.73 for MFG size using a half-sib method in German Friesian cows, which included the records of 233 cows from 21 different sires. However, the methodologies used in the determination of MFG size are out of date and dissimilar to those used presently. Regardless, this finding still supports the notion that MFG size is heritable in dairy cattle. The variation in MFG size observed by Couvreur et al. (2007) and Logan et al. (2014) for cows within a herd may thus be explained in part by genetic differences with or without other unaccounted for on-farm factors. Argov-Argaman et al. (2013) used the ratio of phospholipid to triacylglycerol as an indicator of MFG size and discovered a significant relationship between this ratio and diacylglycerol acyltransferase 1 (*DGAT1*) genotype. The *DGAT1* gene is involved in the last step of the synthesis of triacylglycerol and has a major effect on milk fat content (Grisart et al., 2002) and polar lipid content (Argov-Argaman et al., 2013). The regulation of the overall size distribution of MFG within a milk sample and how the size of any one particular MFG is determined remains unclear. The process by which the mature size of an individual MFG is determined as lipid droplets are transported, coalesced, and secreted is perplexing and requires a comprehensive examination to see the role, if any, of specific genes. Although the results of the present study provide evidence that the average MFG size in a milk sample has a genetic component, the complete genetic architecture of MFG size requires further investigation.

Repeatability estimates were similar for all examined traits (Table 2). Repeatabilities of 0.519 and 0.546 were

estimated for D[4,3] and D[3,2], respectively. Slightly lower and closer to each other repeatabilities were found for pD[4,3] (0.500) and pD[3,2] (0.495). Genetic evaluation of MFG size would therefore benefit from multiple measurements throughout the lactating lifetime of a cow. Because predicted MFG size is coming from the MIR spectra captured during routine DHI milk recording, this trait would be available for every test-date, multiple times during the lactation.

The heritabilities of the predicted MFG size traits were both less than those found for their corresponding gold standard measured trait (0.300 and 0.239 for pD[4,3] and pD[3,2], respectively). The decrease in heritability was more marked between D[3,2] and pD[3,2] than between D[4,3] and pD[4,3]. It is conceivable that more error is introduced into the predicted trait due to the poor performance of the MIR calibration model in the tails of the MFG size distributions, thereby resulting in lower heritabilities. The additive, residual, and phenotypic variance components associated with predicted MFG size were lower than the same components for measured MFG size (Table 3). This finding is attributable to a loss in the total phenotypic variability in the predicted values caused by the prediction models. The relative decline was greater for the additive genetic variance component compared with the permanent environment and residual components, resulting in the reduced heritability estimates. Cecchinato et al. (2009) observed the opposite for measured and MIR-predicted rennet coagulation time and curd firmness, finding that MIR predicted heritabilities were greater than the heritabilities estimated for their measured counterparts. A larger sample size would be required to properly evaluate whether true differences are present in the heritabilities of measured and predicted MFG size.

Genetic Correlations

Genetic correlations between the examined traits are shown in Table 4. A very strong genetic correlation

Table 2. Estimated heritabilities (h^2), their associated standard errors (SE), and repeatabilities (Rep) of milk fat globule volume moment mean (D[4,3]), surface moment mean (D[3,2]), predicted volume moment mean (pD[4,3]), predicted surface moment mean (pD[3,2]), and milk fat and protein percentages

Trait	h^2	SE	Rep
D[4,3]	0.364	0.165	0.519
D[3,2]	0.466	0.163	0.546
pD[4,3]	0.300	0.172	0.500
pD[3,2]	0.239	0.172	0.495
Fat (%)	0.370	0.136	0.370
Protein (%)	0.364	0.144	0.449

Table 3. Estimated additive genetic (σ_a^2), permanent environment (σ_{pe}^2), residual (σ_e^2), and total phenotypic (σ_p^2) variance of milk fat globule volume moment mean (D[4,3]), surface moment mean (D[3,2]), predicted volume moment mean (pD[4,3]), predicted surface moment mean (pD[3,2]), and milk fat and protein percentages

Trait	σ_a^2	σ_{pe}^2	σ_e^2	σ_p^2
D[4,3]	0.068	0.029	0.089	0.186
D[3,2]	0.044	0.008	0.043	0.094
pD[4,3]	0.028	0.019	0.047	0.095
pD[3,2]	0.011	0.012	0.023	0.046
Fat (%)	0.152	0.000	0.258	0.409
Protein (%)	0.025	0.006	0.038	0.070

Table 4. Genetic correlations (SE in parentheses) of milk fat globule volume moment mean (D[4,3]), surface moment mean (D[3,2]), predicted volume moment mean (pD[4,3]), predicted surface moment mean (pD[3,2]), and milk fat and protein percentages

Trait	D[3,2]	pD[4,3]	pD[3,2]	Fat (%)	Protein (%)
D[4,3]	0.980 (0.022)	0.685 (0.209)	0.626 (0.253)	0.538 (0.217)	0.335 (0.297)
D[3,2]		0.798 (0.161)	0.764 (0.171)	0.681 (0.153)	0.524 (0.277)
pD[4,3]			0.985 (0.027)	0.976 (0.159)	0.760 (0.318)
pD[3,2]				0.983 (0.138)	0.734 (0.352)

(0.980) was found between D[4,3] and D[3,2]. These 2 measures of average MFG size are heavily related because they are calculated from the same distribution, but they are biased in different directions. The surface moment mean (D[3,2]) is more sensitive to the presence of small MFG, while D[4,3] is more sensitive to large particles in the globule size distribution. Therefore, a genetic correlation close to 1 was expected. The genetic correlation between D[4,3] and fat percentage was 0.538, while the genetic correlation between D[3,2] and fat percentage was 0.681. These genetic correlations may be explained in part by the relationship between MFG size and the *DGAT1* genotype found by Argov-Argaman et al. (2013) because *DGAT1* has a known effect on milk fat production. A significant phenotypic relationship between average MFG size and milk fat percentage was previously described for this population by Fleming et al. (2017), in which Pearson correlation coefficients of 0.34 and 0.36 were reported for D[4,3] and D[3,2], respectively. A phenotypic relationship between fat production and MFG size has also been noted by Wiking et al. (2004) in Danish Holstein cows. The genetic correlations for D[4,3] and D[3,2] with protein percentage were 0.335 and 0.524, respectively.

A strong genetic correlation (0.685) between measured D[4,3] and its predicted equivalent, pD[4,3], was found. The genetic correlation between D[3,2] and pD[3,2] was marginally higher at 0.764. The greater genetic correlation for D[3,2] measures may be due to the slightly greater R^2_{cv} of the predictive model compared with that for D[4,3]. Cecchinato et al. (2009) presented genetic correlations ranging from 0.71 to 0.87 between measured and MIR predicted curd firmness with prediction R^2 values of 0.46 to 0.52, and from 0.91 to 0.96 between measured and predicted rennet coagulation time with prediction R^2 values of 0.61 to 0.69. They concluded that using these MIR predictions as indicator traits would have equal to slightly lower response to selection compared with direct measurement. Although the MIR prediction equations used for MFG size are not precise enough to accurately quantify MFG size in a milk sample, the strong genetic correlation suggests that selecting animals based on the predicted

values could still have realized effects on true MFG size.

The critical barrier to using the MIR predicted MFG size for selection is revealed in the genetic correlation with fat percentage. The correlation of fat percentage with pD[4,3] and with pD[3,2] were 0.976 and 0.983, respectively, which is notably greater than what was observed for D[4,3] and D[3,2] (Table 4). The increase in the genetic correlation between fat percentage and the predicted MFG size phenotypes is likely an outcome of the prediction equation. Fat content is dominant in the milk MIR spectra, and because a seemingly weak relationship exists between the spectra and average MFG size, the prediction equation is utilizing the indirect correlation with fat content. Eskildsen et al. (2014) advised that phenotypic MIR predictions of individual fatty acids may be very much connected to total fat, and thus if the correlation between fatty acid contents and total fat is inconsistent, the prediction of future samples may be less accurate. This lower accuracy may be exceedingly true for the MFG size MIR predictions. The same phenomenon is again seen, but to a lesser extent, with protein percentage. The genetic correlation with protein percentage increased to 0.760 with pD[4,3] and 0.734 with pD[3,2]. Protein is also a significant component of the milk MIR spectrum, and the phenotypic correlation may be augmented through the prediction equation. Therefore, despite the strong genetic correlation between the measured and predicted MFG size, the predicted values are more genetically associated with fat percentage, and selection based on the predicted MFG size values would be more so, putting selection pressure on fat percentage. Additional work can be done with a larger population having predicted MFG size to determine more precisely the genetic correlation between the predicted values and milk production traits.

Because of the small number of cows and limited samples, large standard errors are associated with most of the estimated genetic parameters, making definitive conclusions difficult. Before the real benefit of a MIR-predicted trait in genetic evaluations can be completely understood, it is important to have knowledge

of the similarity of the genetic relationships between measured values, predicted values, and other correlated traits of interest. A comparison of the genetic parameters for gold standard measured traits and their MIR-predicted equivalents could frequently be restricted by a limited number of gold standard measured records. This limitation may result in genetic parameter estimates with large standard errors, but differences may still be distinguished and tendencies noted. The relationship of measured and predicted traits along with other milk components could reveal additional details of the information used by the prediction models. This information is also important to know if selection based on predicted traits would yield different results in correlated traits than their true values.

CONCLUSIONS

Milk fat globule size is a moderately heritable trait, and it may therefore be possible to alter the average size through selection. Genetic evaluation of this trait is limited by MFG size being arduous to phenotype as part of a milk recording program. Mid-infrared–predicted MFG size, which can be obtained during routine milk recording, could be used as an indicator trait in genetic selection because of the high genetic correlation between measured and MIR predicted average MFG size. However, the very strong genetic correlation that appears between predicted MFG size and fat percentage, which was not as strong between measured MFG size and fat percentage, indicates that MIR-predicted MFG size has little utility in selection programs.

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