Genetics of Milk Coagulation Properties Predicted by Milk Mid-Infrared Spectroscopy Analysis of Irish Dairy Cows

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ABSTRACT: Milk coagulation properties (MCP) are important traits for profitable dairying. To improve MCP through animal breeding large quantity of routinely available data on MCP as well as existence of heritable genetic variation in these traits is required. Objectives of this study were to i) develop prediction equations for MCP through mid-infrared spectroscopy (MIRS) ii) apply equations to a spectra dataset of Holstein-Friesian cows, and iii) estimate variance components. Data for MCP and MIRS were available on 324 samples. Proportion of variation in external validation for RCT, k_{20}, and a_{30} explained by equations was 0.45, 0.41 and 0.27, respectively. Heritability for MCP estimated from 9,867 spectra on 1,104 cows varied from 0.06 to 0.20; coefficient of genetic variation for MCP varied from 5.99 to 10.07, while genetic correlations between MCP and milk quality traits demonstrated positive relationships between MCP and milk composition.
Keywords: milk quality; heritability; milk coagulation properties

Introduction

The European Union (EU) produces approximately half the world's total cheese production. Milk coagulation properties (MCP) are milk quality characteristics which describe the ability of milk to coagulate after rennet addition. Milk coagulation properties can be characterized as rennet coagulation time (RCT, min), curd-firming time (k_{20}, min) and curd firmness within 30 minutes (a_{30}, mm). It was recently suggested by De Marchi et al. (2013) to extend the curd firming test time to 60 minutes (a_{60}, mm), to facilitate the determination of coagulation properties for samples that do not coagulate within 30 minutes. Rennet coagulation time, k_{20}, a_{30}, and a_{60} are measured with reometers, coagulometers, formagraphs and optigraphs (Pretto et al., 2011; Vallas et al., 2010); such gold standard measurements however are time consuming and expensive (De Marchi et al., 2012). Mid infrared spectroscopy (MIRS) is a rapid, low-cost technique routinely used to quantify milk fat, protein, casein, and lactose concentration in milk samples taken during herd testing (De Marchi et al., 2014). Limited studies exist, predominantly from Italy, documenting the ability of MIRS to predict MCP in dairy cows milk (De Marchi et al., 2009, 2013). Therefore, the objective of the present study was to evaluate the ability of MIRS to predict MCP and to estimate genetic parameters for these predicted traits.

Materials and Methods

A total of 324 milk samples from 324 different cows in 5 different Irish research farms were collected between September 2013 and February 2014. Cows were from different breeds, including Holstein-Friesian (HF), Jersey (JER), Norwegian Red (NR), and their respective crosses. Samples were chosen to represent different stages of lactation, milking time (AM vs. PM), parities and experimental treatments/diets. All milk samples were also analyzed using a MilkoScan FT6000 (Foss Electronic A/S, Hillerød, Denmark), and the resulting spectrum, which contains 1,060 wavelength transmittance rates, in the mid-infrared region between 900 and 5,000 cm^{-1}, was stored. The 40 mL milk samples were subsequently preserved with Broad Spectrum Microtab II (D & F Control System Inc., Norwood, MA, USA), stored in a refrigerator and tested for MCP within 5 days of collection.

A 10 mL aliquot from each milk sample was tested for MCP using a Formagraph (Foss Electronic A/S, Hillerød, Denmark). Samples were heated to 35 °C. A solution containing raw rennet and distilled water (1:20) was added to each sample and the test started immediately after rennet addition. Measured traits were RCT (the time taken from rennet addition to the beginning of the coagulation), k_{20} (the time taken from the beginning of the coagulation to the achievement of a 20 mm spread in the graph) as well as curd firmness 30 (a_{30}) and 60 (a_{60}) minutes after the addition of rennet. Values of each milk quality traits were not available for all milk samples.

Milk chemical composition was determined using a MilkoScan FT6000 (Foss Electronic A/S, Hillerød, Denmark) while somatic cell count was assessed by Fossmatic (Foss Electronic A/S, Hillerød, Denmark) and was transformed to somatic cell score (SCS) through the formula $SCS = [3 + \log_2(SCC/100{,}000)]$.

Prediction equations. Spectra data expressed in transmittance were converted to absorbance, by taking the $\log_{10}$ of the reciprocal of the transmittance. Before performing the statistical analysis, the spectra regions between 1,580 and 1,710 cm^{-1} and between 2,990 and 3,670 cm^{-1} were identified as high noise levels regions and thus omitted (Hewavitharana and Brakel, 1997). Principal component analysis (PCA) was performed and one outlying spectral value was discarded. Calibration and validation datasets were generated separately for each of the four MCP traits investigated. A random sample representing 60% of the data was retained for use in the calibration while the remaining 40% compromised the validation dataset.

Prediction equations were generated using partial least squares (PLS) regression analyses in SAS (Statistical

Analysis System, version 9.3; SAS Institute Inc., Cary, NC, USA). The prediction equations developed using the cross-validation in the calibration dataset were those that minimized the predicted residual sum of squares with the lowest number of PLS factors (#L). Prediction equations were then applied to the validation dataset as an external validation.

The goodness of fit of the MIRS models was evaluated through the calculation of R^2_{CV} and R^2_V (coefficient of determination in cross-validation and external validation, respectively), SECcv and SE_V (standard error of cross validation and external validation, respectively) and RPD (ratio performance deviation), calculated as the ratio between standard deviation and SE_V.

Genetic analysis. The prediction equations were applied to the MIRS test-day records of samples analyzed in January, February and between September and December 2013 from HF cows at the Teagasc Moorepark Animal and Grassland Research and Innovation Centre. Variance components for the predicted MCP on 9,867 test-day records from 1,104 cows were estimated using repeatability animal linear mixed models in ASreml (Gilmour et al., 2009). Fixed effects included in the model were experimental treatment, parity-by-days post-calving (days post-calving was fitted as a cubic polynomial regression) and season of calving. Both a direct additive genetic and permanent environmental effects were included in the models as random effects. The genetic correlations between traits were estimated using a series of bivariate animal linear mixed models with the aforementioned fixed and random effects. The pedigree of all animals were traced back at least four generations and included in the generation of the relationship matrix.

Results and Discussion

Table 1 summarizes the descriptive statistics for the coagulation traits in the calibration and validation sets. Comparison of these results with previous studies are difficult, because previous studies were undertaken mainly in Italy and Northern European countries (e.g. Sweden, Estonia), which have a different production system to Ireland. The duration of the analysis in our study was 60 minutes while in most other studies a time threshold of 30 minutes was imposed. Only De Marchi et al. (2013) extended the time of analysis to 60 minutes and they reported an average value of 30.55 min, 9.61 min, and 26.97 mm for RCT, k_{20} and a_{30}, respectively, analyzing individual and bulk milk samples of mainly HF Italian cows. Moreover, De Marchi et al. (2013) reported similar coefficients of variation (47%, 68% and 51% for RCT, k_{20} and a_{30}, respectively) to those observed in the present study. Nevertheless De Marchi et al. (2013) reported lower average values (14.76 mm vs. 35.81 mm) and a greater coefficient of variation (62% vs 37.28%) for a_{60} than observed in the present study.

Prediction models. The fit statistics of the prediction models are in Table 2. The best prediction model was for RCT with a coefficient of determination (R^2) of 0.69 and 0.45 in cross- and external- validation, respectively. The number of factors used in the prediction model (i.e., 13) was less than the 15 reported by De Marchi et al. (2012, 2013) for RCT. The SE_{cv} obtained for RCT in the present study was nonetheless lower than reported by De Marchi et al. (2012, 2013) (5.78 min vs. 7.05 min). The RPD of 1.80 for RCT in the present study is close to the value of two suggested by Sinnaeve et al. (1994) as a threshold to indicate a good prediction model.

Table 1. Descriptive statistics of milk coagulation properties.

Trait	N	Mean	Min	Max	SD	CV
RCT, min	322	20.76	1.75	49.00	10.39	50.03
k_{20}, min	301	5.69	1.25	19.75	4.03	70.92
a_{30}, mm	255	36.08	2.02	74.90	17.61	48.80
a_{60}, mm	320	35.81	8.52	77.04	13.35	37.28

Table 2. Fit statistics[1] of the prediction models for milk coagulation properties.

			Cross Validation				
Trait	#L	N	Mean	SD	R^2_{cv}	SE_{cv}	RPD
RCT, min	13	192	21.17	10.40	0.69	5.78	1.80
k_{20}, min	7	188	5.86	4.17	0.49	2.96	1.41
a_{30}, mm	6	131	3.68	1.85	0.46	1.36	1.36
a_{60}, mm	7	165	35.54	13.62	0.25	11.75	1.16
			External Validation				
Trait	#L	N	Mean	SD	R^2_v	SE_v	RPD
RCT, min	13	130	20.16	10.38	0.45	7.75	1.34
k_{20}, min	7	113	5.39	3.80	0.41	2.92	1.30
a_{30}, mm	6	124	3.54	1.67	0.27	1.57	1.07
a_{60}, mm	7	155	36.1	13.1	0.09	12.68	1.03

[1] #L = number of partial least square factors in the calibration model; R^2_{CV} = Coefficient of determination in cross-validation; SECcv = Standard error of cross validation; R^2_V = coefficient of determination in the external validation; SE_V = Standard error of external validation; RPD = ratio performance deviation.

The prediction models developed for the other three MCP traits were less accurate; results obtained for k_{20} were nevertheless better than those for a_{30} and a_{60}. De Marchi et al. (2012, 2013) reported relatively similar R^2_{cv} of all three MCP traits of 0.76, 0.72 and 0.70 for RCT, k_{20} and a_{30}, respectively. The low accuracy of a_{60} prediction in the present study could be related to the low accuracy of the reference method (Formagraph) for milk samples which coagulate after 30 minutes (Cipolat-Gotet et al., 2012). Moreover, the poorer accuracies in the present study, when compared to other studies, could be explained by the seasonal variation of the Irish dairy production system; in fact many studies have demonstrated the influence of days in milk (DIM) on MCP (Ikoner et al., 2004; Tyriseva et al., 2004). In the present study, the majority of milk samples were obtained from spring calving cows in mid to late

lactation (days 149 to 375 in milk) with a smaller number obtained from a herd of autumn calving cows in early to mid-lactation (days 52 to 156 in milk). Ikoner et al. (2004) and Tyriseva et al. (2004) demonstrated, using milk from HF and Finnish Ayrshire cows, that the best values for MCP were from cows in very early lactation, and these animals were not available for inclusion in the calibration dataset of the present study, due to the period of sampling.

Genetic analysis. Heritability and repeatability estimates for the MCP traits are in Table 3, while genetic correlations among the MCP traits and between the MCP traits and other milk production traits are in Table 4. The heritability for RCT (0.06) and a_{30} (0.17) in the present study were lower than the respective heritability estimates of 0.21 and 0.24 documented by Tiezzi et al. (2013) who applied the predictions equations of De Marchi et al. (2012) to a population of 25,590 Italian HF cows. Heritability for milk yield (0.14) in the present study was nonetheless the same as that reported by Tiezzi et al. (2013). Generally high heritability estimates were obtained for the milk quality traits (0.50 and 0.27 for casein content and SCS, respectively). Repeatability of the MCP traits was generally low. The coefficient of genetic variation of the MCP traits was 5.99, 10.07, and 6.51 for RCT, k_{20} and a_{30}, respectively. These estimates are similar to the coefficient of genetic variation for milk production traits (Berry et al., 2003) signifying that, although the heritability for MCP traits was low, ample exploitable genetic variation still exists in these traits.

Table 3. Heritability ($h^2 \pm$ SE) and repeatability (t ± SE) of predicted MCP, and milk production and milk quality.

Trait	$h^2 \pm$ SE	t ± SE
RCT, min	0.06 ± 0.03	0.19 ± 0.01
k_{20}, min	0.20 ± 0.04	0.27 ± 0.02
a_{30}, mm	0.17 ± 0.05	0.27 ± 0.02
Milk yield, kg/d	0.14 ± 0.05	0.41 ± 0.02
Protein, %	0.58 ± 0.08	0.06 ± 0.02
Casein, %	0.50 ± 0.08	0.57 ± 0.02
Fat, %	0.30 ± 0.07	0.33 ± 0.02
SCS	0.27 ± 0.10	0.58 ± 0.02

Table 4. Genetic correlations between predicted MCP and milk production and quality traits.

Trait	RCT	k_{20}	a_{30}
k_{20}, min	0.55 ± 0.19		
a_{30}, mm	-0.37 ± 0.26	-0.61 ± 0.11	
Milk yield, kg/d	-0.08 ± 0.31	0.41 ± 0.16	-0.24 ± 0.21
Protein, %	-0.17 ± 0.21	-0.74 ± 0.07	0.57 ± 0.11
Casein, %	-0.17 ± 0.21	-0.76 ± 0.06	0.56 ± 0.11
Fat, %	-0.14 ± 0.22	-0.77 ± 0.06	0.40 ± 0.14
SCS	0.24 ± 0.31	-0.31 ± 0.22	0.54 ± 0.19

The genetic correlation between RCT and a_{30} were strong and similar to that reported by Cassandro et al. (2008) and Tiezzi et al. (2013); furthermore a positive genetic correlation was evident between MCP and traditional milk quality traits.

Conclusions

This is the first large scale study of MCP in Irish dairy cows. The MCP qualities of the cows in the present study were comparable to other northern European populations. Moreover, this study confirmed the effectiveness of MIRS in predicting MCP, which when coupled with heritable genetic variation, suggests that MCP could indeed be useful traits in a national breeding program to improve further milk quality.

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