Animal (2013), **7:10**, pp 1592–1599 © The Animal Consortium 2013
doi:10.1017/S1751731113001195

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Heritability and repeatability of milk coagulation properties predicted by mid-infrared spectroscopy during routine data recording, and their relationships with milk yield and quality traits

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(Received 29 January 2013; Accepted 6 May 2013; First published online 2 July 2013)

The aim of this study was to estimate (co)variance components for milk coagulation properties (MCP) predicted by mid-infrared spectroscopy (MIRS) during routine milk recording, and to assess their relationships with yield and quality traits. A total of 63 470 milk samples from Holstein-Friesian cows were analyzed for MCP, pH and quality characteristics using MIRS. Casein to protein and protein to fat ratios were calculated from information obtained by MIRS. Records were collected across 1 year on 16 089 cows in 345 herds. The model used for genetic analysis included fixed effects of parity and stage of lactation, and random effects of herd-test-day, cow permanent environmental, animal additive genetic and residual. (Co)variance components were assessed in a Bayesian framework using the Gibbs Sampler. Estimates of heritabilities were consistent with those reported in the literature, being moderate for MCP (0.210 and 0.238 for rennet coagulation time (RCT) and curd firmness (a_{30}), respectively), milk contents (0.213 to 0.333) and pH (0.262), and low for somatic cell score (0.093) and yield traits (0.098 to 0.130). Repeatabilities were 0.391 and 0.434 for RCT and a_{30}, respectively, and genetic correlations were generally low, with estimates greater than 0.30 (in absolute value) only for a_{30} with fat, protein and casein contents. Overall, results suggest that genetic evaluation for MCP predicted by MIRS is feasible at population level, and several repeated measures per cow during a lactation are required to estimate reliable breeding values for coagulation traits.

Keywords: heritability, genetic correlation, mid-infrared spectroscopy, milk coagulation property, Holstein-Friesian dairy cow

Implications

This paper aimed at assessing parameters which will be useful to estimate the genetic merit of dairy cattle for milk coagulation properties (MCP) predicted by mid-infrared spectroscopy (MIRS) during routine data recording. Estimates of heritability and genetic correlation indicate that selection for coagulation ability is feasible without altering results for milk quality traits. Selection for MCP can be helped, but not substituted, by selection for fat, protein and casein percentages. Repeated measures per cow are needed but MIRS seems to be suitable for genetic purposes.

Introduction

The interest for improving milk coagulation properties (MCP) has increased during last years. The ability of milk to react to the presence of rennet has been intensively studied in cattle (Aleandri *et al.*, 1989; De Marchi *et al.*, 2007). It has been widely demonstrated that milk with desirable clotting characteristics, namely relatively short clotting time, good firming rate and high curd firmness at cut, results in higher cheese yield than poorly coagulating milk (Johnson *et al.*, 2001; Pretto *et al.*, 2013) and increased profitability for the dairy industry (Bynum and Olson, 1982; Formaggioni *et al.*, 2005). The improvement of MCP is strongly advisable to enhance dairy sector efficiency, especially in countries were milk is predominantly destined to cheese production (Geary *et al.*, 2010).

The main parameters describing MCP are the time elapsed between rennet addition and start of casein micelles aggregation (rennet coagulation time (RCT), min), and curd firmness 30 min after enzyme addition (a_{30}, mm). Improvement of these traits can be achieved through selection for correlated traits such as milk protein content and composition (Bonfatti *et al.*, 2011; Jensen *et al.*, 2012), milk acidity (López *et al.*, 1998;

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Nájera *et al.*, 2003) and somatic cell count (SCC; Politis and Ng-Kwai-Hang, 1988; Klei *et al.*, 1998). Moreover, genetic variants at the protein loci have been demonstrated to exert an effect on MCP (Kübarsepp *et al.*, 2005; Comin *et al.*, 2008). In addition, the possibility of improving MCP through selective breeding has been demonstrated in several studies, involving different dairy cattle populations in different countries. Additive genetic variation has been estimated in papers focusing on cow individual measures of MCP and heritabilities for RCT and a_{30} were found to be moderate (Ikonen *et al.*, 2004; Cassandro *et al.*, 2008; Vallas *et al.*, 2010). In addition, cow repeatabilities were moderate to high (Tyrisevä *et al.*, 2003; Vallas *et al.*, 2010).

Genetic correlations among MCP, yield and composition traits revealed that technological properties of milk can be improved without hampering selection for production and quality traits (Pretto *et al.*, 2012). However, direct phenotyping for renneting ability would result in higher genetic gain because both related traits (Pretto *et al.*, 2012) and protein genotypes (Comin *et al.*, 2008; Penasa *et al.*, 2010; Vallas *et al.*, 2012) are not able to give the same genetic change. For instance, genetic correlations of MCP were found negligible with milk yield but moderate to strong with protein and casein percentages and SCC, generally moderate with fat percentage, and strong with milk pH.

The most used instruments to measure MCP are the computerized renneting meter (Ikonen *et al.*, 2004; Cassandro *et al.*, 2008), the Formagraph (Tyrisevä *et al.*, 2003; Cipolat-Gotet *et al.*, 2012) and the Optigraph (Vallas *et al.*, 2010). Unfortunately these methodologies are not applicable at population level, and thus they are not useful for breeding purposes. The use of mid-infrared spectroscopy (MIRS) has been proposed to overcome these limitations and to predict several milk traits such as fatty acid composition (e.g. Soyeurt *et al.*, 2006; De Marchi *et al.*, 2011) and MCP (Dal Zotto *et al.*, 2008; Cecchinato *et al.*, 2009; De Marchi *et al.*, 2009 and 2013). Milk coagulation properties are currently available in several Italian milk laboratories, both for individual and bulk milk samples (De Marchi *et al.*, 2012), since these laboratories have implemented MCP prediction models in Milko-Scan spectrometer (Foss Electric A/S, Hillerød, Denmark). Thus, the use of MIRS to predict MCP could be a valuable technology to collect phenotypes for genetic analyses.

The aims of this study were (i) to estimate heritability and repeatability of MCP predicted by MIRS during routine data collection and (ii) to assess phenotypic and genetic correlations of MCP with pH, quality traits, yields and somatic cell score (SCS) of milk from dairy cows.

Material and methods

From October 2011 to September 2012, 93 605 individual milk samples from 25 590 Holstein-Friesian cows were collected in 449 dairy farms during monthly test-day milk recording. Samples were processed according to International Committee for Animal Recording (ICAR) procedures, combined with preservative immediately after collection (Bronopol; Knoll Pharmaceuticals, Nottingham, UK) and analyzed in the laboratory of the Breeders Association of Veneto region (Padova, Italy). Protein (PP), fat (FP), casein (CP) and lactose (LP) percentages, and pH, RCT and a_{30} were assessed using Milko-Scan FT6000 (Foss Electric A/S). Mid-infrared spectroscopy models were implemented for routine prediction of MCP as reported by De Marchi *et al.* (2012); the authors estimated satisfactory accuracies with coefficients of determination in cross-validation of 0.76 and 0.70 for RCT and a_{30}, respectively. Values of milk pH were obtained using MIRS prediction model implemented by Foss Electric A/S directly on Milko-Scan FT6000 (http://www.foss.dk/industry-solution/products/combifoss-ft). The potential of MIRS to predict milk pH was previously assessed by De Marchi *et al.* (2009), who reported a coefficient of determination in cross-validation of 0.60. SCC was determined with Cell Fossomatic 250 (Foss Electric A/S) and was transformed to SCS according to the formula [$SCS = 3 + \log_2(SCC/100\,000)$]. Besides the aforementioned traits, daily milk yield (MY, kg) was also recorded, and casein to protein (C : P) and protein to fat (P : F) ratios, and daily fat (FY), protein (PY), casein (CY) and lactose (LY) yields were calculated. In the present study, C : P and P : F were included as candidate correlated traits which could improve accuracy of genetic evaluation for MCP.

Only records from cows with known sire and dam were included in the analysis; cows were required to be in parity 1 to 9 and between 5 and 365 days in milk. Records were retained if RCT values were between 5 and 30 min, as this is the range on which the calibration equation was developed. Moreover, records were discarded from the dataset, if they deviated more than 3.5 standard deviations from the mean for each trait. Sires were required to have a minimum of five daughters distributed across at least three herds and cows were required to have a minimum of two herd-test-date (HTD) observations within a lactation. Records from HTD classes with less than three animals were removed from the dataset. After editing, 63 470 records from 16 089 cows in 345 herds were available for statistical analysis. Cows were progeny of 800 sires and the number of daughters per sire ranged from 5 to 417. Data were analyzed using the following linear animal model:

$$y = Xb + Z_h h + Z_p p + Z_a a + e,$$

where y is the vector of phenotypic records for the analyzed trait (RCT, a_{30}, MY, FY, PY, CY, LY, FP, PP, CP, LP, SCS, pH, C : P, P : F), b is the vector of fixed effects of parity (3 classes: parity 1, parity 2 and parities 3 to 9) and stage of lactation (12 monthly classes: 6 to 35 days; 36 to 65 days; 66 to 95 days; 96 to 125 days; 126 to 155 days; 156 to 185 days; 186 to 215 days; 216 to 245 days; 246 to 275 days; 276 to 305 days; 306 to 335 days; 336 to 365 days), h is the vector of solutions for HTD random effect, p is the vector of solutions for cow permanent environmental effect, a is the vector of solutions for animal additive genetic effect and e is the vector of random residuals. X, Z_h, Z_p and Z_a are the respective incidence matrices of the appropriate order.

Variance and covariance components were estimated using univariate and bivariate models implemented in a Bayesian framework using the software GIBBS3F90 (Misztal, available online at: http://nce.ads.uga.edu/%7Eignacy/programs.html). For univariate models, 250 000 iterations were run discarding the first 50 000 samples as burn-in and storing samples every 20 iterations, and for bivariate models chains included a total of 600 000 iterations with the first 100 000 samples as burn-in and a thinning interval of 50 iterations. Heritability (h^2), intra-herd heritability (h^2_{IH}), repeatability (rep) and cow permanent environmental (h_{pe}) and HTD (h_{htd}) effects were defined as

$$h^2 = \frac{\sigma^2_a}{\sigma^2_a + \sigma^2_{pe} + \sigma^2_{htd} + \sigma^2_e}$$

$$h^2_{IH} = \frac{\sigma^2_a}{\sigma^2_a + \sigma^2_{pe} + \sigma^2_e}$$

$$\text{rep} = \frac{\sigma^2_a + \sigma^2_{pe}}{\sigma^2_a + \sigma^2_{pe} + \sigma^2_{htd} + \sigma^2_e}$$

$$h_{pe} = \frac{\sigma^2_{pe}}{\sigma^2_a + \sigma^2_{pe} + \sigma^2_{htd} + \sigma^2_e}$$

$$h_{htd} = \frac{\sigma^2_{htd}}{\sigma^2_a + \sigma^2_{pe} + \sigma^2_{htd} + \sigma^2_e}$$

Genetic (r_{gen}), cow permanent environmental (r_{pe}) and HTD (r_{htd}) correlations for random effects were calculated as:

$$r_{gen} = \frac{cov_a}{\sqrt{\sigma^2_{x,a} + \sigma^2_{y,a}}}, \; r_{pe} = \frac{cov_{pe}}{\sqrt{\sigma^2_{x,pe} + \sigma^2_{y,pe}}},$$

$$r_{htd} = \frac{cov_{htd}}{\sqrt{\sigma^2_{x,htd} + \sigma^2_{y,htd}}}$$

where σ^2_a is the additive genetic variance, σ^2_{pe} is the cow permanent environmental variance, σ^2_{htd} is the HTD variance and σ^2_e is the residual variance. The posterior means of the marginal posterior distributions were considered as estimates of (co)variance components and related parameters, and the 95% highest probability density intervals (HPD95) were calculated as dispersion measure of the posterior distribution.

Results

Descriptive statistics for the analyzed traits are given in Table 1. Milk yield, FY, PY, CY and LY averaged 32.00, 1.18, 1.06, 0.83 and 1.55 kg/day, respectively. Regarding milk components, FP, PP, CP and LP averaged 3.73%, 3.37%, 2.64% and 4.85%, respectively. Mean values for RCT, a_{30}, pH and SCS were 20.61 min, 21.71 mm, 6.62 and 2.93, respectively (Table 1). Somatic cell score showed the highest CV (64.2%), whereas pH the lowest (1%). Rennet coagulation time and a_{30} showed CV of 18.4% and 39.6%, respectively. Coefficient of variation for yield traits ranged from 24.5% (CY) to 28.6% (FY) and for milk contents from 3.7% (LP) to 18.2% (FP). Finally, CV of C : P and P : F were 2.6% and 17.2%, respectively.

Table 1 *Descriptive statistics for milk coagulation properties, production and quality traits* (n = *63 470)*

Trait[a]	Mean	s.d.	CV (%)	Minimum	Maximum
RCT (min)	20.61	3.79	18.4	5.69	29.90
a_{30} (mm)	21.71	8.60	39.6	0.02	55.30
Milk production (kg/day)					
Milk	32.00	8.79	27.5	5.80	59.60
Fat	1.18	0.34	28.6	0.19	2.49
Protein	1.06	0.26	24.6	0.22	1.93
Casein	0.83	0.20	24.5	0.17	1.49
Lactose	1.55	0.44	28.2	0.26	3.06
Milk composition (%)					
Fat	3.73	0.68	18.2	1.80	6.42
Protein	3.37	0.36	10.7	2.34	4.73
Casein	2.64	0.30	11.4	1.80	3.72
Lactose	4.85	0.18	3.7	4.21	5.29
SCS	2.93	1.88	64.2	−1.32	9.86
pH	6.62	0.07	1.0	6.36	6.84
Casein/protein	0.78	0.02	2.6	0.70	0.85
Protein/fat	0.93	0.16	17.2	0.50	1.64

RCT = rennet coagulation time; SCS = somatic cell score.
[a]SCS = $[3 + \log_2(SCC/100\,000)]$; a_{30} = curd firmness 30 min after rennet addition.

Heritabilities, intra-herd heritabilities, repeatabilities and HTD effect are reported in Table 2. The mean of the posterior distribution for each parameter is used as point estimate, and HPD95 are used as error indicator of the estimates. Rennet coagulation time, a_{30} and pH showed heritabilities of 0.210, 0.238 and 0.262, respectively. Heritabilities for daily yields were moderately low and ranged from 0.098 (FY) to 0.130 (MY). Milk constituents expressed as percentage showed higher estimates (0.213 for FP to 0.333 for LP), but with the same pattern of daily yields (Table 2). Finally, SCS showed low heritability (0.093), as well as C : P (0.092) and P : F (0.141).

Intra-herd heritabilities differed from heritabilties only in the presence of a strong HTD effect. Herd-test-day effect was moderately low for MCP, being 0.252 for RCT and 0.199 for a_{30}, leading to intra-herd heritabilites of 0.281 and 0.296, respectively (Table 2). Daily yield traits exhibited a moderately low HTD effect (0.176 for FY to 0.244 for PY) which led to intra-herd heritabilities from 0.119 (FY) to 0.164 (MY). Compared with yield traits, a relatively smaller HTD effect was found for milk composition; it was around 0.145 for FP, PP and CP, and 0.053 for LP. A negligible HTD effect was estimated for SCS (0.046), whereas pH showed the highest intra-herd heritability (0.381), suggesting a certain importance of HTD effect (0.313). Finally, the HTD effect for P : F was low (0.143), and it was high for C : P (0.596). Consequently, intra-herd heritability of C : P was much higher than its heritability (0.228 *v.* 0.092; Table 2).

Repeatabilities for RCT and a_{30} were 0.391 and 0.434, respectively (Table 2), suggesting that the magnitude of cow permanent environmental effects for these traits was similar to

Table 2 *Posterior means and 95% highest probability density intervals (HPD95) for heritability (h^2), intra-herd heritability (h^2_{IH}), repeatability and herd-test-day effect (h_{htd}) of recorded traits*

	h^2		h^2_{IH}		h_{htd}		Repeatability	
Trait[a]	Mean	HPD95	Mean	HPD95	Mean	HPD95	Mean	HPD95
RCT (min)	0.210	0.182; 0.237	0.281	0.244; 0.316	0.252	0.238; 0.267	0.391	0.380; 0.402
a_{30} (mm)	0.238	0.209; 0.271	0.296	0.261; 0.336	0.199	0.187; 0.211	0.434	0.423; 0.445
Milk production (kg/day)								
Milk	0.130	0.104; 0.156	0.164	0.133; 0.198	0.209	0.194; 0.223	0.461	0.448; 0.474
Fat	0.098	0.077; 0.121	0.119	0.093; 0.146	0.176	0.163; 0.189	0.367	0.356; 0.378
Protein	0.110	0.088; 0.134	0.146	0.115; 0.176	0.244	0.228; 0.261	0.412	0.399; 0.424
Casein	0.116	0.092; 0.141	0.150	0.119; 0.180	0.228	0.212; 0.244	0.420	0.407; 0.432
Lactose	0.125	0.100; 0.151	0.159	0.129; 0.192	0.210	0.195; 0.225	0.456	0.443; 0.469
Milk composition (%)								
Fat	0.213	0.184; 0.244	0.250	0.216; 0.286	0.147	0.136; 0.158	0.374	0.364; 0.385
Protein	0.282	0.247; 0.317	0.330	0.292; 0.372	0.146	0.135; 0.156	0.520	0.509; 0.531
Casein	0.283	0.250; 0.320	0.331	0.290; 0.370	0.144	0.134; 0.155	0.527	0.516; 0.539
Lactose	0.333	0.298; 0.373	0.352	0.315; 0.394	0.053	0.047; 0.058	0.562	0.552; 0.572
SCS	0.093	0.067; 0.120	0.097	0.070; 0.125	0.046	0.040; 0.052	0.480	0.470; 0.489
pH	0.262	0.234; 0.291	0.381	0.341; 0.418	0.313	0.297; 0.329	0.389	0.376; 0.401
Casein/protein	0.092	0.079; 0.106	0.228	0.198; 0.259	0.596	0.580; 0.613	0.189	0.180; 0.198
Protein/fat	0.141	0.116; 0.164	0.165	0.136; 0.192	0.143	0.131; 0.153	0.279	0.269; 0.289

RCT = rennet coagulation time; SCS = somatic cell score.
[a]SCS = $[3 + \log_2(SCC/100\,000)]$; a_{30} = curd firmness 30 min after rennet addition.

their heritabilities. Estimates of repeatability for daily yields (0.367 for FY to 0.461 for MY) were comparable to those reported for MCP, although cow permanent environmental effect was higher here. Among milk contents, FP exhibited the lowest repeatability (0.374) and the lowest cow permanent environmental effect. PP, CP and LP exhibited repeatabilities greater than 0.500 (Table 2), and cow permanent environmental effect was lower than their heritabilities. Moderate repeatabilities were also assessed for SCS (0.480) and pH (0.389), and the former trait exhibited the highest cow permanent environmental effect among all studied variables. Finally, C : P (0.189) and P : F (0.279) were less repeatable traits (Table 2).

Table 3 summarizes the genetic, phenotypic, permanent environmental and HTD correlations between RCT and the other traits. Rennet coagulation time and a_{30} were strongly related either on the genetic (−0.910), permanent environmental (−0.900) and phenotypic (−0.807) level, whereas the correlation was slightly weaker at HTD level (−0.657). No significant genetic relationships of RCT with SCS, C : P, P : F, production and quality traits were detected as estimates included '0' in the HPD95, except for LY and LP. Posterior distribution for the genetic relationship between RCT and pH did not include '0', indicating an existing, although low correlation (0.122; Table 3).

Permanent environmental correlations between RCT and the other traits were constantly stronger than genetic ones, and seldom exhibited the same sign. Estimates ranged from −0.242 to −0.151 for production traits, and from −0.398 to 0.105 for milk composition. Only FP was of sign concordant with the genetic correlation, and LP showed the strongest permanent environmental correlation with RCT among all traits (a_{30} excluded). A moderate permanent environmental correlation was found between RCT and SCS (0.339), and a low correlation was found between RCT and pH (−0.190). Again, permanent environmental correlations of RCT with C : P and P : F were higher than the genetic ones (Table 3).

Herd-test-day relationships between RCT and the other traits were moderate to low and not in all cases concordant with genetic and permanent environmental correlations. For yield traits, the posterior distributions were negative and comprised between −0.257 and −0.163. Correlations between RCT and milk contents, SCS and pH were very low, ranging from −0.122 to 0.130, and C : P and P : F showed moderate HTD correlation with RCT (0.279 and −0.153, respectively; Table 3). On the phenotypic level, RCT did not show any strong correlation with production and quality traits, and the highest correlation was with SCS (0.214).

Table 4 summarizes the genetic, phenotypic, permanent environmental and HTD correlations between a_{30} and the other traits. The pattern of the relationships was different than that of RCT. Curd firmness showed stronger and negative genetic correlations with MY (−0.248) and LY (−0.243), although the latter relationship might be an indirect effect of the relationship with MY, being lactose constant in milk. Fat yield, PY and CY were not related to a_{30} on the genetic level (Table 4). Milk contents showed moderate genetic correlations with a_{30}, except for LP, which did not correlate with a_{30}. PP (0.419) and CP (0.416) showed the strongest correlations with a_{30}, whereas FP was slightly less related (0.320). Somatic cell score and pH did not show significant correlations with a_{30} (Table 4). Concerning ratios of milk constituents, only C : P was related to a_{30}.

Permanent environmental correlations between a_{30} and yield traits ranged from 0.037 to 0.206. However, a_{30} was

Table 3 *Posterior means and 95% highest probability density intervals (HPD95) for genetic (r_{gen}), cow permanent environmental (r_{pe}) and herd-test-day (r_{htd}) correlations, and phenotypic relationships (r_{phen}) between rennet coagulation time and milk yield and quality traits*

	r_{gen}		r_{pe}		r_{htd}		
Trait[a]	Mean	HPD95	Mean	HPD95	Mean	HPD95	r_{phen}
a_{30} (mm)	−0.910	−0.929; −0.893	−0.900	−0.916; −0.882	−0.657	−0.685; −0.628	−0.807
Milk production (kg/day)							
Milk	0.115	−0.019; 0.250	−0.171	−0.240; −0.102	−0.256	−0.310; −0.205	−0.146
Fat	0.000	−0.138; 0.149	−0.242	−0.312; −0.171	−0.163	−0.217; −0.107	−0.173
Protein	0.094	−0.049; 0.227	−0.151	−0.218; −0.082	−0.256	−0.309; −0.205	−0.117
Casein	0.094	−0.047; 0.227	−0.164	−0.232; −0.096	−0.212	−0.266; −0.160	−0.111
Lactose	0.143	0.007; 0.280	−0.212	−0.283; −0.146	−0.257	−0.309; −0.205	−0.160
Milk composition (%)							
Fat	−0.100	−0.207; 0.008	−0.154	−0.250; −0.052	0.106	0.051; 0.162	−0.054
Protein	−0.062	−0.165; 0.041	0.105	0.014; 0.194	−0.059	−0.113; −0.004	0.102
Casein	−0.052	−0.157; 0.049	0.053	−0.035; 0.143	0.088	0.032; 0.141	0.108
Lactose	0.110	0.006; 0.217	−0.398	−0.495; −0.302	−0.122	−0.179; −0.058	−0.149
SCS	0.052	−0.102; 0.205	0.339	0.277; 0.405	0.130	0.067; 0.195	0.214
pH	0.122	0.027; 0.223	−0.190	−0.310; −0.081	0.105	0.054; 0.156	−0.032
Casein/protein	0.084	−0.023; 0.200	−0.289	−0.374; −0.205	0.279	0.234; 0.327	0.069
Protein/fat	0.077	−0.048; 0.189	0.262	0.166; 0.362	−0.153	−0.206; −0.096	0.114

SCS = somatic cell score.
[a]SCS = [3 + $\log_2$(SCC/100 000)]; a_{30} = curd firmness 30 min after rennet addition.

Table 4 *Posterior means and 95% highest probability density intervals (HPD95) for genetic (r_{gen}), cow permanent environmental (r_{pe}) and herd-test-day (r_{htd}) correlations, and phenotypic relationships (r_{phen}) between curd firmness 30 min after rennet addition and milk yield and quality traits*

	r_{gen}		r_{pe}		r_{htd}		
Trait[a]	Mean	HPD95	Mean	HPD95	Mean	HPD95	r_{phen}
Milk production (kg/day)							
Milk	−0.248	−0.385; −0.120	0.037	−0.033; 0.111	0.200	0.147; 0.255	−0.057
Fat	0.074	−0.071; 0.216	0.206	0.136; 0.282	0.166	0.109; 0.221	0.066
Protein	−0.013	−0.154; 0.122	0.132	0.063; 0.204	0.237	0.183; 0.289	0.058
Casein	0.006	−0.134; 0.139	0.159	0.090; 0.231	0.226	0.172; 0.280	0.071
Lactose	−0.243	−0.384; −0.115	0.092	0.022; 0.164	0.205	0.152; 0.260	−0.032
Milk composition (%)							
Fat	0.320	0.218; 0.417	0.350	0.258; 0.442	0.010	−0.046; 0.065	0.206
Protein	0.419	0.331; 0.504	0.289	0.204; 0.369	0.202	0.148; 0.254	0.303
Casein	0.416	0.330; 0.500	0.347	0.267; 0.424	0.141	0.087; 0.196	0.314
Lactose	0.031	−0.079; 0.128	0.476	0.385; 0.567	0.168	0.107; 0.226	0.181
SCS	−0.050	−0.215; 0.101	−0.337	−0.401; −0.268	−0.103	−0.167; −0.037	−0.179
pH	−0.100	−0.197; 0.002	0.135	0.017; 0.251	0.059	0.008; 0.112	0.005
Casein/protein	0.178	0.073; 0.282	0.531	0.455; 0.607	−0.085	−0.133; −0.033	0.157
Protein/fat	−0.091	−0.123; 0.028	−0.225	−0.321; −0.118	0.107	0.055; 0.167	−0.040

SCS = somatic cell score.
[a]SCS = [3 + $\log_2$(SCC/100 000)].

more strongly related to milk contents: 0.476 with LP, 0.350 with FP, 0.347 with CP and 0.289 with PP. Similarly to RCT, a_{30} was more strongly related to SCS and pH on the permanent environmental level than on the genetic one (Table 4). HTD correlations of a_{30} with production and quality traits were generally low, particularly with milk composition, SCS, pH, C : P and P : F. Finally, a_{30} scarcely correlated with the other traits at phenotypic level, and the highest correlations were with FP (0.206), PP (0.303) and CP (0.314).

Discussion

Mean (32.00 kg/day) and variation (CV of 27.5%) for MY of Holstein-Friesian from the present study are consistent with values (32.30 kg/day and 31%, respectively) reported by Cassandro *et al.* (2008) in an Italian study conducted on the same breed. Values of FP and PP are slightly lower than findings of Cassandro *et al.* (2008), with 3.73% *v.* 3.89% for FP and 3.37% *v.* 3.45% for PP, respectively, whereas CP is

very similar (2.64% *v.* 2.65%, respectively). Variability for FP, PP and CP is comparable (CV of 18.2% *v.* 20.0%, 10.7% *v.* 12.0% and 11.4% *v.* 11.0%, respectively). The average value of SCS was lower (2.93) than that (3.08) of Cassandro *et al.* (2008), but the variability was of the same magnitude.

Milk coagulation properties were routinely predicted by MIRS; average values for RCT (20.61 min) and a_{30} (21.71 mm) are far from values of Cassandro *et al.* (2008), who reported 16.9 min for RCT and 32.0 mm for a_{30} measured by the computerized renneting meter. However, the variability was quite similar as the CV were 18.4 (*v.* 27%) and 39.6 (*v.* 35%) for RCT and a_{30}, respectively. Average pH predicted by MIRS, showed a lower mean value (6.62 *v.* 6.67) compared with that of Cassandro *et al.* (2008) measured with a pH-meter. Average values for MCP and milk composition are worse than those of Cecchinato *et al.* (2009) in Italian Brown Swiss, who reported means of 15.0 min for RCT and 41.7 mm for a_{30} evaluated using the computerized renneting meter, and by Bonfatti *et al.* (2011) in Italian Simmental, who reported RCT and a_{30} of 16.51 min and 29.08 mm, respectively, using the same mechanical device. Ikonen *et al.* (2004) found 11.80 ± 5.50 min for RCT and 24.0 ± 13.3 mm for a_{30} in Finnish Ayrshire and Tyrisevä *et al.* (2004) reported 11.2 ± 5.0 min for RCT and 26.5 ± 11.8 mm for a_{30} in Finnish Ayrshire, Holstein and crossbred cows. Vallas *et al.* (2010) analyzed log-transformed RCT in Estonian Holstein population and found an average value of 2.3 (CV of 9%) and an average a_{30} of 27.0 mm (CV of 27%). However, direct comparison of MCP among breeds reared in different countries has to be made with caution because dairy populations have different genetic structure, and laboratory analyses are known to differ in terms of instruments used and activity of the coagulant (Pretto *et al.*, 2011).

In the present study, MCP were predicted by MIRS using the calibration model developed by De Marchi *et al.* (2012) and installed by Foss Electric A/S on the laboratory instrument. The model works in the range from 5 to 30 min, and thus it does not consider the so-called noncoagulating (NC) milk, that is, milk with RCT longer than 30 min from rennet addition; however, only 3.5% of samples did not exhibit RCT within 30 min from the beginning of the analysis. The presence of records with RCT longer than 30 min does not necessarily mean that those samples are NC, as they might be because of the inability of the calibration equation to predict RCT when it approaches the 30 min values. The phenomenon of NC milk is associated to several factors such as pH, SCC, mastitis, κ-casein concentration and genotypes, stage of lactation and breed of cow. Besides the aforementioned factors, additive genetic effects seem to influence the occurrence of NC milk (e.g. Ikonen *et al.*, 2004; Tyrisevä *et al.*, 2004). The inclusion of NC information in statistical models aiming at estimating covariance components for MCP may influence the assessment of genetic parameters for coagulation traits (Ikonen *et al.*, 2004), but the impact depends mainly on the proportion of NC samples: the lower the proportion, the lower the influence on covariance components.

The heritabilities and intra-herd heritabilities for RCT and a_{30} predicted by MIRS are moderate, but large enough for genetic improvement of the traits (Table 2). Cassandro *et al.* (2008) estimated heritabilities of 0.25 and 0.15 for RCT and a_{30}, respectively, measured once on Holstein-Friesian cows using the Computerized Renneting Meter (CRM; Polo Trade, Monselice, Italy). Bonfatti *et al.* (2011) reported heritabilities of 0.29 and 0.12 for RCT and a_{30} assessed using the CRM in Italian Simmental. The heritability for a_{30} was much higher in our study than in Cassandro *et al.* (2008) and Bonfatti *et al.* (2011). Cecchinato *et al.* (2009) compared heritabilities of MCP assessed with CRM and Milko-Scan FT120 (Foss Electric A/S) and they reported higher estimates for RCT and a_{30} predicted by MIRS than for measures of these traits obtained with CRM. Ikonen *et al.* (2004) assessed heritability of 0.28 for RCT and 0.39 for a_{30} measured with the CRM on samples from Finnish Ayrshire cows sampled once. In the same study, estimates of herd effect were 0.05 and 0.06 for RCT and a_{30}, respectively, which are lower than values found in the present work. Similarly, Tyrisevä *et al.* (2004) estimated heritabilities of 0.21 and 0.22, and HTD effect of 0.06 and 0.09 for RCT and a_{30}, respectively, in Finnish Ayrshire, Holstein and crossbred cows.

Vallas *et al.* (2010) used repeated measures for MCP assessed using Optigraph in Estonian Holstein cows. The authors reported heritabilities of 0.28 and 0.41 for log-transformed RCT and a_{30}, respectively, repeatabilities of 0.45 and 0.50, and herd effect of 0.04 and 0.03. It is worth noting that in studies which dealt with multiple measures per cow, repeatabilities were higher than heritabilities, proving a consistent cow permanent environmental effect. Repeatability of RCT and a_{30} from the present work is lower than 0.500, and thus it is of undeniable importance to collect repeated measures per cow to obtain reliable estimates of breeding values for MCP. In addition, considering the results, herd environmental effect had moderate impact on MCP.

The present study confirmed the strong genetic and phenotypic correlations between RCT and a_{30}, as previously reported (Tyrisevä *et al.*, 2003; Ikonen *et al.*, 2004; Cassandro *et al.*, 2008). Only Vallas *et al.* (2010) found very weak relationships between a_{30} and log-transformed RCT. On the phenotypic level, MCP did not appear to be related to most of considered traits (coefficients of correlation were lower than 0.25 as absolute value), except for PP and CP, which showed a moderate correlation with a_{30} (0.303 and 0.314, respectively). This result is in agreement with Cassandro *et al.* (2008) who found comparable genetic correlations (0.23 and 0.32, respectively). Similarly, Vallas *et al.* (2010) reported a moderate relationship between PP and a_{30} (0.45). SCS was not phenotypically related to MCP, neither in the present study nor in the literature. On the contrary, in the aforementioned papers milk pH showed moderate phenotypic correlations with MCP, with values often higher than 0.30 as absolute value, indicating that milk characterized by high pH has worse renneting ability (Okigbo *et al.*, 1985; Nájera *et al.*, 2003). The same relationship was not found in the present work, which reported phenotypic correlation

very close to zero between MCP and pH. A possible explanation might be that measures of pH from the present work may depend on the predicting ability of the calibration equation used and further study is needed to investigate strategies to improve the equation for this trait.

Curd firmness was positively and favorably correlated with some milk component traits, particularly with PP, CP and FP, whereas an antagonistic genetic correlation between MY and a_{30} (−0.248) has been assessed. On the contrary, RCT was not strongly related to yield and quality traits. On the cow permanent environmental level, correlations between a_{30} and FP (0.350), PP (0.289) and CP (0.347) reflected the genetic relationships. Surprisingly, LP was correlated both with RCT (−0.398) and a_{30} (0.476). Genetic correlations are partially in agreement with the literature: Cassandro *et al.* (2008) reported moderate relationships of RCT and a_{30} with MY (−0.24 and 0.22, respectively) and CP (−0.22 and 0.53), and of a_{30} with PP (0.44), but all other correlations with milk contents were very low. Vallas *et al.* (2010) estimated moderate to low correlations with PP (0.19 and 0.48 for log-transformed RCT and a_{30}, respectively), and between a_{30} and FP (0.25). Ikonen *et al.* (2004) found weak correlations with MY and milk percentages. Contrarily, Lindström *et al.* (1984) found strong genetic relationships of clotting time with FP (−0.91) and PP (−0.58), and a null genetic correlation with LP (−0.05).

SCS was not genetically related to MCP (0.052 and −0.050 with RCT and a_{30}, respectively), although it was moderately related on the cow permanent environmental level (0.339 and −0.337, respectively). The low genetic correlations are not in agreement with findings from studies that used single cow measurements (Ikonen *et al.*, 2004; Cassandro *et al.*, 2008), but are consistent with Vallas *et al.* (2010), who collected repeated measures per cow and included the cow permanent environmental effect in the model. It is important to underline that only in the case of repeated sampling it is possible to estimate additive genetic and permanent environmental correlations as different entities. In fact, Cassandro *et al.* (2008) assessed genetic correlations of 0.22 and −0.40 between SCS and RCT, and SCS and a_{30}, respectively, and Ikonen *et al.* (2004) found values of 0.29 and −0.45. On the other hand, Vallas *et al.* (2010) estimated genetic correlations of −0.06 and −0.04. Results suggest that SCS is related to MCP when a single measure per cow is considered and the genetic and permanent environmental effect of the cow cannot be disentangled. However, further research is needed as previous results were obtained from different populations.

Milk pH was weakly related to MCP at the genetic (0.122 and −0.100 with RCT and a_{30}, respectively) and cow permanent environmental (−0.190 and 0.135, respectively) level. The results for genetic relationships are much lower than those reported by other authors and this might be related to the predicting ability of the calibration equation used. In Cassandro *et al.* (2008) genetic correlations of pH with RCT and a_{30} were 0.81 and −0.85, respectively; in Bonfatti *et al.* (2011) they were 0.61 and −0.61; and in Ikonen *et al.* (2004) 0.50 and −0.32. However, Vallas *et al.* (2010) estimated genetic relationships of −0.06 between pH and a_{30}, and of 0.69 between pH and log-transformed RCT. Casein to protein and P : F ratios were not related to MCP on the phenotypic and genetic level, whereas a relationship was found on the cow permanent environmental level, particularly in the case of C : P. Results indicate that ratios of milk constituents are not suitable for an indirect selection to improve RCT and a_{30}.

Mid-infrared spectroscopy models used in this study to predict MCP exhibited coefficients of determination of cross-validation equal to 0.76 for RCT and 0.70 for a_{30} (De Marchi *et al.*, 2012); these values can be regarded as a phenotypic relationship between the trait of interest (real milk coagulation ability) and the correlated trait (MIRS-predicted milk coagulation ability). The coefficients obtained here are not optimal, but the lack in prediction is compensated by the higher number of records obtained per cow when MIRS is routinely implemented. Pretto *et al.* (2012) provided an accurate estimation of the genetic progress achievable under different breeding scenarios.

Current selection index for Italian Holstein-Friesian breed includes PY, FY, PP and FP (with higher emphasis on protein than fat and on yields than percentages), and SCS, fertility, functional longevity, type, udder composite index and feet and legs index. In a recent study, Pretto *et al.* (2012) reported that current selection index for Italian-Holstein breed leads to a small genetic improvement of MCP, and thus the direct inclusion of RCT and a_{30} is necessary to genetically enhance coagulation traits. This is the consequence of the rather low genetic correlations between MCP and most production and quality traits (except for a_{30} with PP and CP), as confirmed in the present study. Repeatability of MCP was moderate, suggesting that a single measure per cow is not representative of animal performance. HTD environmental effect has been confirmed to be moderate for MCP; however, a consistent number of herds should be sampled on several months of the year, as differences in MCP are expected to arise from different seasons. HTD correlations between MCP and yield traits were moderate to low, but favorable as shorter RCT and firmer curd were associated to increased yield traits.

Conclusions

Genetic variation for MCP predicted by MIRS exists and can be exploited to improve RCT and a_{30} in dairy cows. Genetic correlations of MCP with production traits, milk composition, SCS, pH, C : P and P : F were generally low, except for the relationship of a_{30} with PP and CP. Overall, milk quality traits measured during routine data collection did not provide a valuable tool to predict MCP nor to enhance the accuracy of breeding value estimation. An exception is represented by PP and CP, which could be included as correlated traits in the estimation of breeding values for a_{30}.

Ackowledgments

The authors thank the laboratory of the Breeders Association of Veneto region (Padova, Italy) for providing data and the

Italian Holstein Friesian Cattle Breeders Association (ANAFI, Cremona, Italy) for pedigree information. The first author gratefully acknowledges the European Social Fund (FSE) for financing the research scholarship.

Authors' contribution

F. Tiezzi performed the statistical analyses and wrote the first draft of the manuscript. D. Pretto and M. Penasa were involved in drafting the paper. M. De Marchi and M. Cassandro designed the research. All authors contributed to the interpretation and discussion of the results.

References

Aleandri R, Schneider JC and Buttazzoni LG 1989. Evaluation of milk for cheese production based on milk characteristics and Formagraph measures. Journal of Dairy Science 72, 1967–1975.

Bonfatti V, Cecchinato A, Gallo L, Blasco A and Carnier P 2011. Genetic analysis of detailed milk protein composition and coagulation properties in Simmental cattle. Journal of Dairy Science 94, 5183–5193.

Bynum DG and Olson NF 1982. Influence of curd firmness at cutting on yield and recovery of milk constituents. Journal of Dairy Science 65, 2281–2290.

Cassandro M, Comin A, Ojala M, Dal Zotto R, De Marchi M, Gallo L, Carnier P and Bittante G 2008. Genetic parameters of milk coagulation properties and their relationships with milk yield and quality traits in Italian Holstein cows. Journal of Dairy Science 91, 371–376.

Cecchinato A, De Marchi M, Gallo L, Bittante G and Carnier P 2009. Mid-infrared spectroscopy predictions as indicator traits in breeding programs for enhanced coagulation properties of milk. Journal of Dairy Science 92, 5304–5313.

Cipolat-Gotet C, Cecchinato A, De Marchi M, Penasa M and Bittante G 2012. Comparison between mechanical and near-infrared methods for assessing coagulation properties of bovine milk. Journal of Dairy Science 95, 6806–6819.

Comin A, Cassandro M, Chessa S, Ojala M, Dal Zotto R, De Marchi M, Carnier P, Gallo L, Pagnacco G and Bittante G 2008. Effects of composite β- and κ-casein genotypes on milk coagulation, quality, and yield traits in Italian Holstein cows. Journal of Dairy Science 91, 4022–4027.

Dal Zotto R, De Marchi M, Cecchinato A, Penasa M, Cassandro M, Carnier P, Gallo L and Bittante G 2008. Reproducibility and repeatability of measures of milk coagulation properties and predictive ability of mid-infrared reflectance spectroscopy. Journal of Dairy Science 91, 4103–4112.

De Marchi M, Dal Zotto R, Cassandro M and Bittante G 2007. Milk coagulation ability of five dairy cattle breeds. Journal of Dairy Science 90, 3986–3992.

De Marchi M, Toffanin V, Cassandro M and Penasa M 2013. Prediction of coagulating and noncoagulating milk samples using mid-infrared spectroscopy. Journal of Dairy Science 96, 4707–4715.

De Marchi M, Penasa M, Tiezzi F, Toffanin V and Cassandro M 2012. Prediction of milk coagulation properties by Fourier transform mid-infrared spectroscopy (FTMIR) for genetic purposes, herd management and dairy profitability. Proceedings of the 38th International Committee for Animal Recording (ICAR) Meeting, 28 May – 1 June 2012, Cork, Ireland. http://www.icar.org/Cork_2012/Manuscripts/Published/Cassandro.pdf.

De Marchi M, Penasa M, Cecchinato A, Mele M, Secchiari P and Bittante G 2011. Effectiveness of mid-infrared spectroscopy to predict fatty acid composition of Brown Swiss bovine milk. Animal 5, 1653–1658.

De Marchi M, Fagan CC, O'Donnell CP, Cecchinato A, Dal Zotto R, Cassandro M, Penasa M and Bittante G 2009. Prediction of coagulation properties, titratable acidity, and pH of bovine milk using mid-infrared spectroscopy. Journal of Dairy Science 92, 423–432.

Formaggioni P, Sandri S, Franceschi P, Malacarne M and Mariani P 2005. Milk acidity, curd firming time, curd firmness and protein and fat losses in the Parmigiano-Reggiano cheesemaking. Italian Journal of Animal Science 4 (suppl. 2), 239–241.

Geary U, Lopez-Villalobos N, Garrick DJ and Shalloo L 2010. Development and application of a processing model for the Irish dairy industry. Journal of Dairy Science 93, 5091–5100.

Ikonen T, Morri S, Tyrisevä A-M, Ruottinen O and Ojala M 2004. Genetic and phenotypic correlations between milk coagulation properties, milk production traits, somatic cell count, casein content and pH of milk. Journal of Dairy Science 87, 458–467.

Jensen HB, Holland JW, Poulsen NA and Larsen LB 2012. Milk protein genetic variants and isoforms identified in bovine milk representing extremes in coagulation properties. Journal of Dairy Science 95, 2891–2903.

Johnson ME, Chen CM and Jaeggi JJ 2001. Effect of rennet coagulation time on composition, yield, and quality of reduced-fat Cheddar cheese. Journal of Dairy Science 84, 1027–1033.

Klei L, Yun J, Sapru A, Lynch J, Barbano D, Sears P and Galton D 1998. Effects of milk somatic cell count on cottage cheese yield and quality. Journal of Dairy Science 81, 1205–1213.

Kübarsepp I, Henno M, Viinalass H and Sabre D 2005. Effect of κ-casein and β-lactoglobulin gentoypes on the milk rennet coagulation properties. Agronomy Research 3, 55–64.

Lindström UB, Antila V and Syväjärvi J 1984. A note on some genetic and non-genetic factors affecting clotting time of Ayrshire milk. Acta Agriculturae Scandinavica 34, 349–355.

López MB, Lomholt SB and Qvist KB 1998. Rheological properties and cutting time of rennet gels. Effect of pH and enzyme concentration. International Dairy Journal 8, 289–293.

Nájera AI, de Renobales M and Barron LJR 2003. Effects of pH, temperature, CaCl2 and enzyme concentrations on the rennet-clotting properties of milk: a multifactorial study. Food Chemistry 80, 345–352.

Okigbo LM, Richardson GH, Brown RJ and Ernstrom CA 1985. Effects of pH, calcium chloride, and chymosin concentration on coagulation properties of abnormal and normal milk. Journal of Dairy Science 68, 2527–2533.

Penasa M, Cassandro M, Pretto D, De Marchi M, Comin A, Chessa S, Dal Zotto R and Bittante G 2010. Short communication: influence of composite casein genotypes on additive genetic variation of milk production traits and coagulation properties in Holstein-Friesian cows. Journal of Dairy Science 93, 3346–3349.

Politis I and Ng-Kwai-Hang KF 1988. Effects of somatic cell counts and milk composition on the coagulating properties of milk. Journal of Dairy Science 71, 1740–1746.

Pretto D, López-Villalobos N, Penasa M and Cassandro M 2012. Genetic response for milk production traits, somatic cell score, acidity and coagulation properties in Italian Holstein-Friesian population under current and alternative selection indices and breeding objectives. Livestock Science 150, 59–66.

Pretto D, De Marchi M, Penasa M and Cassandro M 2013. Effect of milk composition and coagulation traits on Grana Padano cheese yield under field conditions. Journal of Dairy Research 80, 1–5.

Pretto D, Kaart T, Vallas M, Jõudu I, Henno M, Ancilotto L, Cassandro M and Pärna E 2011. Relationships between milk coagulation property traits analyzed with different methodologies. Journal of Dairy Science 94, 4336–4346.

Soyeurt H, Dardenne P, Dehareng F, Lognay G, Veselko D, Marlier M, Bertozzi C, Mayeres P and Gengler N 2006. Estimating fatty acid content in cow milk using mid-infrared spectrometry. Journal of Dairy Science 89, 3690–3695.

Tyrisevä A-M, Ikonen T and Ojala M 2003. Repeatability estimates for milk coagulation traits and non-coagulation of milk in Finnish Ayrshire cows. Journal of Dairy Research 70, 91–98.

Tyrisevä A-M, Vahlsten T, Ruottinen O and Ojala M 2004. Noncoagulation of milk in Finnish Ayrshire and Holstein-Friesian cows and effect of herds on milk coagulation ability. Journal of Dairy Science 87, 3958–3966.

Vallas M, Bovenhuis H, Kaart T, Pärna K, Kiiman H and Pärna E 2010. Genetic parameters for milk coagulation properties in Estonian Holstein cows. Journal of Dairy Science 93, 3789–3796.

Vallas M, Kaart T, Värv S, Pärna K, Jõudu I, Viinalass H and Pärna E 2012. Composite β-κ-casein genotypes and their effect on composition and coagulation of milk from Estonian Holstein cows. Journal of Dairy Science 95, 6760–6769.