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Genetic analysis of rennet coagulation time, curd-firming rate, and curd firmness assessed over an extended testing period using mechanical and near-infrared instruments

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ABSTRACT

The aims of this study were (1) to analyze rennet coagulation time (RCT), curd-firming rate, and curd firmness obtained by extending the standard 30-min testing time to 45 min; (2) to estimate heritabilities of the aforementioned traits determined by mechanical (Formagraph; Foss Electric, Hillerød, Denmark) and near-infrared optical (Optigraph; Ysebaert, Frépillon, France) instruments, and to assess the statistical relevance of their genetic background by using the Bayes factor procedure, the deviance information criterion, and the mean squared error; (3) to estimate phenotypic and genetic relationships between instruments within trait and between traits within instrument; and (4) to obtain correlations for sire rankings based on the used instruments. Individual milk samples were collected from 913 Brown Swiss cows reared in 63 herds located in Trento Province (Italy). Milk coagulation properties (MCP) were measured using 2 different instruments: Formagraph and Optigraph. Both instruments were housed in the same laboratory and operated by the same technician. Each sample was analyzed simultaneously on each instrument. All experimental conditions (milk temperature and the concentration and type of rennet) were identical. For the analysis, univariate and bivariate animal models were implemented using Bayesian methods. Univariate analyses were conducted to test the hypothesis that the traits showed additive genetic determination. Deviance information criterion, Bayes factor, and mean squared error were used as model choice criteria. The main results were that (1) RCT could be measured on all samples by extending the observation time to 45 min, and its genetic parameters ($h^2 = 0.23$) and breeding values could be estimated while avoiding the bias of noncoagulating samples; (2) curd-firming rate could be measured on almost all milk samples, and its genetic parameters could be estimated for the first time on a field data set ($h^2 = 0.21$); (3) for the first time, genetic parameters of curd firmness 45 min after rennet addition ($h^2 = 0.12$) were estimated, and they were compared with curd firmness 30 min after rennet addition ($h^2 = 0.17$); and (4) MCP estimated using the Optigraph appeared to be genetically different from those determined by Formagraph, with the partial exception of RCT (genetic correlation = 0.97). Breeding strategies for the improvement of MCP must be planned with caution. Currently, the high throughput, ease of use, and reduced costs of analysis make predictions obtained from mid-infrared spectroscopy (MIRS) on untreated milk samples a promising alternative to produce relevant data at the population level. The use of mechanical lactodynamographs to establish reference data for MIRS calibrations have been already studied, whereas the use of near-infrared optical lactodynamographs as a reference method for MIRS calibrations needs to be investigated.

Key words: milk coagulation property, mechanical and optical lactodynamograph, heritability, Bayes factor

INTRODUCTION

Measurement of milk coagulation properties (**MCP**) is of special relevance for cheese manufacturing. The most used instruments to assess MCP are lactodynamographs (renneting meters) by which rennet coagulation time (**RCT**, min), curd-firming time ($\mathbf{k_{20}}$, min), and curd firmness are measured after addition of the clotting enzyme to raw milk (Annibaldi et al., 1977; Zannoni and Annibaldi, 1981; McMahon and Brown, 1982). Lactodynamographs record physicochemical changes occurring in milk during the coagulation process when the enzyme hydrolyzes κ-casein aggregates and induces changes in milk viscosity and elasticity (Auldist et al., 2001; O'Callaghan et al., 2002).

Several systems have been adopted to determine MCP in cow's milk. Traditionally, laboratory mechani-

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cal instruments such as the Formagraph (**FRM**; Foss Electric, Hillerød, Denmark) and the Computerized Renneting Meter (Polo Trade, Monselice, Italy) have been used, whereas optical instruments based on infrared technologies have been often used to monitor MCP directly in the cheese-making vats (Payne et al., 1993; Laporte et al., 1998; O'Callaghan et al., 2002). Recently, 2 categories of infrared optical instruments have been adopted to predict MCP at laboratory level. The first includes medium infrared spectrometers (**MIRS**) to predict MCP from raw milk samples analyzed without induction of rennet coagulation (Dal Zotto et al., 2008; De Marchi et al., 2009). The second includes lactodynamographs such as the Optigraph (**OPT**; Ysebaert, Frépillon, France), which has been proposed to determine MCP through induction of rennet coagulation of milk (Panari et al., 2002; Kübarsepp et al., 2005). The 2 types of lactodynamographs record the same parameters on coagulating samples but using different principles. Mechanical measures are based on continuous recording of the movement, after the immersion of small loop pendulum in linearly oscillating samples of coagulating milk, induced by minute forces applied to the pendulum as a consequence of the milk coagulation (McMahon and Brown, 1982). The optical instrument continuously measures the optical signal in the near-infrared (NIR) region (820 nm) and estimates the MCP by means of specific calibration equations. Recently, a phenotypic study by Cipolat-Gotet et al. (2012) demonstrated that FRM and OPT yield different results with the partial exception of RCT.

Several studies have been carried out to estimate genetic parameters of MCP measured by mechanical lactodynamographs (e.g., Lindström et al., 1984; Ikonen et al., 2004; Cecchinato et al., 2012), and recently one study dealt with OPT (Vallas et al., 2010). No direct comparisons between genetic parameters of MCP obtained from mechanical and optical instruments are currently available. Moreover, past research on genetic aspects of MCP dealt with renneting parameters determined for 30 min after the addition of the clotting enzyme, and faced the problem of milk samples that do not coagulate within the testing time of 30 min (the so-called noncoagulating samples, **NC**) and of the potential bias in the estimation of genetic parameters of MCP and breeding values of animals (Cecchinato and Carnier, 2011). A significant fraction of milk samples do not usually attain curd firmness of 20 mm within the 30-min testing time; hence, k_{20} is often excluded from genetic analyses. Only a few studies have estimated genetic parameters for k_{20}, and they were based on a small number of cows reared on experimental farms (Tervala et al., 1985; Ikonen et al., 1997).

Therefore, the objectives of this study were (1) to analyze RCT, k_{20}, and curd firmness obtained by extending the standard 30-min testing time ($\mathbf{a_{30}}$) to 45 min ($\mathbf{a_{45}}$); (2) to estimate heritabilities of the aforementioned traits measured by FRM and OPT instruments, and to assess the statistical relevance of their genetic background by using the Bayes factor (**BF**) procedure, the deviance information criterion (**DIC**), and the mean squared error (**MSE**); (3) to estimate phenotypic and genetic relationships between instruments within trait and between traits within instrument; and (4) to obtain correlations for sire rankings based on the instruments used.

MATERIALS AND METHODS

Field Data

In total, 913 Brown Swiss cows from 63 herds located in Trento Province (Italy) were sampled once during the evening milking between April 2010 and February 2011. Within a given day, only one herd was sampled. Two milk subsamples per cow were collected and immediately refrigerated at 4°C without preservative. One random subsample was transported to the Milk Quality Laboratory of the Breeders Federation of Trento Province (Trento, Italy) for composition analysis. The other subsample was transferred to the cheese-making laboratory of the Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE) of the University of Padova (Legnaro, Italy) for MCP analysis. All samples were processed within 20 h of collection. Information on cows and herds were provided by the Breeders Federation of Trento Province (Italy). Pedigree information was supplied by the Italian Brown Swiss Cattle Breeders Association (ANARB, Verona, Italy) and included cows with phenotypic records for the investigated traits and all their known ancestors.

Analysis of Milk Quality

Individual milk subsamples were analyzed for fat, protein, and casein contents using MilkoScan FT6000 (Foss). Somatic cell count was obtained from the Fossomatic FC counter (Foss) and was then converted to SCS by means of logarithm transformation (Ali and Shook, 1980). The pH of the subsamples was measured before MCP analysis, using a Crison Basic 25 electrode (Crison, Barcelona, Spain).

Analysis of Milk Coagulation Properties

Milk coagulation properties were determined using FRM and OPT. Both instruments were housed in the

same laboratory and operated by the same technician. Each subsample was analyzed simultaneously on each instrument. All experimental conditions (milk temperature and the concentration and type of rennet) were identical. Two racks containing 10 cuvettes (one rack per instrument) were prepared; milk samples (10 mL) were heated to 35°C and 200 μL of rennet solution (Hansen Standard 160, with 80 ± 5% chymosin and 20 ± 5% pepsin; Pacovis Amrein AG, Bern, Switzerland) diluted to 1.6% (wt/vol) in distilled water, to yield 0.051 international milk clotting units (IMCU)/mL, was added to samples after milk heating. Both instruments yield the width (mm) of the oscillatory graph during testing: the OPT records a datum every 6 s and the FRM every 15 s. The observation period continued for 90 min after rennet addition but, for the purposes of the present work, only the first 45 min were considered. Variations in absorbance, as detected by OPT, were transformed by instrument software using an appropriate calibration equation to mimic the shape of the graph afforded by traditional mechanical instruments (Kübarsepp et al., 2005). This means that MCP can be calculated using either device. The MCP recorded were (1) the time from addition of enzyme to the beginning of visible coagulation (gelification) within a time interval of 45 min (RCT, min); (2) the interval from gelification (RCT) to the time at which the width of the graph attained 20 mm (k_{20}, min); (3) the firmness of the curd at 30 min from rennet addition (a_{30}, mm); and (4) the firmness of the curd at 45 min from rennet addition (a_{45}, mm). Samples that did not coagulate within 30 min were classified as NC (Ikonen et al., 1999), although extension of analysis allowed RCT and k_{20} to be recorded for all samples.

Statistical Analysis

Nongenetic Effects. Nongenetic effects to be included in mixed models to estimate genetic parameters for MCP determined by FRM and OPT were identified through preliminary analysis based on the GLM procedure (SAS Inst. Inc., Cary, NC). For all traits, the model accounted for the effects of herd (63 levels), DIM (class 1: <60 d, class 2: 60–120 d, class 3: 121–180 d, class 4: 181–240 d, class 5: 241–300 d, and class 6: >300 d), parity (1 to 4 or more), and renneting meter sensor (10 levels) of the lactodynamograph, being the pendula of FRM and the monochromators of OPT. All these effects were important sources of variation ($P < 0.05$) except for the renneting meter sensor for RCT and k_{20}.

Univariate Models for Testing the Hypothesis of Additive Genetic Determination. The genetic background of the MCP (**y**) was investigated by analyzing data under the following hierarchical model:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z}_1\mathbf{h} + \mathbf{Z}_2\mathbf{a} + \mathbf{e}, \quad [1]$$

where **y** was the vector of phenotypic records with dimension n; **X**, $\mathbf{Z}_1$, and $\mathbf{Z}_2$ were appropriate incidence matrices for systematic effects (**b**), herd-date effects (**h**), and polygenic additive genetic effects (**a**), respectively; and **e** was the vector of residual effects. Specifically, **b** included nongenetic effects of DIM, parity, and renneting meter sensor (only for a_{30} and a_{45}).

All models were analyzed under a standard Bayesian approach. The joint distribution of the parameters in the model was proportional to

$$p\left(\mathbf{b},\mathbf{h},\mathbf{a},\sigma_e^2,\sigma_h^2,\sigma_a^2 \mid \mathbf{y}\right) \propto p\left(\mathbf{y} \mid \mathbf{b},\mathbf{h},\mathbf{a},\sigma_e^2\right)p\left(\sigma_e^2\right)p\left(\mathbf{b}\right) \times p\left(\mathbf{h} \mid \sigma_h^2\right)p\left(\sigma_h^2\right)p\left(\mathbf{a} \mid \mathbf{A},\sigma_a^2\right)p\left(\sigma_a^2\right),$$

where **A** was the numerator relationship matrix between individuals (Wright, 1922), and σ_e^2, σ_h^2, and σ_a^2 were the residual, herd-date, and additive genetic variances, respectively. The a priori distributions of **h** and **a** were assumed multivariate normal, as follows:

$$p\left(\mathbf{h} \mid \sigma_h^2\right) \sim N\left(0,\mathbf{I}\sigma_h^2\right) \text{and}$$
$$p\left(\mathbf{a} \mid \sigma_a^2\right) \sim N\left(0,\mathbf{A}\sigma_a^2\right),$$

where **I** was an identity matrix with dimensions equal to the number of elements in **h**. Priors for **b** and variance components were assumed to be flat.

The univariate model was used to test for additive genetic determination of each trait. Different criteria were used for this purpose. The DIC (Spiegelhalter et al., 2002) was computed both for the model including the additive genetic effect and for the reduced model without this effect; differences in DIC of more than 7 units were considered important (Spiegelhalter et al., 2002). The BF (Kass and Raftery, 1995; García-Cortés et al., 2001; Casellas et al., 2010) was computed as a pair-wise comparison by calculating the ratio between the posterior probabilities of 2 competing models, taking any positive value between >0 and $+\infty$. In this case, a linear mixed model with additive polygenic effects (numerator model) was compared against a model without additive polygenic effects (denominator model), where >1 BF favored the numerator model and <1 BF favored the denominator model. In this report, the BF results were discussed within the context of the Jeffreys (1984) discrete scale of evidences. This scale classifies the BF according to 6 levels of evidence for the numerator model, objectively classifying the BF as follows: denominator model supported, not worth more than a bare mention, substantial evidence, strong

evidence, very strong evidence, and decisive evidence. From now on, this terminology will be systematically used when referring to the BF. The MSE between real and predicted phenotypic records was also used to compare models (i.e., the one with additive polygenic effects and the same model without additive polygenic effects). For all MCP traits, the expectation of the predictive distribution of a given record was computed as in Varona et al. (1999):

$$\hat{y}_{MCPi} = \mathbf{x}_i\hat{\beta} + \mathbf{z}_{1i}\hat{\mathbf{h}} + \mathbf{z}_{2i}\hat{\mathbf{a}} - \hat{\mathbf{e}}_i,$$

where $\hat{y}_{MCPi}$ is the expectation for the ith MCP record, $\mathbf{x}_i$, $\mathbf{z}_{1i}$, and $\mathbf{z}_{2i}$ are the ith rows of the incidence matrices that link systematic, herd-date, and additive genetic effects, and $\hat{\mathbf{e}}_i$ are the residuals for the ith MCP record. Note that $\hat{\beta}$, $\hat{\mathbf{h}}$, $\hat{\mathbf{a}}$, and $\hat{\mathbf{e}}_i$ are posterior median estimates. The MSE was defined as

$$\text{MSE} = \frac{\sum_{i=n}^{n}\left(y_i - \hat{y}_i\right)^2}{n}.$$

Bivariate Models for Estimating Correlations Between Traits. To estimate genetic correlations between MCP variables, a set of bivariate analyses was conducted, implementing model [1] in its multivariate version. In this case, the involved traits were assumed to jointly follow a multivariate normal (**MVN**) distribution as well as the additive genetic, herd-date, and residual effects. For these effects, the corresponding prior distributions were

$$\begin{aligned}\mathbf{a} \mid \mathbf{G}_0, \mathbf{A} &\sim MVN\left(0, \mathbf{G}_0 \otimes \mathbf{A}\right),\\ \mathbf{h} \mid \mathbf{H}_0 &\sim N\left(0, \mathbf{H}_0 \otimes \mathbf{I}_n\right),\\ \mathbf{e} \mid \mathbf{R}_0 &\sim N\left(0, \mathbf{R}_0 \otimes \mathbf{I}_m\right),\end{aligned}$$

where $\mathbf{G}_0$, $\mathbf{H}_0$, and $\mathbf{R}_0$ were the corresponding variance-covariance matrices between the involved traits, and $\mathbf{a}$, $\mathbf{h}$, and $\mathbf{e}$ were vectors of dimension equal to the number of animals in the pedigree (n and m) times the number of traits considered.

Gibbs Sampler. Marginal posterior distributions of unknown parameters were estimated by performing numerical integration using the Gibbs sampler (Gelfand and Smith, 1990). This was used to obtain auto-correlated samples from the joint posterior distributions and subsequently from the marginal posterior distributions of all unknowns in the model. The lengths of the chain and the burn-in period were assessed by visual inspection of trace plots, as well as by the diagnostic tests of Geweke (1992) and Gelman and Rubin (1992). After a preliminary run, we decided to construct a single chain consisting of 850,000 iterations and to discard the first 50,000 iterations as a very conservative burn-in. Subsequently, 1 of every 200 successive samples was retained, to store draws that were more loosely correlated. Thus, 4,000 samples were used to determine posterior distributions of unknown parameters. The lower and upper bounds of the highest 95% probability density regions (**HPD95%**) for parameters of concern were obtained from the estimated marginal densities. The posterior median was used as the point estimate for all parameters. Auto-correlations between samples and estimates of Monte Carlo standard error (Geyer, 1992) were calculated.

Heritability was computed as follows:

$$h^2 = \frac{\sigma_A^2}{\sigma_A^2 + \sigma_H^2 + \sigma_E^2},$$

where σ_A^2, σ_H^2, and σ_E^2 are additive genetic, herd-date, and residual variances, respectively.

Additive genetic correlations (r_A) were estimated as

$$r_A = \frac{\sigma_{A1,A2}}{\sigma_{A1} \cdot \sigma_{A2}},$$

where $\sigma_{A1,A2}$ is the additive genetic covariance between trait 1 and 2, and σ_{A1} and σ_{A2} are the additive genetic standard deviations for traits 1 and 2, respectively.

RESULTS

Milk Coagulation Properties and Phenotypic Variation

Descriptive statistics for MCP from FRM and OPT are summarized in Table 1. A comprehensive discussion on the phenotypic pattern of milk coagulation and curd firming from mechanical and NIR instruments has been reported by Cipolat-Gotet et al. (2012). In general, the average RCT from OPT was slightly shorter (1 min) than the value from FRM. Despite this, a_{30} was approximately 3 mm smaller when assessed by OPT than by FRM. Curd-firming time was notably longer when evaluated by OPT (8.16 min) than by FRM (5.36 min). Finally, a_{45} was about 8 mm greater when assessed by OPT (41.49 mm) than by FRM (33.65 mm). Rennet coagulation time and a_{30} from OPT were less variable than the corresponding MCP from FRM, whereas the opposite was found for a_{45}. Variances of MCP from FRM and OPT were statistically different according to Levene's test ($P < 0.05$; data not shown) with the

Table 1. Descriptive statistics (n = 913) of milk coagulation properties assessed using Formagraph (Foss Electric, Hillerød, Denmark) and Optigraph (Ysebaert, Frépillon, France) instruments[1]

	Formagraph				Optigraph			
Trait[2]	Mean	SD	P1	P99	Mean	SD	P1	P99
RCT (min)	19.95	5.81	10.31	38.00	18.91	4.40	11.40	31.70
k_{20} (min)	5.36	3.12	1.45	17.30	8.16	2.97	3.70	17.80
a_{30} (mm)	30.09	11.34	0.40	51.04	27.23	10.80	2.35	55.79
a_{45} (mm)	33.66	8.43	9.03	52.20	41.49	11.54	11.87	70.62

[1]P1 = 1st percentile; P99 = 99th percentile.

[2]RCT = rennet coagulation time of samples coagulating within 45 min from enzyme addition; k_{20} = curd-firming time of samples reaching 20 mm of firmness within 45 min from enzyme addition; a_{30} = curd firmness at 30 min after enzyme addition; a_{45} = curd firmness at 45 min after enzyme addition.

exception of k_{20}. In fact, despite mean values being notably different between instruments, k_{20} values exhibited similar standard deviations. Consequently, the proportion of NC samples at 30 min (samples without a detectable a_{30}) was 6.57% for FRM and 2.08% for OPT ($P < 0.05$; data not shown). The extension of MCP analysis up to 45 min allowed the RCT recording for all samples and k_{20} recording for most late-coagulating milks. The late-coagulating milks also affected the distribution of a_{30} and k_{20}, whereas a_{45} showed close to Gaussian distribution.

Heritability of Milk Coagulation Properties

Point estimates (median of the marginal posterior density of the parameter) for the additive genetic, herd-date, and residual variances, and heritabilities of MCP measured by FRM and OPT are shown in Table 2, and the estimated posterior densities of the heritabilities are depicted in Figure 1. All Monte Carlo standard errors were very small, and a lack of convergence was not detected by the Geweke test (data not shown; Geweke, 1992). Marginal posterior distributions were approximately normal; thus, mode, mean, and median were similar, and only the posterior median is reported.

Rennet coagulation time was moderately heritable, and estimates were very similar between instruments (0.230 and 0.241 for FRM and OPT, respectively) with HPD95% between 0.10 and 0.40 both for FRM and OPT. The posterior densities were symmetric either for FRM and OPT and their shape was similar and almost overlapping. Even though heritability estimates were similar, the additive genetic, herd-date, and residual variances were 41.4, 51.1, and 43.5% lower when RCT was determined by OPT than by FRM.

Posterior density distribution and point estimates of heritability for k_{20} yielded by FRM were very close to those obtained for RCT, whereas the corresponding features from OPT data were much different. Heritability of k_{20} from OPT was almost twice the value found for the corresponding MCP of FRM and of RCT of both instruments. Moreover, the posterior densities (Figure 1) of the heritability for k_{20} obtained using OPT were more dispersed (HPD95% between 0.15 and 0.61) than those obtained by FRM, indicating more uncertainty in the estimation of this parameter. The high value of heritability was the consequence of the notably higher additive genetic and lower residual variance of OPT compared with FRM (+48.8% and −33.4%, respectively; Table 2). Estimates of variance due to herd-date effects on this MCP were also higher for OPT than for FRM (+26.9%).

Point estimates of heritability for a_{30} were slightly lower than those estimated for RCT and were comparable between instruments (0.171 for FRM and 0.205 for OPT; Table 2 and Figure 1). In addition, the HPD95% were similar between instruments, varying from 0.04 to 0.40. Estimates of additive genetic and residual variance were not very different between instruments (+13.2% and −14.3%, respectively), whereas herd-date variance was much higher (+64.1%) for OPT than for FRM.

Results for a_{45} were extremely variable and inconsistent between instruments. Point estimate of additive genetic variance from OPT was almost 5 times that from FRM. This explains the large differences in heritability for a_{45} assessed by FRM (0.120) and OPT (0.309), also in terms of variation of the point estimate (HPD95% from 0.02 to 0.27 for FRM, and 0.13 to 0.51 for OPT) as clearly depicted in Figure 1. Even in this case, estimates of heritability for a_{45} obtained using OPT were characterized by more uncertainty.

The inclusion of the additive polygenic effect improved the goodness of fit of the model for all MCP, regardless of the instrument used to assess MCP (Table 3). In particular, DIC and MSE decreased when the additive polygenic effect was accounted for in the analysis, suggesting a better fitting. The decrease in DIC ranged between 60 and 144 units. The BF confirmed the relevance of including the polygenic effect in the model, particularly in the analysis of RCT, and of a_{30}

Table 2. Features of marginal posterior densities of heritability (h^2), additive genetic $\left(\sigma_a^2\right)$, herd/date $\left(\sigma_h^2\right)$, and residual variances $\left(\sigma_e^2\right)$ of milk coagulation properties assessed using Formagraph (Foss Electric, Hillerød, Denmark) and Optigraph (Ysebaert, Frépillon, France) instruments

	Formagraph		Optigraph		
Trait[1]/parameter	Median[2]	HPD95%[3]	Median	HPD95%	Δ%[4]
RCT (min)					
σ_a^2	7.09	3.12; 13.02	4.16	2.01; 7.32	−41.4
σ_h^2	4.34	2.52; 7.41	2.12	1.17; 3.63	−51.1
σ_e^2	19.44	14.46; 23.65	11.00	8.37; 13.29	−43.5
h^2	0.230	0.10; 0.41	0.241	0.12; 0.41	+4.8
k_{20} (min)					
σ_a^2	2.10	0.74; 4.33	3.13	1.29; 5.52	+48.8
σ_h^2	0.29	2.52; 7.40	0.36	0.07; 0.82	+26.9
σ_e^2	7.54	5.73; 9.06	5.02	3.12; 6.71	−33.4
h^2	0.212	0.07; 0.41	0.368	0.15; 0.61	+73.6
a_{30} (mm)					
σ_a^2	21.03	5.01; 46.23	23.82	5.94; 49.52	+13.2
σ_h^2	6.42	1.85; 13.57	10.54	5.08; 19.44	+64.1
σ_e^2	95.33	74.01; 114.2	81.67	61.01; 100.3	−14.3
h^2	0.171	0.04; 0.36	0.205	0.05; 0.40	+19.9
a_{45} (mm)					
σ_a^2	8.37	1.74; 19.84	38.11	16.36; 67.22	+355.4
σ_h^2	12.54	7.78; 20.02	9.80	4.57; 18.32	−21.9
σ_e^2	49.0	39.38; 57.34	75.34	52.33; 96.87	+53.9
h^2	0.120	0.02; 0.27	0.309	0.13; 0.51	+157.5

[1]RCT = rennet coagulation time of samples coagulating within 45 min from enzyme addition; k_{20} = curd-firming time of samples reaching 20 mm of firmness within 45 min from enzyme addition; a_{30} = curd firmness at 30 min after enzyme addition; a_{45} = curd firmness at 45 min after enzyme addition.

[2]Median = median of the marginal posterior density of the parameter.

[3]HPD95% = lower and upper bounds of the 95% highest posterior density.

[4]Median of the marginal posterior density of the difference between variance components and heritabilities for milk coagulation properties assessed using Formagraph and Optigraph.

and a_{45} assessed using OPT. The BF between models with and without a genetic component, in fact, gave values >1 for all traits, providing evidence that the model was preferable when additive polygenic effects were included. The BF >100 indicated "decisive evidence" of genetic determinism for RCT yielded by both lactodynamographs, and for a_{30} and a_{45} obtained with OPT.

Relationships Among Milk Coagulation Properties

Point estimates (posterior medians) and HPD95% for genetic (r_g) and phenotypic (r_p) correlations between the same MCP trait assessed using FRM and OPT are reported in Table 4. The estimated phenotypic correlations were moderate to high and ranged from 0.426 (a_{45}) to 0.806 (RCT). The estimated genetic relationships were always high and were between 0.764 (k_{20}) and 0.974 (RCT).

Within instrument, the phenotypic correlations between the MCP were moderate to high, with some differences between the 2 instruments (Table 5). Genetic correlations between traditional MCP (RCT, k_{20}, and a_{30}) were very high, with the only exception being the relationship between RCT and k_{20} yielded by OPT (0.415). Curd firmness measured at 45 min from rennet addition yielded very low (and opposite in sign) genetic correlations with RCT with both instruments. The correlations of a_{45} with k_{20} and a_{30} were both low in the case of FRM and very high in the case of OPT.

Relationships Between Sire Rankings

The relationships between sire rankings based on EBV for each MCP measured using FRM and OPT are depicted in Figure 2. The sire ranking for RCT was only marginally affected by the instrument used to assess the trait, as the correlation between EBV based

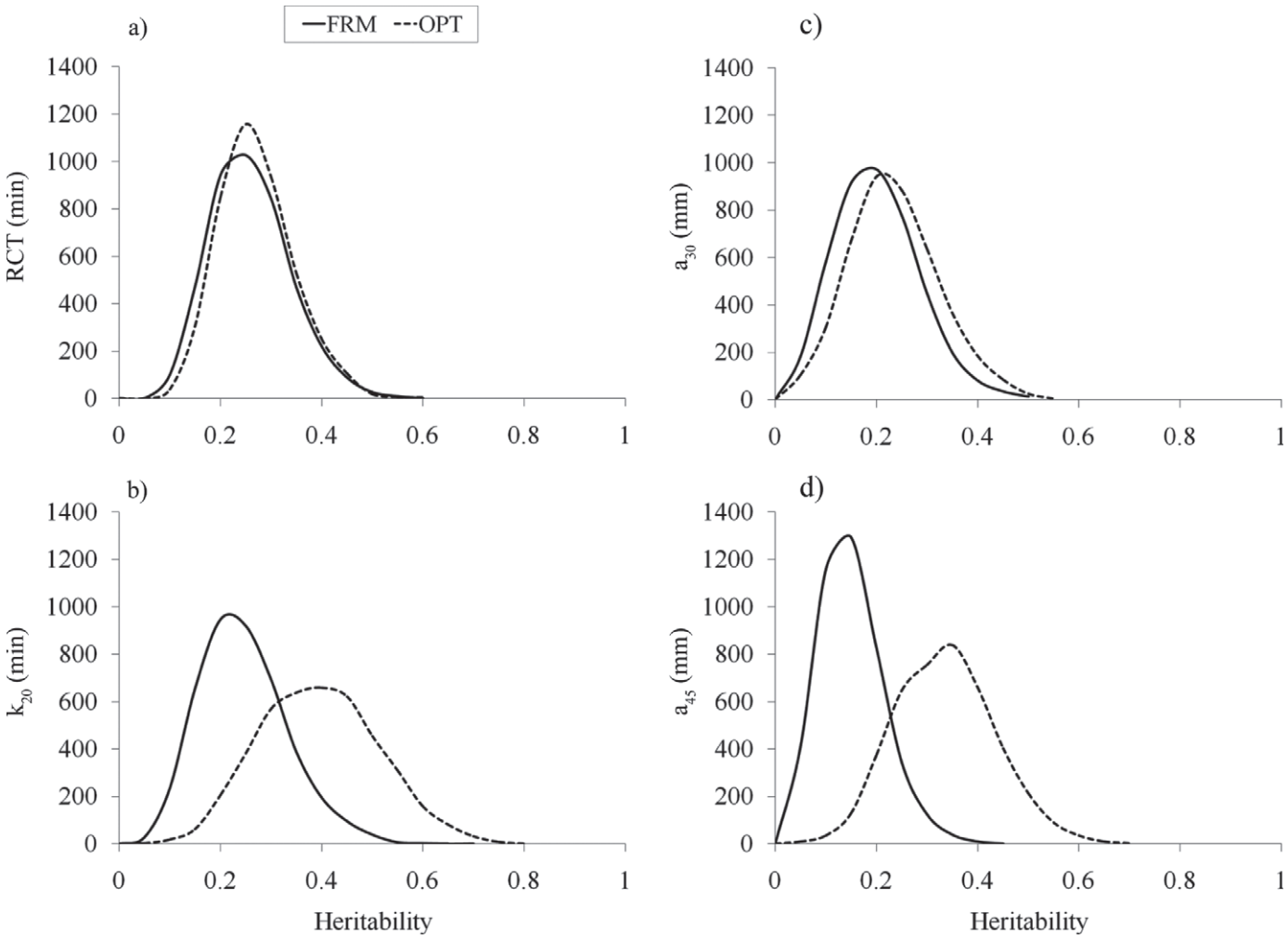

Figure 1. Marginal posterior distributions of the heritability for measures of rennet coagulation time of samples coagulating within 45 min from rennet addition (RCT, min), curd-firming time measured within 45 min from rennet addition (k_{20}, min), curd firmness at 30 min after rennet addition (a_{30}, mm), and curd firmness at 45 min after rennet addition (a_{45}, mm) assessed using Formagraph (FRM; Foss Electric, Hillerød, Denmark) and Optigraph (OPT; Ysebaert, Frépillon, France) instruments.

on measures of RCT determined by the 2 instruments was 0.99. For a_{30} (r = 0.95), a_{45} (r = 0.94), and k_{20} (r = 0.87), reranking was more pronounced.

DISCUSSION

Heritability of RCT Measured over an Extended Testing Period

To our knowledge, this is the first study dealing with estimation of genetic parameters of MCP obtained on a testing period extended to 45 min to avoid NC and to allow calculation of k_{20} on almost all milk samples. Most estimates of genetic parameters for RCT found in the literature have been obtained after discarding NC samples. As the risk of NC milk is higher for cows and progeny characterized by a slow coagulation process, it is clear that both genetic parameters and EBV for RCT can be biased. This risk is particularly high for breeds characterized by slowly coagulating milk, such as Holstein-Friesian and some Scandinavian breeds (Ikonen et al., 1997; Tyrisevä et al., 2004; De Marchi et al., 2007).

To account for the NC samples, Cecchinato and Carnier (2011) compared different statistical models (linear, right-censored linear, survival, and threshold) and concluded that the best approach is to treat NC samples as censored records. They used individual RCT data from 1,025 Holstein-Friesian cows determined through a 30-min testing-period analysis and found that both additive genetic and error variances estimated using a right-censored linear model were approximately twice

Table 3. Deviance information criterion (DIC), mean squared error (MSE), and Bayes factor[1] (BF) for analysis of milk coagulation properties under the model with (M+) and without (M−) additive polygenic effects[2]

	Formagraph		Optigraph	
Item[3]	M+	M−	M+	M−
RCT (min)				
DIC	5,403.7	5,479.5	4,901.5	4,986.3
MSE	13.7	24.4	7.7	14.1
BF	239.8		12,193.2	
k_{20} (min)				
DIC	4,512.5	4,565.3	4,258.1	4,402.4
MSE	5.6	9.1	3.1	7.4
BF	11.9		4.8	
a_{30} (mm)				
DIC	6,796.2	6,841.7	6,670.9	6,730.9
MSE	70.3	102.1	58.7	94.1
BF	15.8		214.6	
a_{45} (mm)				
DIC	6,161.2	6,189.6	6,655.8	6,767.4
MSE	38.64	52.2	49.1	102.7
BF	3.75		494.4	

[1]Bayes factor of the model with additive polygenic effects against the same model without additive polygenic effects following García-Cortés et al. (2001).

[2]Formagraph from Foss Electric (Hillerød, Denmark); Optigraph from Ysebaert (Frépillon, France).

[3]RCT = rennet coagulation time of samples coagulating within 45 min from enzyme addition; k_{20} = curd-firming time of samples reaching 20 mm of firmness within 45 min from enzyme addition; a_{30} = curd firmness at 30 min after enzyme addition; a_{45} = curd firmness at 45 min after enzyme addition.

the values found when ignoring NC samples and using a linear model. Consequently, the heritability estimates were very similar.

Cecchinato et al. (2011) applied a right-censored linear model to RCT data from Brown Swiss cows and compared the results with findings from a previous study that was based on the same data but that ignored NC samples and used a linear instead of a censored-linear model to analyze the records (Cecchinato et al., 2009). The use of the right-censored linear model led to an increase in the additive genetic variance (4.96 to 5.39 min^2) and to a even more pronounced increase of the residual variance, so that heritability estimate decreased from 0.34 (Cecchinato et al., 2009) to 0.24 (Cecchinato et al., 2011).

Milk from Holstein-Friesian is usually characterized by a slower coagulation process than milk from Brown Swiss cows, even if the incidence of NC samples is seldom reported in literature (Malacarne et al., 2005, 2006). It is worth mentioning here that milk protein genetic variants play an important role in explaining the additive genetic variance of MCP (Penasa et al., 2010) and that this effect, and consequently the differences among breeds, depends on the relative frequencies of the genetic variants, especially those relative to κ-casein alleles (Ikonen et al., 1999; Auldist et al., 2002; Bittante et al., 2012). A summary of literature on the effects of genetic variants of milk protein fractions on MCP has recently been reviewed by Bittante et al. (2012), whereas an extensive discussion on the role of κ-casein gene allelic variants on MCP has been reported by Bonfatti et al. (2010).

In the present study, extending the observation period to 45 min allowed us to obtain RCT values for all samples, even if 6.67% of milks coagulated after 30 min from rennet addition. As expected, the estimate of genetic variance of RCT measured by FRM for 45 min (7.09 min^2) was higher than those (4.40–5.48 min^2) obtained from a linear model on different subsamples of the same breed, but determined for 30 min (Cecchinato et al., 2009). Moreover, the estimate of the additive genetic variance was higher than that previously reported by Cecchinato et al. (2011) on the same breed using the right-censored linear model. A possible explanation is that the distribution of RCT of the entire population measured extending the observation period is not perfectly Gaussian, showing a skewness due to a larger-than-expected right tail. As a result, the assumption of normality of the right-censored linear model can lead to underestimation of the contribution of both additive genetic and residual variances induced by slowly coagulating samples. The heritability estimate of RCT

Table 4. Additive genetic (r_g) and phenotypic (r_p) correlations within milk coagulation properties assessed using Formagraph (Foss Electric, Hillerød, Denmark) and Optigraph (Ysebaert, Frépillon, France) instruments[1]

	r_g		r_p	
Trait[2]	Median	HPD95%	Median	HPD95%
RCT (min)	0.974	0.90; 0.99	0.806	0.78; 0.83
k_{20} (min)	0.764	0.31; 0.99	0.518	0.46; 0.57
a_{30} (mm)	0.917	0.61; 0.99	0.731	0.69; 0.76
a_{45} (mm)	0.847	0.45; 0.99	0.426	0.36; 0.49

[1]Median = median of the marginal posterior density of the parameter; HPD95% = lower and upper bounds of the 95% highest posterior density.

[2]RCT = rennet coagulation time of samples coagulating within 45 min from enzyme addition; k_{20} = curd-firming time of samples reaching 20 mm of firmness within 45 min from enzyme addition; a_{30} = curd firmness at 30 min after enzyme addition; a_{45} = curd firmness at 45 min after enzyme addition.

Table 5. Additive genetic (r_g) and phenotypic (r_p) correlations between milk coagulation properties within instrument[1] [Formagraph (Foss Electric, Hillerød, Denmark) or Optigraph (Ysebaert, Frépillon, France)]

	Formagraph				Optigraph			
	r_g		r_p		r_g		r_p	
Trait[2]	Median	HPD95%	Median	HPD95%	Median	HPD95%	Median	HPD95%
RCT with								
k_{20}	0.792	0.43; 0.95	0.675	0.63; 0.71	0.415	−0.06; 0.74	0.416	0.34; 0.47
a_{30}	−0.856	−0.98; −0.64	−0.854	−0.87; −0.83	−0.769	−0.91; −0.50	−0.821	−0.84; −0.79
a_{45}	0.162	−0.41; 0.75	−0.213	−0.29; −0.12	−0.131	−0.53; 0.32	−0.397	−0.46; −0.32
k_{20} with								
a_{30}	−0.979	−0.99; −0.85	−0.847	−0.86; −0.82	−0.953	−0.99; −0.75	−0.757	−0.78; −0.72
a_{45}	−0.284	−0.68; 0.39	−0.583	−0.63; −0.52	−0.966	−0.99; −0.83	−0.825	−0.84; −0.80
a_{30} with								
a_{45}	0.269	−0.62; 0.79	0.476	0.40; 0.53	0.774	0.49; 0.92	0.798	0.76; 0.82

[1]Median = median of the marginal posterior density of the parameter; HPD95% = lower and upper bounds of the 95% highest posterior density.
[2]RCT = rennet coagulation time of samples coagulating within 45 min from enzyme addition; k_{20} = curd-firming time of samples reaching 20 mm of firmness within 45 min from enzyme addition; a_{30} = curd firmness at 30 min after enzyme addition; a_{45} = curd firmness at 45 min after enzyme addition.

determined for 45 min was very similar to the estimate found by Cecchinato et al. (2011) from RCT measured for 30 min and with NC samples treated as censored.

Genetic Parameters of Curd-Firming Time

Curd-firming time is valuable at the industry level because it defines the optimal moment for curd cutting, limiting the fines losses with early cutting and the excess moisture of the curd with late cutting (Janhøj and Qvist, 2010). Only 2 studies estimated heritability for k_{20}: Tervala et al. (1985) reported very low heritability (0.021) using 319 milk samples from Finnish Ayrshire, Finnish Friesian, and Finncattle cows reared in an experimental farm and sampled once, whereas Ikonen et al. (1997) found very high estimates using 174 samples from 59 Finnish Ayrshire (0.540) and 155 samples from 55 Finnish Friesian (0.660) cows, again from an experimental farm.

No estimates are available on field data primarily because not only NC samples but also many slowly coagulating samples do not reach curd firmness of 20 mm within the usual testing time of 30 min, so that large biases can be expected from both genetic parameters and EBV.

Heritability of k_{20} from FRM (0.212; Table 2) was close to the average heritability of the other MCP and was intermediate between findings from Tervala et al. (1985) and Ikonen et al. (1997). Tervala et al. (1985) defined k_{20} differently from our research and from Ikonen et al. (1997). Moreover, the data used by Ikonen et al. (1997) were measured on samples reaching the target value (20 mm) within 30 min from gelification, whereas the time limit was 45 min in the present study. Differences in type and activity of coagulant and in statistical analysis were also found in the studies, making difficult the comparison among them.

No estimates of genetic correlations between k_{20} and other MCP or milk production or composition traits are available in the literature. In the present study, k_{20} showed high positive phenotypic and genetic correlations with RCT and very high negative correlations with a_{30}, confirming that late-coagulating samples are characterized by slow firming rate and low a_{30}. With a genetic correlation of −0.979, k_{20} seems to add no valuable information, from a genetic point of view, beyond that yielded by a_{30}.

Genetic Parameters of Curd Firmness Evaluated 30 Minutes After Rennet Addition

In addition to RCT, a_{30} is also affected by the problem of NC samples. Samples that do not coagulate within 30 min from coagulant addition do not have a curd firmness value over the baseline, which is assumed to be zero. Most published studies report estimates of genetic parameters for a_{30} that were obtained without the inclusion of NC samples. On the contrary, Ikonen et al. (1999) and Tyrisevä et al. (2004) reported estimates of genetic parameters that were obtained with the inclusion of NC samples. Again, Ikonen et al. (2004) faced this problem comparing 2 approaches. The first was based on the inclusion of NC samples attributing to a_{30} a zero value; results showed a higher heritability estimate compared with that found using only coagulated samples (from 0.22 to 0.39, respectively). The second approach treated a_{30} as a binary trait (occurrence of coagulation); heritability (0.26) was higher than that obtained excluding NC samples but much lower than that found including them as zero values.

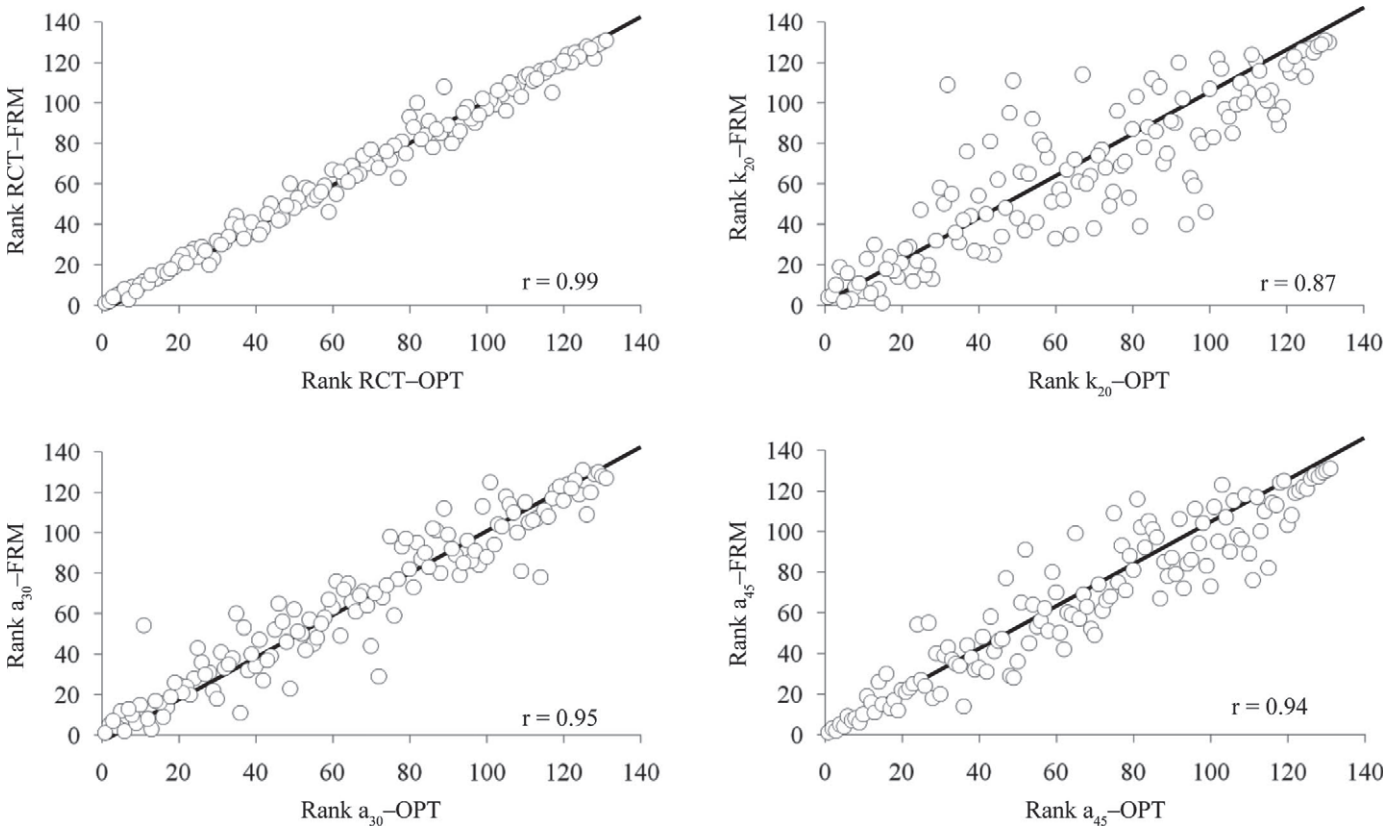

Figure 2. Relationships between sire rankings based on EBV for measures of rennet coagulation time of samples coagulating within 45 min from rennet addition (RCT, min), curd-firming time measured within 45 min from rennet addition (k_{20}, min), curd firmness at 30 min after rennet addition (a_{30}, mm), and curd firmness at 45 min after rennet addition (a_{45}, mm) assessed using Formagraph (FRM; Foss Electric, Hillerød, Denmark) and Optigraph (OPT; Ysebaert, Frépillon, France) instruments.

The inclusion of samples coagulating after 30 min (with a zero value) led to an estimate of additive genetic variance slightly larger than that found in the same breed by Cecchinato et al. (2009, 2011) excluding NC samples, but the estimate of the residual variance was even larger so that heritability estimate was relatively low (0.171), similar to the value found by Cassandro et al. (2008) in Holstein-Friesians but lower than the estimates of previous studies in the same breed (Oloffs et al., 1992; Ikonen et al., 1997) and other northern breeds (Tervala et al., 1985; Oloffs et al., 1992; Tyrisevä et al., 2004).

Moreover, despite the different definition of RCT and the use of the zero a_{30} for NC samples, the present work confirmed the very high genetic and phenotypic correlations between the 2 MCP traits found in most previous studies. It is clear that the longer the RCT, the shorter the time available for curd firming and the smaller the curd firmness measured at a fixed time, thus confirming the poor informative value of a_{30} beyond RCT information and the need for new modeling of the curd-firming process and traits that are less interdependent (Bittante, 2011).

Genetic Parameters of Curd Firmness Evaluated 45 Minutes After Rennet Addition

To overcome the problem of NC samples and define more independent traits, some researchers have extended the interval between enzyme addition and curd firmness measurement to 45 min (Mariani et al., 1997; Cecchi and Leotta, 2002) or 60 min (O'Brien et al., 2002; Auldist et al., 2004), but no estimates of genetic parameters for a_{45} or a_{60} are available in the literature.

The a_{45} measured by the mechanical instrument is more independent than a_{30} from the other MCP (RCT and k_{20}), both at the phenotypic and genetic levels. Moreover, the correlations between curd firmness measured 30 and 45 min after rennet addition are relatively low, at both the genetic (+0.269) and phenotypic (+0.476) levels. This low dependence from other traditional MCP is probably because curd firmness tends to increase after gelification to a maximum value and later tends to decrease due to syneresis. The time at which maximum curd firmness is reached can differ greatly in different samples (Bittante et al., 2012), so that a_{45} can be measured in the ascending or descending phase

of the curd-firmness curve. Consequently, the same a_{45} value can be observed in very late coagulating samples as in very early coagulating samples (characterized by rapid syneresis). Because of these considerations, a_{45} does not seem very useful at the industry level, but more research on this topic is needed.

Heritability of MCP Measured by NIR Lactodynamograph

In previous research (Cipolat-Gotet et al., 2012), MCP determined by mechanical and NIR lactodynamographs on the same samples, in the same laboratory, and by the same technician, have been shown to be different traits, with the partial exception of RCT. Differences were observed in mean values (especially for k_{20}), variability (with the exception of k_{20}), distribution of the data, and correlations with other MCP and with milk yield and composition traits.

The present study highlighted that the optical device yields MCP that are different from the mechanical device, from the genetic point of view (Table 2). It is worth noting that the NIR lactodynamograph does not measure curd firmness; rather, it predicts curd firmness based on an optical signal that is modified by chemical changes that happen mainly during the first phase of coagulation process (before gelification), not during the second phase, when the physical properties (firmness) change dramatically (O'Callaghan et al., 2002). Thus, the ability of OPT to mimic mechanical instruments is expected to decrease after gelation.

Vallas et al. (2010) found heritability of 0.28 for log-transformed RCT and 0.41 for a_{30} in Estonian Holstein-Friesian cows. Comparison with the present study is not very useful because the Estonian study used a microbial enzyme (instead of calf rennet) at a very high activity level. As expected, within 30 min of coagulant addition, Vallas et al. (2010) obtained very short RCT but a_{30} similar to that found in the present research using a much lower enzyme activity. The use of a high concentration of enzyme is not advisable at the industry level because extra enzyme would reduce cheese yield, change the balance between coagulation time and acidification, and increase the production of bitter peptides beyond the capacity of the enzymes of the cheese microflora to degrade them (Law, 2010). This is particularly true for the production of Protected Designation of Origin cheeses, where production processes cannot be altered to address milk defects and, thus, such cheese-makers require (and pay for) milk of high technological quality (Bertoni et al., 2005; Bittante et al., 2011a,b).

It is worth noting that optical measurements have been used without inducing and monitoring rennet coagulation of milk samples, by predicting MCP on the basis of the MIRS spectra of fresh milk samples through a proper calibration with MCP measured with mechanical lactodynamographs (Dal Zotto et al., 2008; De Marchi et al., 2009). Cecchinato et al. (2009), comparing the genetic parameters of MCP measured by a mechanical lactodynamograph with those of MCP predicted by MIRS spectra, found results similar to those obtained from the present study, with a slight increase of heritability for RCT and a more pronounced increase of heritability for a_{30}.

Relationships Between MCP Measured by Mechanical and Optical Lactodynamographs

The genetic correlations between FRM and OPT for the same MCP were much higher than the corresponding phenotypic relationships (Table 4). A similar result was obtained by Cecchinato et al. (2009) for both RCT and a_{30} predicted by MIRS or measured by mechanical lactodynamograph.

The genetic correlation between RCT yielded by FRM and OPT was very high (0.974), as was the rank correlation of EBV of sires, and the genetic correlation is only slightly higher than that found in the same breed by Cecchinato et al. (2009), who compared measured and MIRS-predicted RCT (0.93, average of 4 subsets). We can assume that the use of MIRS prediction on fresh noncoagulated milk samples is almost as efficient as, but much less expensive and time consuming than, NIR lactodynamograph estimates in a breeding program aimed at improving RCT as an alternative to the use of traditional mechanical lactodynamographs. The genetic correlation between a_{30} from FRM and OPT (0.917) was lower than the corresponding value exhibited by RCT on the same instrument (0.974), but much higher than the genetic correlation between measured and MIRS predicted a_{30} obtained by Cecchinato et al. (2009). The genetic correlations between k_{20} and a_{45} from FRM and OPT are lower than for RCT and a_{30} (Table 4), but no studies are currently available in literature for comparison.

The genetic correlation between RCT and a_{30} yielded by OPT was slightly lower than that obtained by FRM (-0.769 vs. -0.856, respectively), but indeed very high and much different from the genetic correlation (-0.160) estimated by Vallas et al. (2010) comparing log-transformed RCT and a_{30} obtained by OPT. No literature comparisons are possible for the other MCP.

CONCLUSIONS

Extending the standard 30-min testing time to 45 min allowed us to measure RCT of all milk samples

and k_{20} for most late-coagulating milks, avoiding NC records. The use of all RCT data (included those larger than 30 min) led to higher additive genetic and residual variances compared with those found in literature, but the heritability remained almost unchanged. For FRM, heritability of k_{20} was similar to that of RCT, but the genetic correlations with both RCT and a_{30} were very high, so that the value of k_{20} for breeding purposes, beyond RCT, is questionable. The relevance of a_{30} is also questionable because of the high genetic correlation with RCT. Genetic parameters for a_{45} have been estimated for the first time; this trait exhibited a lower correlation coefficient with RCT than a_{30}, but compared with a_{30} it was characterized by lower heritability (only for FRM). The MCP estimated by OPT appeared to be different traits from those measured by FRM with the exception of RCT. Breeding strategies for the enhancement of MCP must be planned with caution. Presently, the high throughput, ease of use, and reduced costs of analysis make predictions obtained from MIRS on untreated milk samples a promising alternative for the generation of relevant data at the population level. The use of mechanical lactodynamographs to establish the reference data for MIRS calibrations have been already studied (De Marchi et al., 2009), whereas the use of NIR optical lactodynamographs as reference method for MIRS calibrations needs to be investigated.

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