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Genetic Parameters of Milk Coagulation Properties and Their Relationships with Milk Yield and Quality Traits in Italian Holstein Cows

M. Cassandro,*[1] A. Comin,* M. Ojala,† R. Dal Zotto,* M. De Marchi,* L. Gallo,* P. Carnier,* and G. Bittante*
*Department of Animal Science, University of Padova, 35020 Legnaro (PD), Italy
†Department of Animal Science, University of Helsinki, PO Box 28, 00014 Helsinki University, Finland

ABSTRACT

Milk coagulation properties (MCP) are an important aspect in assessing cheese-making ability. Several studies showed that favorable conditions of milk reactivity with rennet, curd formation rate, and curd strength, as well as curd syneresis, have a positive effect on the entire cheese-making process and subsequently on the ripening of cheese. Moreover, MCP were found to be heritable, but little scientific literature is available about their genetic aspects. The aims of this study were to estimate heritability of MCP and genetic correlations among MCP and milk production and quality traits. A total of 1,071 Italian Holstein cows (progeny of 54 sires) reared in 34 herds in Northern Italy were sampled from January to July 2004. Individual milk samples were collected during the morning milking and analyzed for coagulation time (RCT), curd firmness (a_{30}), pH, titratable acidity, fat, protein, and casein contents, and somatic cell count. About 10% of individual milk samples did not coagulate in 31 min, so they were removed from the analyses. Estimates of heritability for RCT and a_{30} were 0.25 ± 0.04 and 0.15 ± 0.03, respectively. Estimates of genetic correlations between MCP traits and milk production traits were negligible except for a_{30} with protein and casein contents (0.44 ± 0.10 and 0.53 ± 0.09, respectively). Estimates of genetic correlations between MCP traits and somatic cell score were strong and favorable, as well as those between MCP and pH and titratable acidity. Selecting for high casein content, milk acidity, and low somatic cell count might be an indirect way to improve MCP without reducing milk yield and quality traits.
Key words: heritability, genetic correlation, milk coagulation property, Holstein dairy cow

INTRODUCTION

Milk coagulation properties (**MCP**) are an important aspect in cheese-making production, especially in those countries where dairy industry is based on traditional products and is market-oriented (Annibaldi et al., 1977; Aleandri et al., 1989; Cassandro, 2003). In Italy, more than 70% of the overall milk production (ISTAT, 2005) is used in the manufacture of cheese, and 55% of the total milk production is processed for PDO (Protected Designation of Origin) cheeses like Parmigiano-Reggiano, Grana Padano, Provolone, Asiago, Gorgonzola, and Mozzarella.

The evaluation of cheese-making ability can be performed using 2 milk coagulation traits as coagulation time (**RCT**, min) and curd firmness ($\mathbf{a_{30}}$, mm), determined by a coagulometer (Annibaldi et al., 1977; Zannoni and Annibaldi, 1981; MacMahon and Brown, 1982). Indeed, favorable conditions of milk reactivity with rennet, curd formation rate, and curd strength, as well as curd syneresis have a positive effect on the entire cheese-making process and subsequently on the ripening of cheese (Mariani and Battistotti, 1999). A few studies have estimated the genetic parameters for those traits, showing that genetic improvement of MCP could be one way to improve cheese yield (Ikonen, 2000; Ojala et al., 2005). Estimates of heritability for MCP traits were about 0.30 to 0.40 (Ikonen et al., 1999; Bittante et al., 2002) and estimates of repeatability ranged from 0.53 to 0.68 (Schaar, 1984; Caroli et al., 1990; Tyrisevä et al., 2003), suggesting that only few MCP measurements per cow and lactation would be sufficient for a reliable genetic evaluation. However, the lack of suitable equipment for routine determination of MCP has restricted the possibility of implementing MCP in a milk recording system for genetic evaluation of animals. Alternatively, indirect selection for some traits strongly associated with MCP could be considered. Concerning a phenotypic point of view, milk with a medium-to-high casein content, good colloidal calcium phosphate content, the correct degree of titratable acidity (**TA**), moderate SCC, and an adequate fat-to-casein ratio was shown to be ideal for cheese-making (Mariani and Battistotti, 1999). From a genetic point of view, several studies have reported a positive genetic correlation among MCP and low pH and SCS, and high casein and protein content (Lindström et al., 1984; Oloffs et al.,

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[1]Corresponding author: martino.cassandro@unipd.it

1992; Ikonen et al., 1999, 2004). Unfortunately, the literature concerning genetic correlations among MCP and other traits measured in routine milk recording systems (test-day milk yield, fat and protein contents, and SCC), and other quality traits such as TA are scarce; moreover, it is difficult to utilize these values in the Italian dairy sector because of diversity in cattle breeds and management conditions. In the last few years the selection program for Italian Holstein-Friesians has focused on production traits such as milk yield (**MY**) and composition (ANAFI, 2006) causing a deterioration of MCP, as reported by some authors (Mariani et al., 1992; Sandri et al., 2001).

The aim of this study was to estimate genetic parameters of MCP and milk production and quality traits in the Italian Holstein-Friesian cattle breed.

MATERIALS AND METHODS

Data Collection

A total of 1,071 Holstein-Friesian cows were sampled from January to July 2004. Cows were the offspring of 54 AI sires and were reared in 34 herds located in the Northern Italy. Individual milk samples were collected during the morning milking of a test day. After collection, milk samples without any preservative were stored in portable refrigerators (4°C), transferred to the milk quality lab of Veneto Agricoltura Institute (Thiene, Italy) and analyzed within 3 h after collection. Measures of MCP were obtained using a computerized renneting meter (Polo Trade, Monselice, Italy). Milk samples (10 mL) were heated to 35°C, and 200 μL of rennet (Hansen standard 190 with 63% of chymosin and 37% of pepsin, Pacovis Amrein AG, Bern, Switzerland) diluted 1.6% in distilled water, was added to milk. Measurement of MCP ended within 31 min after the addition of the clotting enzyme. This analysis provided measures of 2 milk coagulation traits: RCT, the time between the addition of the clotting enzyme and the beginning of the coagulation process, and a_{30}, measured 31 min after addition of the clotting enzyme, because the curd is usually cut 30 min after the addition of the clotting enzyme to the milk. Milk samples that did not coagulate within 31 min were classified as not coagulating (**NC**) following the approach of Ikonen et al. (1999).

In the same laboratory, fat and protein contents (Combi Foss 6000 FC, Foss Electric A/S, Hillerød, Denmark), casein content (Cell Fossomatic 250, Foss Electric A/S), pH, TA expressed in Soxhlet-Henkel degrees (Crison Compact D, Crison Instruments SA, Alella, Spain), and SCC (Cell Fossomatic 250) were determined. Values of SCC were converted by logarithm transformation to SCS [$SCS = 3 + \log_2(SCC/100{,}000)$]. Information on cows and herds were provided by Provincial Breeders Associations of Veneto. Pedigree information was supplied by the Italian Holstein-Friesian Cattle Breeders Association (ANAFI, Cremona, Italy) and included all known ancestors of sampled cows.

Statistical Analyses

Milk samples that did not coagulate within 31 min (n = 102 records; 9.7% of the total) were excluded from the statistical analyses because, during cheese-making, curd is usually cut 30 min after the addition of rennet to the milk. It is expected that some of these samples would have coagulated after 31 min, but in this study the analysis was stopped after 31 min. Data editing aimed to discard records with sampling or recording errors, such as protein and fat contents beyond the range of mean ± 4 standard deviation units. Seven records were discarded for missing information about parity. At the end of the editing process 1,042 records remained. (Co)variance components for MCP, daily MY and quality (contents of fat, protein, and casein, SCS, pH, and TA) traits were estimated by a 7-trait animal model using the program VCE 4.0 (version 4.2.5, Groeneveld, 1998). For all traits, the following linear model was used:

$$y_{ijklm} = \mu + Herd_i + DIM_j + Parity_k + anim_l + \varepsilon_{ijklm}$$

where y_{ijklm} is a measure of a trait (e.g., daily MY, milk quality, and MCP traits); μ is the general mean of the model; $Herd_i$ (i = 1, ..., 34) is the fixed effect of herd; DIM_j (j = 1, ..., 14) is the fixed effect of DIM; $Parity_k$ (k = 1, 2, 3) is the fixed effect of parity; $anim_l$ is the random additive genetic effect of an animal l, $N(\mathbf{0}, \mathbf{A}\sigma^2_a)$; and ε_{ijklm} is a random residual effect, $N(0, \mathbf{I}\sigma^2_\varepsilon)$. Animal and residual effects were assumed to be independent. The 1,042 cows with records were daughters of 54 sires with an average number of daughters per sire of 19 and a range from 3 to 87. The pedigree information consisted of at least 3 generations for each cow with a record, and the total number of animals in the statistical analyses was 7,387.

In the model, the herd and test-day effects were confounded because cows in each herd were sampled only once, all on the same test day. Days in milk of each cow were grouped into 10 monthly classes from 5 to 305 d after calving, 3 bimonthly classes from 306 to 486 d, and 1 class for records collected after 486 d. Parity was classified into 3 classes for first, second, and third to seventh calving.

RESULTS AND DISCUSSION

Table 1 shows the descriptive statistics of MCP, milk production, and quality traits. Fat, protein, and casein

Table 1. Descriptive statistics for milk coagulation, production, and quality traits

Trait[1]	n	Mean	CV, %	Minimum	Maximum
RCT, min	960	16.9	27	7.2	29.4
a_{30}, mm	960	32.0	35	4.0	60.0
MY, kg/d	1,042	32.3	32	6.0	65.7
Fat, %	1,030	3.89	20	1.56	6.83
Protein, %	1,035	3.45	12	2.43	5.50
Casein, %	1,037	2.65	11	1.64	4.10
SCS[2]	1,038	3.08	63	–1.64	9.11
pH	1,034	6.67	2	6.33	7.18
TA, SH°/50 mL	1,034	3.26	13	1.55	4.70

[1]RCT = milk coagulation time; a_{30} = curd firmness; MY = milk yield; TA = titratable acidity (Soxhlet Henkel °/50 mL).

[2]SCS = [3 + $\log_2$ (SCC/100,000)].

contents were in agreement with the advanced lactation stage of the cows (227 d). Milk yield averaged 32.3 kg/d, which is comparable to the average yield of Holstein cows registered in the National Herdbook (ANAFI, 2006). Somatic cell score was the trait with the greatest coefficient of variation (63%), whereas pH had the lowest coefficient of variation (2%). Variation of TA was greater than for pH; however, acidity traits are not yet available in routine milk recording systems.

In total, 9.7% of samples were NC, which was in accordance with the literature, where the presence of NC samples varied in relation to cattle breeds: 13.2% (Ikonen et al., 2004) and 8.6% (Tyrisevä et al., 2004) in Finnish Ayrshire, 1.3% in the Finnish Holstein (Tyrisevä et al., 2004), and 7.5% in the Finnish Ayrshire (Ikonen et al., 1999).

The average RCT value was 16.9 min, which is close to the optimal value recommended by the renneting classification of milk proposed by Zannoni and Annibaldi (1981). However, RCT was variable (coefficient of variation = 27%) with respect to RCT values of other studies, even though comparison among studies was difficult because measurements were based on different breeds or on bulk milk instead of individual samples. Chiofalo et al. (2000) reported average RCT values of 15.4 and 10.5 min in Italian Holstein and Modicana (a local cattle breed of Sicilia region) individual milk samples, respectively. Ikonen et al. (1999) obtained an average RCT of 12.3 min (CV = 41%) for milk produced by Finnish Ayrshire and Finnish Friesian cows; these values were consistent with those reported by Tyrisevä et al. (2004) for the same breeds and by Ikonen et al. (2004) in a study of Ayrshire cows. The considerable difference between Finnish dairy cows and Italian Holstein cows in relation to RCT might be attributed to specific features of these cattle populations as well as to different herd management and feeding conditions.

The average a_{30} value was 32 mm, which is considered a low value for the milk renneting classification proposed by Zannoni and Annibaldi (1981). However, mean a_{30} values reported for Finnish dairy cattle were even lower than this and ranged from 25 to 27 mm (Ikonen et al., 1999, 2004; Tyrisevä et al., 2004).

Additive Genetic Variation and Heritability of Traits

Table 2 shows estimates of additive genetic standard deviations and heritability of the analyzed traits. The additive genetic standard deviation for MCP traits was moderately high, ranging from 12.7 to 13.1% of the phenotypic mean of the trait for a_{30} and RCT, respectively. This variation was greater than for other traits, which ranged from 0.75 (pH) to 11% (fat content) of the phenotypic mean of the trait. The high genetic variance estimated for MCP supports possible genetic improvement of milk coagulation ability in dairy cattle. Such a plan would require the development of alternative instruments for the measurement of RCT and a30 to avoid the limitation of number of analyses per day (almost 100) of the coagulometer used in this study. A promising alternative technology might be the use of infrared spectrometry, as reported by Laporte et al. (1998), Barbano and Lynch (2006), and Fagan et al. (2007).

Estimates of heritability ($h^2 \pm$ SE) obtained in this study for daily MY (0.09 ± 0.03), fat content (0.39 ±

Table 2. Estimates of additive genetic standard deviation (σ_a) and heritability (h^2) for milk coagulation, production, and quality traits

Trait[1]	σ_a	$h^2 \pm$ SE
RCT, min	2.22	0.25 ± 0.04
a_{30}, mm	4.06	0.15 ± 0.03
MY, kg/d	1.95	0.09 ± 0.03
Fat, %	0.42	0.39 ± 0.04
Protein, %	0.17	0.30 ± 0.03
Casein, %	0.15	0.35 ± 0.03
SCS	0.47	0.07 ± 0.02
pH	0.05	0.21 ± 0.04
TA, SH°/50 mL	0.15	0.17 ± 0.03

[1]RCT = milk coagulation time; a_{30} = curd firmness; MY = milk yield; TA = titratable acidity (Soxhlet Henkel °/50 mL).

Table 3. Phenotypic correlations (above diagonal) and genetic correlations with SE (below diagonal) among milk coagulation, production, and quality traits

Trait[1]	RCT	a_{30}	MY	Fat	Protein	Casein	SCS	pH	TA
RCT, min	—	–0.76	–0.11	–0.11	–0.07	–0.19	0.17	0.52	–0.43
a_{30}, mm	–0.89 ± 0.04	—	0.03	0.16	0.23	0.32	–0.14	–0.46	0.41
MY, kg/d	–0.24 ± 0.12	0.22 ± 0.12	—	–0.21	–0.36	–0.28	–0.22	–0.14	0.02
Fat, %	–0.05 ± 0.09	0.14 ± 0.10	–0.73 ± 0.06	—	0.45	0.49	0.11	–0.13	0.25
Protein, %	–0.08 ± 0.08	0.44 ± 0.10	–0.40 ± 0.11	0.70 ± 0.07	—	0.95	0.06	–0.11	0.41
Casein, %	–0.22 ± 0.09	0.53 ± 0.09	–0.44 ± 0.10	0.77 ± 0.06	0.98 ± 0.01	—	0.00	–0.22	0.50
SCS	0.25 ± 0.18	–0.40 ± 0.18	–0.30 ± 0.20	0.32 ± 0.12	0.04 ± 0.15	–0.03 ± 0.14	—	0.18	–0.13
pH	0.81 ± 0.07	–0.85 ± 0.07	–0.19 ± 0.11	–0.16 ± 0.09	–0.24 ± 0.08	–0.36 ± 0.08	0.44 ± 0.19	—	–0.70
TA	–0.50 ± 0.08	0.66 ± 0.09	0.19 ± 0.10	0.49 ± 0.09	0.58 ± 0.08	0.65 ± 0.07	–0.08 ± 0.19	–0.68 ± 0.07	—

[1]RCT = milk coagulation time; a_{30} = curd firmness; MY = milk yield; TA = titratable acidity (Soxhlet Henkel °/50 mL).

0.04), protein content (0.30 ± 0.03), and SCS (0.07 ± 0.02) were less than those reported for the Italian Holstein population (Samorè et al., 2002). This difference can be partially attributed to the availability, in the current study, of only a single test-day record per cow instead of multiple records per lactation.

The heritability estimates (h^2 ± SE) were moderate for pH (0.21 ± 0.04) and TA (0.17 ± 0.03), and high for casein content (0.35 ± 0.03), consistent with observations by Ikonen et al. (2004).

Estimates of heritability (h^2 ± SE) for MCP traits were intermediate (RCT: 0.25 ± 0.04) or moderate (a_{30}: 0.15 ± 0.03), but greater than the estimate for MY (0.09 ± 0.03). The estimates of heritability for MCP traits were larger than those obtained for traits that are already included in the current breeding goal for the Italian Holstein cattle population and offer an opportunity of being exploited in selection programs aiming at MCP enhancement.

The estimate of heritability for RCT was in agreement with those reported by other studies in Ayrshire and Holstein-Friesian populations (Ikonen et al., 1999; Tyrisevä et al., 2004), but lower than estimates reported for Ayrshire (Lindström et al., 1984; Ikonen et al., 2004) and Angler cows (Oloffs et al., 1992). The estimated heritability of a_{30} obtained in this study was lower than other estimates available in the literature. Oloffs et al. (1992) reported estimates of heritability for a_{30} ranging from 0.30 (Holstein) to 0.39 (Angler). Ikonen et al. (1999) estimated a heritability value of 0.40 for a_{30} from a sample of Finnish Ayrshire and Holstein-Friesian, whereas in a study on Ayrshire cows, Ikonen et al. (2004) obtained estimates of heritability for a_{30} ranging from 0.22 to 0.39. An estimate of heritability of 0.22 for a_{30} was obtained by Tyrisevä et al. (2004) from a sample of Finnish Ayrshire and Holstein-Friesian cows.

Phenotypic Correlations

Table 3 shows the phenotypic correlations among MCP, MY, and quality traits. The RCT and a_{30} traits had a negative and strong phenotypic correlation (–0.76), as reported by Lindström et al. (1984) and Ikonen et al. (2004), confirming that when RCT decreases, a_{30} increases. Favorable MCP were associated with low pH (0.52 and –0.43 with RCT and a_{30}, respectively) and high acidity (–0.46 and 0.41 with RCT and a_{30}, respectively). This type of association was also reported by Ikonen et al. (2004) for pH trait. As reported by Lindström et al. (1984), RCT and protein content were not correlated (–0.07), but high a_{30} values were associated with high protein and casein content (0.23 and 0.32, respectively). The phenotypic correlations of MCP with the other milk traits were small.

Genetic Correlations

Table 3 also shows the genetic correlations of MCP and other milk production and quality traits. As expected, RCT and a_{30} were highly correlated because coagulation and firming are consecutive steps of the same process. If milk takes a short time to coagulate, it leaves more time for curd firming and obviously has better coagulation ability in general; thus, the final curd will be firmer. Conversely, if milk takes a long time to coagulate, the curd will have less time to firm and it will be weaker.

The genetic correlations between MCP and MY were of moderate magnitude (–0.24 and 0.22 for RCT and a_{30}, respectively) and associated with large standard errors (±0.12). This is consistent with results of previous studies in which weak genetic correlations among MCP and MY were estimated for Finnish Ayrshire (Ikonen et al., 1999, 2004) and Friesian cows (Oloffs et al., 1992). Genetic correlations between RCT and fat or protein content were almost null (–0.05 or –0.08, respectively). Conversely, genetic correlations between a_{30} and protein and casein content were moderate to high (0.44 and 0.53, respectively). Literature estimates for genetic correlations between MCP and protein or casein content are not consistent. In several studies, short RCT was genetically associated with high protein content (Linds-

tröm et al., 1984; Ikonen et al., 2004), but in other studies RCT correlated positively (Oloffs et al., 1992; Ikonen et al., 1999) or did not correlate at all with milk protein content (Oloffs et al., 1992). Likewise, genetic correlations between a_{30} and protein or casein contents were found to be positive by Oloffs et al. (1992), negative by Ikonen at al. (1999), and null in a successive study by Ikonen et al. (2004). Several factors might explain these inconsistencies, such as sample size, the investigated breeds, models and methods of estimations, and variation across laboratories.

An interesting genetic relationship was detected between MCP and SCS (0.25 and –0.40 with RCT and a_{30}, respectively) although standard errors (±0.18) were large. These results were in agreement with findings by Ikonen et al. (2004), who concluded that one way to genetically improve the MCP and to reduce the occurrence of noncoagulating milk could be selection for low SCC.

In this study, desirable MCP (i.e., short coagulation time and high curd firmness) were markedly associated with acidity of milk, measured both as pH and TA, and these relationships were in agreement with results from previous studies (Lindström et al., 1984; Oloffs et al., 1992; Ikonen et al., 1999, 2004). Because pH and TA can be measured more easily than MCP, enhancement of MCP could be achieved through indirect selection based on these indicator traits.

Genetic correlations among the production and quality traits showed values in agreement with the extensive literature available for these traits (Samorè et al., 2002; Ikonen et al., 2004; Ojala et al., 2005; Interbull, 2007).

CONCLUSIONS

Cheese is recognized as one of the major agro-feeding products worldwide. A favorable step to improve the dairy cattle population would be the selection of animals for improved MCP. Because of the moderate heritability of MCP and small genetic correlations with milk production traits, it should be possible to improve MCP with negligible correlated effects on MY. Adding another trait to the selection objective would, however, decrease the response of the existing traits in the index. However, because of the lack of suitable instruments to measure MCP in milk recording systems, it is currently difficult to select for MCP in the whole population. Hence, strategies that might be used to select for MCP traits are 1) recording MCP directly, as done in this study, only in a random sample of available daughters per sire; 2) recording MCP directly, which would require the use of devices more efficient than those previously or currently available; 3) recording MCP indirectly using genetically correlated traits such as protein and casein contents, acidity, and SCC. Moreover, a combination of the abovementioned strategies might be adopted even if not all indirect MCP traits are currently available in routine milk recording systems (e.g., acidity traits).

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