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Selection for milk coagulation properties predicted by Fourier transform infrared spectroscopy in the Italian Holstein-Friesian breed

S. Chessa,*[1] O. Bulgari,† R. Rizzi,‡ L. Calamari,§ P. Bani,§ S. Biffani,* and A. M. Caroli†
*Institute of Agricultural Biology and Biotechnology, National Research Council (CNR), via Einstein, 26900 Lodi, Italy
†Dipartimento di Medicina Molecolare e Traslazionale, Università degli Studi di Brescia, 25121 Brescia, Italy
‡Dipartimento di Scienze Veterinarie e Sanità Pubblica, Università degli Studi di Milano, 20133 Milano, Italy
§Istituto di Zootecnica, Università Cattolica del Sacro Cuore, 29122 Piacenza, Italy

ABSTRACT

Milk coagulation is based on a series of physicochemical changes at the casein micelle level, resulting in formation of a gel. Milk coagulation properties (MCP) are relevant for cheese quality and yield, important factors for the dairy industry. They are also evaluated in herd bulk milk to reward or penalize producers of Protected Designation of Origin cheeses. The economic importance of improving MCP justifies the need to account for this trait in the selection process. A pilot study was carried out to determine the feasibility of including MCP in the selection schemes of the Italian Holstein. The MCP were predicted in 1,055 individual milk samples collected in 16 herds (66 ± 24 cows per herd) located in Brescia province (northeastern Italy) by means of Fourier transform infrared (FTIR) spectroscopy. The coefficient of determination of prediction models indicated moderate predictions for milk rennet coagulation time (RCT = 0.65) and curd firmness (a_{30} = 0.68), and poor predictions for curd-firming time (k_{20} = 0.49), whereas the range error ratio (8.9, 6.9, and 9.5 for RCT, k_{20}, and a_{30}, respectively) indicated good practical utility of the predictive models for all parameters. Milk proteins were genotyped and casein haplotypes (α_{S1}-, β-, α_{S2}-, and κ-casein) were reconstructed. Data from 51 half-sib families (19.9 ± 16.4 daughters per sire) were analyzed by an animal model to estimate (1) the genetic parameters of predicted RCT, k_{20}, and a_{30}; (2) the breeding values for these predicted clotting variables; and (3) the effect of milk protein genotypes and casein haplotypes on predicted MCP (pMCP). This is the first study to estimate both genetic parameters and breeding values of pMCP, together with the effects of milk protein genotypes and casein haplotypes, that also considered k_{20}, probably the most important parameter for the dairy industry (because it indicates the time for the beginning of curd-cutting). Heritability of predicted RCT (0.26) and k_{20} (0.31) were close to the average heritability described in literature, whereas the heritability of a_{30} was higher (0.52 vs. 0.27). The effects of milk proteins were statistically significant and similar to those obtained on measured MCP. In particular, haplotypes including uncommon variants showed positive (*B-I-A-B*) or negative (*B-A^1-A-E*) effects. Based on these findings, FTIR spectroscopy-pMCP is proposed as a potential selection criterion for the Italian Holstein.
Key words: milk coagulation properties, Fourier transform infrared spectroscopy, prediction

INTRODUCTION

The Italian dairy market system is characterized by transformation of milk into cheese; 75% of milk is processed for cheese production. In the rest of Europe and in North America, about 50% of milk is used to produce cheese (International Dairy Federation, 2011). The European cheese market is growing, although at a declining rate, and an annual growth of 0.6% is expected between 2011 and 2015. In the United States, per capita consumption of natural cheese increased by 0.16 kg in 2011, and the largest consumption increase was for Italian-type cheeses (Rabobank, 2011). Cheese making requires the coagulation of milk, a process based on a series of physicochemical changes at the level of the casein micelle, resulting in formation of a gel. The most useful strategy to test the cheese-making properties of milk is to analyze milk coagulation properties (**MCP**). The MCP of herd bulk milk are periodically analyzed and used to reward or penalize producers of Protected Designation of Origin (PDO) cheeses (Calamari et al., 2005; Bittante et al., 2011). The economic importance of improving MCP fully justifies the need to account for this trait in the selection process.

The most common approach to determine MCP is lactodynamography (reviewed by Bittante et al., 2012), where MCP are measured by mechanical equipment, such as the Formagraph (Foss Electric, Hillerød, Den-

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[1]Corresponding author: chessa@ibba.cnr.it

mark) or computerized renneting meter (CRM; Polo Trade, Monselice, Italy), or with an optical instrument (Optigraph; Ysebaert, Frépillon, France). Formagraph analysis, the most frequently used method, consists of registering the behavior of small-loop pendula immersed in linearly oscillating cuvettes containing rennet-added milk maintained at a fixed temperature. Before gel formation, the pendula do not move, but as gel formation begins, the increase in viscosity causes synchronous motion of the pendula. The registration of such movements is translated into 2 outputs: a typical fork-shaped diagram (McMahon and Brown, 1982) and the measures of MCP parameters. The Optigraph, instead, uses a single near-infrared reflectance wavelength to monitor coagulation (Kübarsepp et al., 2005). This device estimates MCP by analyzing optical information acquired in real time and transforming it to simulate traditional parameters.

The 3 MCP parameters usually registered are (1) rennet coagulation time (**RCT**, min)—the interval from the addition of rennet to milk to the time at which the pendula start oscillating; (2) the time from RCT to a curd firmness of 20 mm ($\mathbf{k_{20}}$, min)—the interval from the start of gel formation to the time at which the width between the 2 arms of the fork-shaped diagram becomes 20 mm; and (3) the curd firmness at 30 min after enzyme addition ($\mathbf{a_{30}}$, mm)—measured as the distance between the 2 arms of the fork-shaped diagram at that time. Different researchers (Kübarsepp et al., 2005; Pretto et al., 2011; Cipolat-Gotet et al., 2012; Cecchinato et al., 2013) have analyzed MCP using both mechanical and optical instruments, and found high correlations between the measures for RCT (0.81 phenotypic and 0.97 genetic) and a_{30} (0.73 phenotypic and 0.92 genetic; Cecchinato et al., 2013).

Optical technologies can also be used to predict MCP directly from milk without inducing coagulation. Mid-infrared reflectance spectroscopy (**MIRS**) was used to indirectly predict the MCP of milk using appropriate calibration algorithms (Dal Zotto et al., 2008) and a mathematical pretreatment of spectra (De Marchi et al., 2009). Cecchinato et al. (2009) found moderate phenotypic correlations between measured and predicted MCP (**pMCP**; 67 and 51% for RCT and a_{30}, respectively) but high genetic correlations (93% for RCT and 77% for a_{30}, respectively). Thus, because MCP measures can be predicted by analyzing the spectra obtained from current milk recording for milk, fat, and protein analyses without further measurements (Barbano and Lynch, 2006), MIRS could be routinely used to achieve genetic improvements in cheese-making properties of milk. Moreover, when better calibration and prediction models are obtained, spectra could easily be reanalyzed. For this reason, we carried out a pilot study to evaluate the possibility of using MCP predicted by means of Fourier transform infrared (**FTIR**) spectroscopy as selection criteria for the Italian Holstein breed. Additionally, we evaluated the effects of β-lactoglobulin (***LGB***) A and B variants and of casein haplotypes on the clotting properties of milk.

MATERIALS AND METHODS

Samples, Data Collection, and Genotyping

Milk coagulation properties were evaluated in 1,055 individual milk samples collected in 16 herds (66 ± 24 cows per herd) located in Brescia province (northeastern Italy) from May 16 to June 15, 2012. Because milk tends to have better, but more variable, MCP at the beginning and the end of lactation (Cipolat-Gotet et al., 2012), only cows within 90 and 300 DIM were sampled to reduce the variability. Milk samples were immediately refrigerated at 4°C without preservative and transported to the Agrifood Laboratory of the University of Brescia (Brescia, Italy), where they were immediately subdivided into 2 aliquots: 0.02% sodium azide (NaN_3) was added to one aliquot for milk protein genotyping before freezing and the second aliquot was deep-frozen without preservative and sent to the Laboratory of the Istituto di Zootecnica of the Università Cattolica del Sacro Cuore (Piacenza, Italy) for prediction of MCP by FTIR spectroscopy. A subsample of 1,017 cows from 51 half-sib families (19.9 ± 16.4 cows per sire), including only families with at least 5 daughters per bulls, was then selected for the following analyses. Families included 36 sires with at least 10 daughters, of which 19 sires had at least 20 daughters.

Milk proteins were genotyped by isoelectric focusing as described by Rignanese et al. (2009); the β-CN *I* allele was distinguished from the A^2 allele by bidirectional allele specific-PCR (Chessa et al., 2013).

Pedigree information together with data on cows and herds were supplied by the Italian Holstein-Friesian Cattle Breeders Association (ANAFI, Cremona, Italy) and the Italian Breeders Association (AIA, Roma, Italy).

Analysis of Milk Quality and MCP

Mid-infrared spectra (in the range 5,012–926 cm^{-1}) from individual milk subsamples (2 replicates of each sample) were obtained using a MilkoScan FT 120 (Foss Electric, Hillerød, Denmark), and MCP (RCT, k_{20}, and a_{30}) were predicted by using internal calibration curves developed by the Istituto di Zootecnica of the Università Cattolica del Sacro Cuore (Piacenza). The predic-