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Phenotypic and genetic associations of milk traits with milk coagulation properties

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ABSTRACT

The aim of this study was to examine milk composition and rennet-induced coagulation properties of milk from 892 individual Danish Holstein and Danish Jersey cows and determine the genetic influences on these properties by determining heritability and genomic correlations with single nucleotide polymorphisms identified by the bovine HD Beadchip (Illumina Inc., San Diego, CA). Despite no signs of clinical mastitis, milk from cows with somatic cell counts >500,000 cells/mL showed altered milk composition, indicating impaired barrier between the milk and the blood. Curd-firming rate (CFR) and rennet coagulation time (RCT) were used to describe milk coagulation properties (MCP). These traits describe the second phase of milk coagulation and were mutually negatively correlated, but only to some extent associated with the same compositional traits. In both breeds, CFR were highly correlated with protein content, whereas longer RCT were primarily associated with lower milk pH. Estimated heritabilities for milk production and compositional traits ranged from 0.09 for yield to 0.82 for citric acid in Danish Jersey cows, and from 0.21 for yield to 0.59 for citric acid in Danish Holstein cows. Heritabilities for MCP traits varied considerably between breeds, and were estimated to be 0.28 for RCT and 0.75 for CFR in Danish Holstein cows and 0.45 for RCT and 0.15 for CFR in Danish Jersey cows. This difference was further reflected in the genomic correlations between RCT and CFR which was −0.90 in Danish Holstein and 0.06 in Danish Jersey. These data suggest that potential for changing MCP through breeding exists, but the genetic background of the MCP traits might be different in different breeds; therefore, using Danish Holstein as background for Danish Jersey is not trivial. Thereby, the study underlines the need for breed-specific models.

Key words: coagulation, genomic heritability, genomic correlation, somatic cell count

INTRODUCTION

Genetic and environmental factors affect raw milk quality and cause variations in nutritional and functional properties of milk within and among cow breeds. Moreover, the physiological state and health of cows affect milk composition, and milk from individual cows measured at different times during lactation can vary notably (Palmquist et al., 1993; Jõudu et al., 2007).

Rennet-induced milk coagulation is central to the initial phases of cheese manufacturing. In Denmark, 47% of milk is used for cheese production (Danish Agricultural and Food Council, 2012), and improved cheese milk is therefore of interest to industry. Variation in milk coagulation properties (**MCP**) among individual cows is primarily related to the quantities and relative proportions of caseins (α_{S1}-, α_{S2}-, β-, and κ-CN) and whey proteins (Jõudu et al., 2008), and the distribution of micellar and soluble minerals, especially calcium and phosphorus (Hallén et al., 2010; Jensen et al., 2012). These components influence the size and stability of casein micelles. For example, high levels of κ-CN are associated with smaller micelles, and high calcium content leads to increased micellar-bound CN, which increases cheese yield (Gaucheron, 2005; Glantz et al., 2010a). Posttranslational modifications of CN also play roles, and more heavily phosphorylated proteins are associated with poor coagulation (Frederiksen et al., 2011; Jensen et al., 2012), whereas a higher proportion of glycosylated κ-CN seems to be associated with shorter rennet coagulation times (**RCT**; Bonfatti et al., 2014).

Another important factor that determines milk coagulation is milk pH. Acidification of milk initiates aggregation of CN micelles. By adding starter cultures to milk, cheese producers can ensure better cheese yield and provide the pH needed for optimal rennet activity. Altered pH levels in raw milk from individual cows can reflect mastitis, where an impaired barrier between blood and milk raises milk pH toward the higher pH of

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the blood. Lower milk pH induces dissolution of micellar calcium phosphate (Gaucheron, 2005) and thereby influences the MCP. In addition to pH, milk constituents with chelating activities can decrease micellar-bound calcium (Gaucheron, 2005). For example, citrate in milk can form salts with calcium, which reduces the stability of CN micelles, and increased citrate levels in skim milk from individual cows has previously been associated with poor MCP (Frederiksen et al., 2011; Sundekilde et al., 2011). Moreover, clinical and subclinical mastitis and secretions of constituents from blood or interstitial fluids through the paracellular pathway can impair milk quality by changing milk composition (Shennan and Peaker, 2000).

Several studies have documented reasonable heritabilities for milk production, quality, and coagulation traits. According to a recent review by Bittante et al. (2012), the estimated heritabilities for RCT and curd firmness across cow populations were 0.26 and 0.27, respectively. Generally, heritability estimates for curd firmness vary more than for RCT, and its variation is more prone to the analytic method used in the study, the rennet concentration, or differences in statistical models used (Bittante et al., 2012). The moderate to large heritabilities for MCP traits suggested that they are genetically regulated and may be included in future breeding objectives.

Previously, we have demonstrated differences in the MCP of milk from 3 Scandinavian cow breeds and documented strong associations with major genetic variants of the CN genes (Poulsen et al., 2013). The objective of the present study was to investigate the associations between milk composition traits and MCP on pH-normalized milk from healthy, mid lactation Danish Holstein (**DH**) and Danish Jersey (**DJ**) cows and estimate heritability for these traits as well as the genomic correlations between them.

MATERIALS AND METHODS

Samples

Our study was conducted as part of the Danish-Swedish Milk Genomics Initiative. Ear tissue and morning milk samples were collected from 456 DH cows from 20 dairy herds (October–December 2009) and 436 DJ cows from 22 dairy herds (February–April 2010) in mid lactation. Care was taken to minimize potential sources of environmental variation and to ensure low genetic relatedness among cows in the sample populations. Samples were collected at 1 morning milking, and all cows were from conventional (nonorganic) herds. All cows were housed in loose housing systems, fed according to standard practices, and milked 2 or, rarely, 3 times daily (see also Poulsen et al., 2012). Parity and lactation stage were recorded for all cows. Milk yield at each milking was recorded, and representative milk samples were at least 0.5 L. Samples were placed on ice during transport to the laboratory.

Individual milk samples were analyzed for SCC using flow cytometry (Fossomatic 5000, Foss Analytical, Hillerød, Denmark) at the Eurofins Laboratory (Holstebro, Denmark). Milk samples were also analyzed for fat, protein, lactose, CN, citric acid, and urea content by infrared spectroscopy (MilkoScan FT2, Foss Analytical).

In addition to infrared measurements, pH and conductivity were measured in skim milk samples, before coagulation, with a PHM 220 pH meter (RadioMeter, Copenhagen, Denmark) and an LDM 210 conductivity meter (RadioMeter). Immediately after sampling, milk samples were aliquoted, skimmed (centrifuged for 30 min at 2,643 × g at 4°C), and refrigerated at 5°C for up to 6 h without preservatives before rheological analyses.

Rheological Analyses for Determination of MCP

Rennet-induced coagulation of skim milk samples was determined by a ReoRox4 rheometer (MediRox AB, Nyköping, Sweden), as outlined in Poulsen et al. (2013). Briefly, milk samples were adjusted to pH 6.5 with 10% (vol/vol) lactic acid and preincubated for 30 min at 33°C before the rheological analysis. Hereafter, each milk sample was set into free oscillation and amplitude damping and frequency changes were measured continuously for 1 h after addition of chymosin to a final concentration of 0.04 international milk clotting units (IMCU) per mL. Each milk sample was measured as technical duplicates. The MCP for individual samples were described as RCT and curd-firming rate (**CFR**) with the ReoRox software (version 1.5.0.1055). The RCT was defined as the amount of time from chymosin addition to when the phase angle reached 45° ($\theta = 45°$); CFR was calculated from consecutive points of the linear (lin) part of the gelation profile $[\Delta G'/\Delta t]_{lin}$, where G' is the storage modulus and t is time in minutes.

SNP Markers and Genotyping

Genomic DNA extracted from ear tissue resulted in total, 371 DH and 302 DJ cows that were genotyped by the bovine HD Beadchip (Illumina Inc., San Diego, CA; Van Tassell et al., 2008). From these animals, 777,962 SNP markers were assayed, with a median interval of 2.68 kb between SNP (www.illumina.com). The Illumina Infinium II Multisample assay platform was used. The SNP chips were scanned by iScan and analyzed with