Genetic analysis of detailed milk protein composition and coagulation properties in Simmental cattle

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ABSTRACT

The objective of this study was to estimate genetic parameters for milk protein fraction contents, milk protein composition, and milk coagulation properties (MCP). Contents of α_{S1} -, α_{S2} -, β -, γ -, and κ -casein (CN), β -lactoglobulin (β -LG), and α -lactalbumin (α -LA) were measured by reversed-phase HPLC in individual milk samples of 2,167 Simmental cows. Milk protein composition was measured as percentage of each CN fraction in CN (α_{S1} -CN%, α_{S2} -CN%, β -CN%, γ -CN%, and κ -CN%) and as percentage of β -LG in whey protein (β -LG%). Rennet clotting time (RCT) and curd firmness (a_{30}) were measured by a computerized renneting meter. Heritabilities for contents of milk proteins ranged from 0.11 (α -LA) to 0.52 (κ -CN). Heritabilities for α_{S1} -CN%, κ -CN%, and β -CN% were similar and ranged from 0.63 to 0.69, whereas heritability of α_{S2} -CN%, γ -CN%, and β -LG% were 0.28, 0.18, and 0.34, respectively. Effects of *CSN2-CSN3* haplotype and *BLG* genotype accounted for more than 80% of the genetic variance of α_{S1} -CN%, β -CN%, and κ -CN% and 50% of the genetic variance of β -LG%. The genetic correlations among the contents of CN fractions and between CN and whey protein fractions contents were generally low. When the data were adjusted for milk protein gene effects, the magnitude of the genetic correlations among the contents of milk protein fractions markedly increased, indicating that they undergo a common regulation. The proportion of β -CN in CN correlated negatively with κ -CN% ($r = -0.44$). The genetic relationships between CN and whey protein composition were trivial. Low milk pH correlated with favorable MCP. Genetically, contents and proportions of α_{S1} - and α_{S2} -CN in CN were positively correlated with RCT. The relative proportion of β -CN in CN exhibited a genetic correlation with RCT of -0.26 . Both the content and the relative proportion of κ -CN in CN did not correlate with RCT. Weak curds

were genetically associated with increased proportions in CN of α_{S1} - and α_{S2} -CN, decreased contents of β -CN and κ -CN, and decreased proportion of κ -CN in CN. Negligible effects on the estimated correlations between a_{30} and κ -CN contents or proportion in CN were observed when the model accounted for milk protein gene effects. Increasing β -CN and κ -CN contents and relative proportions in CN and decreasing the content and proportions of α_{S1} -CN and α_{S2} -CN and milk pH through selective breeding exert favorable effects on MCP.

Key words: milk protein composition, milk coagulation property, heritability, genetic correlation

INTRODUCTION

Milk protein polymorphisms and composition affect variation of milk coagulation properties (MCP; Jöndu et al., 2008; Bonfatti et al., 2010a,b) and changes in relative concentrations of milk protein fractions seem to be the primary cause of the effects of milk protein genetic polymorphisms on MCP (Bonfatti et al., 2010a) and cheese yield (Bonfatti et al., 2011). For this reason, milk protein composition might play an important role for the profitability of the dairy industry.

Genetic variation of MCP measures has been investigated by many authors (Ikonen et al., 1999; Cassandro et al., 2008; Cecchinato et al., 2009) and improvement of MCP based on selective breeding has been proposed (Ikonen et al., 2004). In addition, gene-assisted selection, exploiting polymorphisms at milk protein genes, has been suggested (Heck et al., 2009).

Only 8 studies (summarized by Bobe et al., 1999; Graml and Pirchner, 2003; Schopen et al., 2009) investigated the genetic variation of milk proteins because of analytical difficulties of quantifying simultaneously the major milk proteins using large samples. Five studies reported estimates of the genetic correlations for milk protein composition, 2 of them in peer-reviewed journals (Renner and Kosmack, 1975; Schopen et al., 2009). The genetic relationships between milk protein composition and MCP have never been published in a peer-reviewed journal. To evaluate the opportunity of altering milk protein composition and MCP through

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selective breeding and to elucidate the relationships between milk protein composition and MCP, genetic parameters for milk protein fraction contents, milk protein composition and MCP need to be estimated. The aim of this study was to estimate genetic parameters for milk protein fraction contents, milk protein composition, and MCP in a population of Simmental cows.

MATERIALS AND METHODS

Animals and Milk Sampling

Individual milk samples of 2,167 Simmental cows reared in 47 commercial herds in the north of Italy were collected from November 2007 to December 2008. Milk sampling occurred once per animal, during the morning or evening milking, concurrently with the monthly milk recording of the herd. Each herd was sampled once and all lactating cows were sampled. Milk was added with preservative (Bronopol, 0.6:100 vol/vol; Grunenthal Prodotti & Farmaceutici Formenti, Milan, Italy) immediately after collection and stored at -40°C until reversed-phase HPLC (RP-HPLC) analysis. Pedigree information was supplied by the Italian Simmental Cattle Breeders Association (ANAPRI, Udine, Italy) and included all known ancestors of sampled cows. Each sampled cow had at least 4 generations of known ancestors and the pedigree file included 16,602 animals.

Milk Protein Composition and Genotyping

Contents of α_{S1} -CN, α_{S2} -CN, β -CN, γ -CN, κ -CN, β -LG, and α -LA were measured using the RP-HPLC method proposed by Bonfatti et al. (2008). Genotypes of cows for *CSN2*, *CSN3*, and *BLG* were also obtained through RP-HPLC. A detailed description of the RP-HPLC technique used in this study can be found in Bonfatti et al. (2008). Although peaks of β -CN genetic variants and the phosphorylated forms of α_{S1} -CN and α_{S2} -CN are better resolved using capillary zone electrophoresis, RP-HPLC has the advantage over the capillary zone electrophoresis method used by Heck et al. (2008) of providing a more precise quantification of κ -CN because it does not co-elute with β -CN.

Total CN content (TCN, g/L) was computed as the sum of α_{S1} -CN, α_{S2} -CN, β -CN, γ -CN, and κ -CN contents. Total whey protein content (WH, g/L) was calculated as the sum of α -LA and β -LG contents. Protein composition (i.e., the relative proportions of protein fractions) was computed as the percentage ratio of α_{S1} -CN (α_{S1} -CN%), α_{S2} -CN (α_{S2} -CN%), β -CN (β -CN%), γ -CN (γ -CN%), and κ -CN (κ -CN%) to TCN and as the percentage ratio of β -LG to WH (β -LG%). Because WH was the sum of α -LA and β -LG contents,

the percentage of α -LA was not included because it can be derived from β -LG%.

Milk Coagulation Properties

Rennet coagulation time (RCT) and curd firmness (a_{30}) of individual milk samples were measured by the computerized renneting meter (CRM-48; Polo Trade, Monselice, Italy) within 3 h after sample collection. Details on the equipment and procedures used to assess MCP have been reported by Dal Zotto et al. (2008), including repeatability and reproducibility of measures. Milk samples that did not coagulate within 31 min (6.3% of samples) were classified as noncoagulating milk and were not included in the statistical analysis. The inclusion of noncoagulating milk in the estimation of genetic parameters of MCP has limited effects on the magnitude of the estimated parameters when the proportion of noncoagulating milk is low (Cecchinato and Carnier, 2011) as in the Simmental population. The potential relationship between noncoagulation of milk and milk protein composition will be the subject of a future paper. Measures of milk pH (pH-Burette 24; Crison, Barcelona, Spain) were also obtained.

Statistical Analysis

For all traits, the model of analysis included the polygenic effect of the animal as well as non-genetic sources of variation: the effects of herd test day (47 levels), parity of the cow (first, second, third, and fourth and later), and DIM class (12 classes of 30-d intervals, with the exception of the last class, which included samples collected at DIM 330 or greater). Parity $\times$ DIM class effects were trivial and were not considered in the analysis. As an alternative, a mixed inheritance model including, besides the above mentioned effects, the effects of *CSN2*-*CSN3* haplotype (as regressions on haplotype probabilities), and *BLG* genotype (AA, AB, and BB) was used. The probability for each *CSN2*-*CSN3* possible haplotype inherited by a sampled cow was estimated using the method of Boettcher et al. (2004). Computation of haplotype probabilities was carried out by assuming no recombination between CN genes. Details on the estimation of haplotype probabilities and allele frequencies can be found in Bonfatti et al. (2010b).

Estimation of (Co)variance Components and Genetic Parameters

Estimation of (co)variance components for protein fraction contents, protein composition, and MCP was performed through bivariate Bayesian analyses. The general model, in matrix notation, was

$$\begin{bmatrix} \mathbf{y}_1 \\ \mathbf{y}_2 \end{bmatrix} = \begin{bmatrix} \mathbf{X}_1 & \mathbf{0} \\ \mathbf{0} & \mathbf{X}_2 \end{bmatrix} \begin{bmatrix} \boldsymbol{\beta}_1 \\ \boldsymbol{\beta}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{U}_1 & \mathbf{0} \\ \mathbf{0} & \mathbf{U}_2 \end{bmatrix} \begin{bmatrix} \mathbf{q}_1 \\ \mathbf{q}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{Z}_1 & \mathbf{0} \\ \mathbf{0} & \mathbf{Z}_2 \end{bmatrix} \begin{bmatrix} \mathbf{a}_1 \\ \mathbf{a}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{e}_1 \\ \mathbf{e}_2 \end{bmatrix},$$

where $\mathbf{y}_1$ and $\mathbf{y}_2$ are vectors of phenotypic records for trait 1 and 2, respectively; $\boldsymbol{\beta}_1$ and $\boldsymbol{\beta}_2$ are vectors of non-genetic effects and, in the case of the mixed inheritance model, effects of *CSN2-CSN3* haplotype and *BLG* genotype, which are assumed to follow a flat distribution; $\mathbf{X}_1$ and $\mathbf{X}_2$ are known incidence matrices relating effects in $\boldsymbol{\beta}_1$ and $\boldsymbol{\beta}_2$ to $\mathbf{y}_1$ and $\mathbf{y}_2$, respectively; and $\mathbf{q}_1$ and $\mathbf{q}_2$ are vectors of herd test-day effects considered to be normally distributed. Pairs of records from different individuals are assumed to be conditionally independent given the parameters, but a correlation between effects acting on the same individual is allowed. Hence, sorting the data by individual, the herd test-day (co)variance matrix can be written as $\mathbf{Q} \otimes \mathbf{I}$, with $\mathbf{Q}$ being the (co)variance matrix between herd test-day effects on both traits and $\mathbf{I}$ an identity matrix of the same order as the number of individuals. Matrices $\mathbf{U}_1$ and $\mathbf{U}_2$ are known incidence matrices relating herd test-day effects in $\mathbf{q}_1$ and $\mathbf{q}_2$ to $\mathbf{y}_1$ and $\mathbf{y}_2$, respectively; $\mathbf{a}_1$ and $\mathbf{a}_2$ are vectors of additive genetic effects of animals assumed to follow a multivariate normal distribution. Sorting the data by individual, the animal (co)variance matrix can be written as $\mathbf{G} \otimes \mathbf{A}$, where $\mathbf{G}$ is the (co)variance matrix for animal genetic effects on both traits and $\mathbf{A}$ is the numerator of Wright's relationship matrix. Matrices $\mathbf{Z}_1$ and $\mathbf{Z}_2$ relate additive genetic effects in $\mathbf{a}_1$ and $\mathbf{a}_2$ to $\mathbf{y}_1$ and $\mathbf{y}_2$, respectively; $\mathbf{e}_1$ and $\mathbf{e}_2$ are vectors of residual effects assumed to follow a multivariate normal distribution. Sorting the data by individual, the residual (co)variance matrix can be written as $\mathbf{R} \otimes \mathbf{I}$, where $\mathbf{R}$ is the (co)variance between residual effects for both traits. Inverse Wishart prior distributions, which are very vague priors (Blasco, 2001), were used for all (co)variances in matrices $\mathbf{Q}$, $\mathbf{G}$, and $\mathbf{R}$.

Marginal posterior distributions of parameters were estimated by performing numerical integration through the Gibbs sampler, as implemented in the program TM (available on request from the author at andres.legarra@toulouse.inra.fr). A unique Gibbs chain of 5,000,000 iterations was run for each bivariate analysis. Samples were saved every 250 iterations. The length of the burn-in period was determined in a preliminary analysis by visual inspection of the chains. Based on the results of the preliminary analysis, the length of the burn-in period was set to 50,000 iterations. The posterior median was used as a point estimate of variance components, heritabilities, and correlations. Lower and upper bounds of the 95% highest posterior density interval (HPD) were obtained from the estimated marginal densities. The heritability was defined as

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_h^2 + \sigma_e^2},$$

where σ_a^2 , σ_h^2 , and σ_e^2 were the additive genetic, herd test day, and residual variance, respectively. The posterior probability of the heritability being greater than 0.1 was computed. To evaluate the relevance of genetic correlations, the probability of positive correlations being greater than 0.1 and of negative correlations being lower than -0.1 was also calculated.

RESULTS AND DISCUSSION

Descriptive statistics for the contents of protein fractions, protein composition, and MCP are reported in Table 1. Genotype, allele, and haplotype frequencies for the data used in this study can be found in Bonfatti et al. (2010b).

Heritability of Protein Fraction Content

Features of the estimated marginal posterior densities of variance components and heritabilities for the contents of milk protein fractions, milk protein composition, and MCP are in Table 2. Heritabilities of milk protein fraction contents ranged from 0.11 (α -LA) to 0.53 (κ -CN). The size of HPD for these estimates ranged from 0.13 (α -LA) to 0.26 (κ -CN). Because traits with heritability lower than 0.1 can be considered traits for which selection progress would be particularly slow and difficult to achieve, the threshold of relevance for the heritability was set to 0.1. The posterior probability of being greater than 0.1 was greater than 95% for all heritability estimates, the only exception being the heritability of α -LA. The largest estimates for protein fraction contents were those of κ -CN and β -CN whose synthesis is mainly regulated by *CSN3* and *CSN2*. The content of β -LG, which is affected to a large extent by *BLG* genotype effects, exhibited a heritability of 0.37. The heritability estimates for protein fraction contents were similar (α_{S1} -CN, α_{S2} -CN, and β -LG) or greater (κ -CN and β -CN) than those obtained by Graml and Pirchner (2003). The low estimate for α -LA may be partly ascribed to the lower accuracy of its quantification relative to that of the other fractions. Despite some difficulties of quantification due to multiple peaks and limited resolution, the estimated heritability for γ -CN content was similar to that obtained for TCN and contents of α_{S1} -CN and α_{S2} -CN. The estimated heritabilities indicate that it might be possible to modify the content of milk protein fractions through selective breeding. Interestingly, TCN and WH were less heritable than κ -CN or β -CN and β -LG contents,

Table 1. Descriptive statistics for milk protein fractions, protein composition, and coagulation properties¹

Trait	Mean	SD	Minimum	Maximum
Protein, g/L	38.78	4.54	19.58	59.16
CN, g/L	33.88	3.40	16.92	51.76
Whey protein, g/L	4.91	0.71	2.58	7.63
Milk protein fraction, g/L				
α_{S1} -CN	11.38	1.41	5.82	17.38
α_{S2} -CN	4.23	0.74	1.74	7.24
β -CN	12.99	2.02	5.99	22.83
γ -CN	1.62	0.45	0.23	4.51
κ -CN	3.66	0.85	1.29	6.84
α -LA	1.25	0.23	0.53	2.10
β -LG	3.66	0.61	1.76	6.13
Milk protein composition, %				
α_{S1} -CN%	35.61	2.65	28.00	45.46
α_{S2} -CN%	12.11	1.60	5.39	18.36
β -CN%	37.14	2.90	27.31	46.02
γ -CN%	4.68	1.33	0.77	12.56
κ -CN%	10.46	1.95	4.70	17.57
β -LG%	74.49	3.79	61.25	87.38
Milk coagulation property				
RCT, min	16.51	4.63	4.02	30.12
a_{30} , mm	29.08	7.51	5.00	46.00
pH	6.72	0.08	6.29	7.05

¹Contents of all protein fractions were measured by reversed-phase HPLC on skim milk; CN = α_{S1} -CN + α_{S2} -CN + β -CN + γ -CN + κ -CN; whey protein = β -LG + α -LA; protein = CN + whey protein; CN number = (CN/protein) $\times$ 100; α_{S1} -CN%, α_{S2} -CN%, β -CN%, γ -CN%, and κ -CN% are measured as percentages of total CN content; β -LG% is measured as percentage of total whey protein content; RCT = rennet coagulation time; a_{30} = curd firmness.

respectively, indicating that selection focusing on these protein fractions would be more effective than selection for altering TCN and WH.

Heritability of Milk Protein Composition

With the exceptions of γ -CN and β -LG, heritabilities for the relative proportions of protein fractions were greater than those for contents. Heritabilities for α_{S1} -CN%, κ -CN%, and β -CN% were similar, ranging from 0.63 to 0.69. These results evidence a tight relation between the relative proportions of these protein fractions depending on the CN haplotype (Bobe et al., 1999; Bonfatti et al., 2010b). The heritability of α_{S2} -CN% was moderate. Conversely, Schopen et al. (2009) reported that the heritability of α_{S2} -CN% was large and similar to estimates for α_{S1} -CN% and κ -CN%, whereas the heritability of β -CN% was moderate. As suggested by those authors, heritability estimates may differ across studies depending on the accuracy of analytical methods used to quantify contents of milk protein fractions. For example, Kroeker et al. (1985), using PAGE combined with densitometry, estimated heritabilities for milk protein composition that were not different from zero. Schopen et al. (2009) quantified the glyco-free portion of κ -CN only because the glycosylated form, almost one-half of the total κ -CN content, co-eluted with β -CN due to features of the analytical method used. This may partly explain the marked difference between

the β -CN% heritability obtained in that study relative to our estimates. Use of different breeds and statistical models, and differences in allele frequencies across populations contribute to inconsistencies in heritability estimates observed across studies.

The heritability of β -LG% was moderate and lower than the estimate obtained by Schopen et al. (2009), but in agreement with estimates presented by Bobe et al. (1999). Across-population variations in the extent of allele differential expression of heterozygous animals have been reported (Ng-Kwai-Hang and Kim, 1996) and might be responsible of the inconsistencies that β -LG% heritability estimates exhibit when studies are compared. In Heck et al. (2009) and Bobe et al. (1999), the additive deviation of *BLG* genotypes for the percentage of β -LG expressed on total protein was approximately 1.5%, whereas in Bonfatti et al. (2010b), it was approximately 2%. However, Bonfatti et al. (2010b) measured β -LG% as percentage of total whey protein. Hence, the effect reported by Heck et al. (2009) and Bobe et al. (1999) was roughly 6-fold greater than that reported by Bonfatti et al. (2010b).

Proportion of Phenotypic Variation Due to Herd Test-Day Effects

In agreement with Schopen et al. (2009), the proportion of phenotypic variation of milk protein traits attributable to herd test-day effects was small. The

Table 2. Features of the marginal posterior densities for variance components and heritability of milk protein fraction contents, milk protein composition, and coagulation properties¹

Trait ²	Polygenic model ³				Mixed inheritance model ⁴				Genetic variance explained ⁵	
	h^2	HPD95%	σ_a^2	σ_h^2	h^2	HPD95%	σ_a^2	σ_h^2	%	P (%)
CN, g/L	0.23	0.14, 0.34	3.05	2.41	0.19	0.09, 0.29	2.23	2.30	27	73
Whey protein, g/L	0.29	0.20, 0.38	0.12	0.11	0.20	0.11, 0.29	0.08	0.11	36	90
Protein fraction content, g/L										
α_{S1} -CN	0.24	0.14, 0.34	0.42	0.30	0.18	0.08, 0.27	0.29	0.31	30	77
α_{S2} -CN	0.22	0.14, 0.31	0.11	0.16	0.19	0.12, 0.28	0.09	0.16	16	60
β -CN	0.45	0.34, 0.56	1.53	0.47	0.19	0.10, 0.29	0.41	0.43	73	100
γ -CN	0.23	0.15, 0.32	0.05	0.02	0.04	0.00, 0.08	0.01	0.02	88	100
κ -CN	0.53	0.40, 0.66	0.35	0.06	0.27	0.16, 0.38	0.07	0.06	80	100
α -LA	0.11	0.05, 0.18	0.006	0.02	0.09	0.04, 0.15	0.004	0.02	18	60
β -LG	0.37	0.27, 0.46	0.11	0.06	0.25	0.16, 0.34	0.06	0.06	40	97
Milk protein composition, %										
α_{S1} -CN%	0.68	0.57, 0.80	4.17	0.59	0.28	0.17, 0.39	0.68	0.55	84	100
α_{S2} -CN%	0.28	0.19, 0.38	0.74	0.79	0.24	0.15, 0.33	0.60	0.81	19	67
β -CN%	0.69	0.58, 0.81	5.27	0.32	0.17	0.09, 0.25	0.42	0.31	92	100
γ -CN%	0.18	0.10, 0.26	0.31	0.24	0.05	0.01, 0.09	0.06	0.26	80	100
κ -CN%	0.63	0.50, 0.76	2.43	0.42	0.24	0.15, 0.34	0.30	0.45	88	100
β -LG%	0.34	0.24, 0.43	4.78	4.67	0.19	0.11, 0.28	0.26	4.49	53	100
Milk coagulation property										
RCT, min	0.29	0.19, 0.40	5.68	1.88	0.23	0.13, 0.33	4.18	2.02	27	76
a_{30} , mm	0.12	0.04, 0.21	5.72	5.43	0.11	0.03, 0.18	4.50	5.48	21	59
pH	0.18	0.10, 0.26	0.001	0.002	0.17	0.09, 0.25	0.001	0.002	6	45

¹ h^2 = median of the marginal posterior density of the heritability; HPD95% = bounds of the 95% high posterior density interval; σ_a^2 = median of the marginal posterior density of the additive genetic variance; σ_h^2 = median of the marginal posterior density of the herd test-day variance.

² α_{S1} -CN%, α_{S2} -CN%, β -CN%, γ -CN%, and κ -CN% are measured as percentages of total CN content; β -LG% is measured as percentage of total whey protein content; RCT = rennet coagulation time (min); a_{30} = curd firmness (mm).

³The model included polygenic effects of animals.

⁴The model included polygenic effects of animals, regressions on *CSN2-CSN3* haplotype probabilities, and *BLG* genotype effects.

⁵The proportion of the additive genetic variance estimated by the polygenic model due to milk protein genes; P = probability of the proportion of the additive genetic variance explained by *CSN2-CSN3* haplotypes and *BLG* genotypes being greater than 10%.

only exceptions were α_{S2} -CN and α -LA for which herd test-day effects were responsible for approximately one-third of phenotypic variation. These results suggest that herd conditions and feeding, in particular, exert limited influences on milk protein composition.

Proportion of Genetic Variance Explained by *CSN2-CSN3* Haplotype and *BLG* Genotype

The contribution of milk protein genes to variation of traits was investigated by estimating the change in the polygenic variance due to the fitting of the *CSN2-CSN3* haplotype and *BLG* genotype effects with the mixed inheritance model. Accounting for variation due to milk protein genes decreased considerably the estimated polygenic variance for α_{S1} -CN, β -CN, γ -CN, κ -CN, and β -LG traits, but the heritability of the majority of these traits was close to 0.20 or larger (Table 2). In agreement with Bobe et al. (1999), milk protein genes explained more than 80% of the genetic variance of α_{S1} -CN%. Conversely, Schopen et al. (2009) reported

that genotypes at *CSN2*, *CSN3* and *BLG* had no effect on the polygenic variance of α_{S1} -CN.

Despite a marked effect of the *BLG* genotype on β -LG% (Bonfatti et al., 2010a), the residual polygenic variance of this trait estimated with the mixed inheritance model remained noteworthy, indicating that other genes or polymorphisms, besides those considered, are responsible of the genetic variation of this protein fraction. Good candidates might be within the 33 new polymorphisms in the promoter region of *BLG* described by Ganai et al. (2009).

Heritabilities of contents and relative proportions of milk protein fractions were less variable when estimated with the mixed inheritance model, suggesting that the synthesis of milk proteins is regulated by protein-specific genes (CN and *BLG* genes), but also by genes that regulate the overall production of milk protein. Polymorphisms dramatically affect the levels of expression of milk protein genes, but their effects on TCN or WH are less pronounced. One hypothesis is that when expression of one CN gene is downregulated, expres-

Table 3. Additive genetic correlations for the contents (g/L) of total CN (TCN), total whey protein (WH), and milk protein fractions estimated without (above diagonal) or with (below diagonal) taking into account *CSN2-CSN3* haplotypes and *BLG* genotype effects¹

Protein	TCN	WH	α_{S1} -CN	α_{S2} -CN	β -CN	γ -CN	κ -CN	α -LA	β -LG
TCN		0.42 (100)	0.41 (97)	0.43 (100)	0.80 (100)	0.51 (100)	0.45 (100)	0.29 (86)	0.38 (100)
WH	0.68 (100)		0.26 (88)	0.43 (100)	0.26 (93)	0.46 (100)	0.05 (33)	0.33 (94)	0.98 (100)
α_{S1} -CN	0.91 (98)	0.67 (100)		0.22 (79)	-0.07 (43)	-0.14 (60)	0.12 (56)	0.09 (47)	0.26 (90)
α_{S2} -CN	0.47 (97)	0.47 (100)	0.26 (81)		0.14 (62)	0.50 (100)	0.11 (54)	0.38 (96)	0.35 (100)
β -CN	0.95 (100)	0.53 (100)	0.82 (100)	0.30 (88)		0.39 (100)	0.08 (42)	0.14 (60)	0.25 (93)
γ -CN	0.23 (64)	0.27 (70)	0.08 (47)	0.35 (80)	0.21 (62)		0.08 (43)	0.50 (100)	0.36 (98)
κ -CN	0.75 (100)	0.55 (100)	0.61 (100)	0.31 (91)	0.66 (98)	0.15 (55)		0.11 (53)	0.06 (33)
α -LA	0.19 (65)	0.54 (100)	0.25 (75)	0.32 (87)	0.03 (36)	0.24 (63)	0.12 (54)		0.10 (51)
β -LG	0.70 (100)	0.97 (100)	0.67 (98)	0.44 (98)	0.58 (98)	0.22 (64)	0.58 (100)	0.32 (89)	

¹Median of the marginal posterior density of the additive genetic correlation; the posterior probability (%) for positive correlations greater than 0.1 or for negative correlations lower than -0.1 is given within parentheses.

sion of the others can be upregulated to compensate (Leroux et al., 2003).

Inconsistent results for the detected proportion of variation due to milk protein genes might arise from different extents of linkage disequilibrium between these genes and polymorphisms in their promoter region. Bovenhuis et al. (1992) suggested that conflicting results for the effects of mutations at CN genes might be due to linkage between mutations at different CN genes as well as to different models used in statistical analyses. They proposed a multi-gene model as an alternative to single-gene models. As mutations affecting variation of quantitative traits occur in exons, introns, promoters, and other regulatory sequences, some of these functional mutations might not be within the set of mutations considered for genotyping.

Heritability of Milk Coagulation Properties

Estimated heritabilities for RCT and pH were similar to previous reports (Ikonen et al., 1999, 2004; Tyrisev et al., 2004; Cassandro et al., 2008). The estimate for a_{30} was similar to those obtained by Tyrisev et al. (2004) and Cassandro et al. (2008), but lower than estimates reported by other authors (Ikonen et al., 1999, 2004; Cecchinato et al., 2009).

Effects of the *CSN2-CSN3* haplotype and *BLG* genotype did not affect variation of pH, but accounted for 27 and 21% of the genetic variance of RCT and a_{30} , respectively. In a study by Ikonen et al. (1999) the estimated additive genetic variance for RCT and a_{30} decreased by 20 and 24%, respectively, when records were adjusted for *CSN2-CSN3* and *BLG* genotype effects.

Genetic Correlations for Milk Protein Fraction Contents

Point estimates and probabilities of relevance of the additive genetic correlations for the contents of milk protein fractions are in Table 3. All phenotypic correla-

tions were low and positive with the exception of those involving γ -CN, which were slightly negative or close to zero (data not presented). As γ -CN is a product of β -CN proteolysis, a negative phenotypic relationship between contents of β -CN or TCN and γ -CN and weak associations between γ -CN and the other fraction contents were expected. The size of phenotypic correlations between TCN or WH and milk protein fraction contents approximately reflected the relative proportion of each fraction in CN or WH. For example, β -CN, which quantitatively was the most important CN, exhibited the largest phenotypic correlation with TCN.

A genetic relationship was observed between TCN and WH, supporting the suggestion that the regulation of CN and whey proteins partially involves the same co-factors, hormones, and transcription factors (Groenen and van der Poel, 1994). The genetic correlations for the contents of the 5 CN fractions ranged from -0.14 to 0.50. The genetic correlations of κ -CN with the other CN were not relevant. The largest correlation was the one between α_{S2} -CN and γ -CN. This CN fraction exhibited a positive genetic association with the content of β -CN also. With the only exception of κ -CN, contents of CN fractions, in particular of α_{S2} -CN and γ -CN, were also positively correlated with WH, suggesting that these CN and whey proteins are partly subjected to a common regulatory system. These results indicate that contents of CN, in particular κ -CN, are rather independent genetically. Casein genes are located very closely, within a 250-kb region of chromosome 6 (Threadgill and Womack, 1990) and homology between the promoter region of α_{S1} -CN, α_{S2} -CN, and β -CN genes (Groenen and van der Poel, 1994) might elucidate possible co-regulation mechanisms. As suggested by Schopen et al. (2009), weak genetic relationships among the contents of milk proteins might arise from a differential post-transcriptional regulation (Bevilacqua et al., 2006). The size of HPD for the estimated correlations for milk protein fraction contents ranged from 0.03 (genetic correlation between β -LG and WH content) to 0.7 (genetic

correlation between α_{S1} -CN and α -LA content). The size of most HPD was close to 0.35.

The estimated genetic correlations among the milk protein fraction contents markedly increased and many became relevant when the statistical model accounted for *CSN2-CSN3* haplotype and *BLG* genotype effects (Table 3). The genetic correlations for the contents of α_{S1} -CN, β -CN, and κ -CN from very low or slightly negative became large and positive. In particular, the genetic correlation between α_{S1} -CN and β -CN changed from -0.07 to 0.82 . This suggests that the synthesis of all protein fractions undergo a common regulation. However, ranging from moderate to large, these correlations indicate that other genes or other factors, such as variable stability of mRNA, might affect the synthesis of specific protein fractions. Previous studies (Bobe et al., 1999; Bonfatti et al., 2010b) suggested that α_{S1} -CN and β -CN are subjected to competitive synthesis. Results for these correlations might be due to close linkage between alleles with favorable effects on synthesis of β -CN and alleles of the promoter region of *CSN1S1* with unfavorable effects on α_{S1} -CN synthesis (Kuss et al., 2005). Chanat et al. (1999) investigated transport of CN from the endoplasmic reticulum (ER) to the Golgi apparatus in mammary epithelial cells and suggested that α_{S1} -CN interacts with the other CN for efficient transportation to the Golgi. Accumulation of β -CN (or κ -CN and β -CN mixtures) was observed in the ER of mammary epithelial cells of α_{S1} -CN-deficient goats. Preformed CN complexes emerge from the ER via secretory vesicles for efficient transit to the Golgi apparatus. Because α_{S1} -CN can reduce aggregated species, it allows the associated particles to escape the ER. This property of α_{S1} -CN may elucidate the tight positive association of its content with contents of β - and κ -CN detected when accounting for *CSN* and *BLG* effects.

Genetic Correlations for Milk Protein Composition

Point estimates and probabilities of relevance of the additive genetic correlations for milk protein composition are in Table 4. The genetic correlations for the relative proportions of CN fractions ranged from -0.68 (correlation between α_{S1} -CN% and β -CN%) to 0.37 (correlation between α_{S2} -CN% and γ -CN%). These results are not in agreement with those obtained by Schopen et al. (2009) who reported that the largest genetic correlations were the ones between α_{S1} -CN% and α_{S2} -CN% and between α_{S1} -CN% and κ -CN%. Differences with estimates by Schopen et al. (2009) might be ascribed to use of different breeds, analytical procedures for measurement of milk protein composition, and trait definition.

On the basis of the estimated correlations, selecting for increased TCN is expected to increase the relative proportion of β -CN, to decrease α_{S1} -CN% and α_{S2} -CN%, and to exert limited effects on κ -CN% and β -LG%. Casein composition is genetically independent on whey protein composition, as genetic correlations between CN relative proportions and β -LG% are trivial. When *CSN2-CSN3* haplotype and *BLG* genotype effects were accounted for by the model, the largest changes were for the genetic correlations between α_{S2} -CN% or β -CN% and α_{S1} -CN%, indicating that milk protein genes affect the additive genetic covariance between these traits.

Genetic Correlations Between Milk Proteins and MCP

Point estimates and probabilities of relevance of the additive genetic correlations of protein fraction contents or protein composition with RCT, a_{30} , and milk pH are in Table 5. The size of HPD for these estimates ranged from 0.36 (genetic correlation between RCT and pH)

Table 4. Additive genetic correlations for total CN (TCN) and whey protein content (WH; g/L) and milk protein composition (α_{S1} -CN%, α_{S2} -CN%, β -CN%, γ -CN%, κ -CN%, and β -LG%) estimated without (above diagonal) or with (below diagonal) taking into account *CSN2-CSN3* haplotypes and *BLG* genotype effects¹

Protein ²	TCN	WH	α_{S1} -CN%	α_{S2} -CN%	β -CN%	γ -CN%	κ -CN%	β -LG%
TCN		0.42 (100)	-0.48 (100)	-0.23 (82)	0.39 (100)	0.17 (65)	0.15 (66)	0.15 (66)
WH	0.68 (100)		-0.10 (50)	0.14 (63)	0.04 (25)	0.36 (95)	-0.10 (50)	0.70 (100)
α_{S1} -CN%	0.02 (33)	0.08 (45)		0.04 (23)	-0.68 (100)	-0.46 (100)	-0.19 (86)	0.08 (43)
α_{S2} -CN%	-0.14 (60)	0.05 (38)	-0.65 (100)		-0.43 (100)	0.37 (97)	-0.13 (62)	0.05 (9)
β -CN%	0.18 (66)	-0.22 (76)	-0.09 (47)	-0.56 (100)		0.08 (41)	-0.44 (100)	-0.00 (27)
γ -CN%	-0.73 (100)	-0.32 (81)	-0.45 (93)	0.38 (87)	-0.23 (68)		-0.13 (58)	-0.03 (31)
κ -CN%	0.20 (62)	0.19 (73)	-0.24 (93)	-0.11 (51)	-0.33 (96)	-0.15 (83)		-0.01 (20)
β -LG%	0.58 (100)	0.56 (100)	0.07 (42)	-0.15 (64)	0.07 (43)	-0.46 (95)	0.28 (33)	

¹Median of the marginal posterior density of the additive genetic correlation; the posterior probability (%) for positive correlations greater than 0.1 or for negative correlations lower than -0.1 is given within parentheses.

² α_{S1} -CN%, α_{S2} -CN%, β -CN%, γ -CN%, and κ -CN% are measured as percentages of total CN content; β -LG% is measured as percentage of total whey protein content.

to 0.8 (genetic correlation between α -LA content and a_{30}). The size of most HPD was close to 0.55. A large negative genetic correlation between RCT and a_{30} was expected because these traits are measured in consecutive steps of the milk coagulation process and the time of analysis is restricted to 31 min. In agreement with Oloffs et al. (1992), Ikonen et al. (1999, 2004), and Cassandro et al. (2008), desirable MCP correlated with low pH of milk.

The genetic correlation between RCT and TCN was slightly unfavorable and had a probability of relevance of 0.74. The genetic relationship between a_{30} and TCN was positive and greater than 0.10 with a probability of 0.8. Estimates for the genetic correlations between MCP and protein or CN content are not consistent across studies. Cassandro et al. (2008) reported that short RCT correlated with high TCN. In previous studies (Oloffs et al., 1992; Ikonen et al., 1999, 2004), RCT was either negatively correlated or uncorrelated with protein content and TCN. Genetic correlations between a_{30} and protein content or TCN were found to be positive by Oloffs et al. (1992) and Cassandro et al. (2008), negative by Ikonen et al. (1999), or negligible by Ikonen et al. (2004). These results indicate that protein content and TCN are not suitable indicator traits for indirect genetic improvement of MCP. Contents of some protein

fractions were more tightly associated with MCP than TCN: α_{S1} -CN and α_{S2} -CN exhibited positive relationships with RCT and κ -CN was favorably associated with a_{30} .

Within the milk protein fractions, β -CN exhibited the most favorable association with RCT. Increased relative proportion of β -CN in CN correlated with short RCT and the posterior probability of the genetic correlation being lower than -0.1 was 0.94. Studies on the structure of milk protein micelles evidenced that most, if not all, κ -CN molecules are located on the micelle surface (Dalglish et al., 1989). However, on the basis of the measured size of CN micelles and the amount of κ -CN, only one-third of the micellar surface can be covered by κ -CN (Dalglish, 1998). Both α_S -CN and β -CN may share the micelle surface with κ -CN, but properties of β -CN and α_S -CN have been less studied and are not fully understood. Investigations on the rate of hydrolysis of different proteins in micelles treated with trypsin suggest that β -CN is the protein closest to the surface (Dalglish, 1998). Cooling makes β -CN dissociate from micelles. When micelles are warmed and β -CN re-associates with them, it is unlikely that β -CN penetrates deeply into the micelle. Thus, during re-association, the dissociated β -CN may alter the structure of the surface of the CN micelle (Dalglish, 1998). This

Table 5. Additive genetic correlations between milk protein fraction contents or milk protein composition and milk coagulation properties [rennet coagulation time (RCT), curd firmness (a_{30}), and pH]¹

Trait ²	RCT		a_{30}		pH	
	Polygenic model ³	Mixed inheritance model ⁴	Polygenic model	Mixed inheritance model	Polygenic model	Mixed inheritance model
CN, g/L	0.20 (74)	0.34 (91)	0.27 (80)	0.18 (67)	0.22 (76)	0.15 (61)
Whey protein, g/L	-0.02 (27)	0.02 (34)	0.12 (55)	0.30 (82)	-0.00 (27)	0.01 (29)
Protein fraction content, g/L						
α_{S1} -CN	0.46 (100)	0.25 (80)	-0.13 (53)	0.22 (70)	0.09 (46)	0.07 (43)
α_{S2} -CN	0.37 (97)	0.34 (92)	-0.13 (53)	-0.07 (44)	0.26 (84)	0.07 (43)
β -CN	-0.11 (54)	0.32 (88)	0.33 (92)	0.20 (69)	0.16 (66)	0.20 (71)
γ -CN	0.19 (74)	0.40 (86)	-0.11 (50)	-0.48 (88)	0.15 (62)	0.34 (79)
κ -CN	0.08 (43)	0.16 (64)	0.43 (98)	0.38 (92)	-0.00 (23)	-0.00 (27)
α -LA	0.24 (78)	0.09 (50)	0.06 (45)	0.12 (54)	0.25 (81)	0.04 (40)
β -LG	-0.06 (36)	0.01 (20)	0.10 (49)	0.30 (84)	-0.08 (42)	-0.01 (29)
Protein composition, %						
α_{S1} -CN%	0.21 (84)	-0.15 (60)	-0.31 (91)	0.10 (50)	-0.12 (56)	-0.05 (38)
α_{S2} -CN%	0.25 (88)	0.14 (61)	-0.32 (87)	-0.24 (72)	0.11 (53)	-0.04 (36)
β -CN%	-0.26 (94)	0.08 (46)	0.17 (67)	0.04 (42)	0.09 (44)	0.17 (65)
γ -CN%	0.14 (61)	0.06 (44)	-0.22 (72)	-0.48 (90)	0.10 (51)	0.18 (61)
κ -CN%	0.02 (25)	-0.08 (46)	0.36 (95)	0.34 (89)	-0.04 (32)	-0.10 (50)
β -LG%	-0.19 (79)	-0.10 (50)	0.03 (34)	0.20 (68)	-0.21 (81)	-0.07 (43)
a_{30}	-0.78 (100)	-0.83 (100)				
pH	0.61 (100)	0.73 (100)	-0.61 (100)	-0.66 (100)		

¹Median of the marginal posterior density of the additive genetic correlation; the posterior probability (%) for positive correlations greater than 0.1 or for negative correlations lower than -0.1 is given within parentheses.

² α_{S1} -CN%, α_{S2} -CN%, β -CN%, γ -CN%, and κ -CN% are measured as percentages of total CN content; β -LG% is measured as percentage of total whey protein content.

³The model included polygenic effects of animals.

⁴The model included polygenic effects of animals, regressions on *CSN2-CSN3* haplotype probabilities, and *BLG* genotype effects.

might partly explain the association of β -CN% with RCT. The genetic correlations between RCT and the content or the relative proportion of κ -CN were trivial.

As expected on the basis of the estimated genetic correlation between RCT and a_{30} , the genetic correlations between milk protein composition and a_{30} were of reverse sign relative to those obtained for RCT. Genetically, weak curds were associated with increased proportions of α_{S1} - and α_{S2} -CN and with decreased proportions of κ -CN in CN. The latter association is attributable to the decreased size of micelles for milk with increased κ -CN content and proportion in CN, allowing for more compact arrangements of para-CN micelles and an increased number of intermicellar bonds per unit of surface area (Horne, 1998). No nonlinear pattern between milk MCP and milk protein composition was observed. The genetic relationships between protein fraction contents or proportions and pH were weak or negligible.

Genetic Correlations Between Milk Proteins and MCP when Accounting for Milk Protein Genes Effects

When the *CSN2-CSN3* haplotype and *BLG* genotype were accounted for by the model, the additive genetic correlations between RCT and the contents of β -CN and γ -CN increased, becoming positive and relevant, whereas the correlation between RCT and α_{S1} -CN content decreased. The probability of relevance was low for the correlations between RCT and milk protein composition, particularly β -CN%. A possible explanation is that β -CN% is genetically related to RCT because of effects due to *CSN2* alleles. Variation at *CSN2* has been reported to affect both RCT and β -CN%, albeit *CSN2* effects on RCT were not attributable to the influence of the gene on β -CN% (Bonfatti et al., 2010a). Accounting for variation at *CSN3* did not influence the estimated genetic correlations between κ -CN content or relative proportion in CN and MCP. Bonfatti et al. (2010a) reported that associations between alleles at *CSN3* and MCP are to be attributed exclusively to variation in κ -CN content induced by *CSN3* alleles. Changes were detected for the estimated correlations between a_{30} and γ -CN content or relative proportion in CN, which became unfavorable. Kelly and McSweeney (2002) reported that proteolytic enzymes are released in milk from lysosomes of somatic cells. Plasmin is the most important enzyme and can rapidly cleave β -CN into γ -CN and smaller polypeptides (Auld et al., 1996). Increased plasmin activity is the main factor responsible for CN degradation, increased γ -CN content, changes in functionality of milk proteins affecting milk coagulation

(Zachos et al., 1992), and impaired coagulation features (Lucey, 1996; Srinivasan and Lucey, 2002).

Using the mixed inheritance model, the estimated correlations between β -LG content or relative proportion in whey protein and a_{30} became favorable. An allele-specific expression has been described for *BLG* (Heck et al., 2009; Bonfatti et al., 2010a). Allele A is associated with greater β -LG content and relative proportion in whey protein and favorable effects on MCP relative to *BLG* B (Ikonen et al., 1999; Bonfatti et al., 2010a). This might explain the changes of genetic correlation between β -LG content or β -LG% and MCP when accounting for milk protein gene effects.

Even when accounting for variation induced by CN and *BLG* genes, genetic correlations between milk protein traits and pH were trivial. This was expected, as milk protein genes did not affect the additive genetic variation of milk pH. However, an increase of the genetic correlation between γ -CN and pH was observed, which partially elucidates the marked unfavorable correlations of γ -CN with MCP.

For populations under selection, posterior distributions of genetic parameters are not the same as posterior distributions without selection, unless all data used for selection are included in the analysis (Sorensen and Gianola, 2002). Although selection was not performed on the investigated traits, a correlated response for these traits is envisaged due to selection for yield and contents of protein and fat in the Simmental population. In this study, we neglected that animals came from a population under selection and some sources of inaccuracy associated to the selection process might have occurred. A multivariate analysis using all data since the initial generation of selection is needed to obtain accurate estimates of the genetic parameters. We have performed this analysis for the TCN and CN fractions using the historical data set of protein yield of the Simmental population and the correlated effect of selection on the estimated parameters was negligible. This analysis is difficult to perform because populations are selected over a long time and not always under the same criteria. Hence, it is common to accept this source of inaccuracy (Boettcher et al., 2007). The effect of selection on the estimated parameters is due to several causes. For example, the Bulmer effect decreases variances with selection. Moreover, selection induces genetic disequilibrium, leading to correlated infinitesimal effects of genes, to a non-normal distribution of the additive effects, and to the impossibility of advocating the central limit theorem. Also, the selection process decreases the sampling space, producing the same effect of having missing data in an analysis, and leading to different posterior distributions.

CONCLUSIONS

The heritability for milk protein composition ranged from moderate to large. Milk protein genes explained a considerable part of the additive genetic variance of some protein fractions. However, additional genetic variation exists in the rest of the genome and can be exploited to alter protein composition by selective breeding. Selection for increasing total CN content of milk is not useful for the enhancement of coagulation properties. Increasing β -CN and κ -CN contents and decreasing the content of α_{S1} -CN and milk pH through selective breeding are more attractive alternatives. The heritability of MCP was still appreciable after adjustment for CN haplotypes and *BLG* genotype effects, suggesting that phenotype recording for these traits cannot be replaced by genotyping of animals for milk protein variants. The lack of rapid and automatic analytical methods that allow the quantification of milk protein fractions on a large scale limit the opportunity of exploiting genetic variation of milk protein composition for the improvement of milk coagulation properties through selective breeding. Although the development of easy immunoassay is currently under investigation, as well as the use of near-infrared spectroscopy for the prediction of milk protein composition, gene-assisted selection to change the frequency of alleles associated with expression of proteins fractions of interest also could be a possible strategy. The genetic relationships among milk protein composition, coagulation properties, cheese yield, and traits in the current breeding goal of dairy cattle populations need to be investigated.

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