Effect of β– and κ– casein genotypes on milk coagulation properties, milk production and content, and milk quality traits in Italian Holstein cows

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Introduction

Cheese-making ability of milk plays a fundamental role in cheese production, especially for the hard and long ripened ones. This quality has a great impact in Italy, where the manufacture of cheese employs about 70 % of the overall milk production (ISTAT, 2005). In milk-to-cheese phases, the most important and sensitive one is the coagulation of milk, both because it occurs as first and because it affects the following phases in the process. Thus, evaluation of milk for cheese production can effectively be based on coagulability of milk measured with Formagraph (Aleandri et al., 1989), an analysis which provides three traits, together called Milk Coagulation Properties (MCP) in this text.

Genetic improvement of MCP is a confirmed option to improve efficiency of cheese production (Ikonen, 2000; Bittante et al., 2002; Ojala et al., 2005) but the lack of suitable equipment for routine determination of them would restrict the possibility of direct selection of breeding animals for the trait. Alternatively, an indirect selection to improve MCP might be to favour alleles in milk protein loci that are associated with better coagulability of milk (Ikonen, 2000).

According to the literature, β–lactoglobulin (β-LG) genotype seems to affect casein percentage (Mariani et al, 1976, Ikonen et al., 1997), casein number (Ng-Kwai-Hang et al., 1986; Ikonen et al., 1997), fat content and cheese yield (Aleandri et al., 1990). α_{s1}-casein (α_{s1}-CN) was found to influence significantly only protein content (Aleandri et al., 1986; Ng-Kwai-Hang et al., 1986). κ-casein (κ-CN) genotypes are known to have effects on casein content (Mariani et al., 1984; Ng-Kwai-Hang et al., 1984 and 1986), protein content (Ng-Kwai-Hang et al., 1984; Aleandri et al., 1986 and 1990; Ikonen et al. 1999b) and cheese yield (Mariani et al.,1976; Aleandri et al. 1990) as well as on curd firmness (Marzali et al., 1986; Davoli, 1990; Ikonen et al., 1999a). β-casein (β-CN) genotypes have been found to be associated with fat percentage, fat and protein yield (Ng-Kwai-Hang et al., 1984) and curd firmness (Politis et al, 1988; Ikonen et al., 1999b).

Physical mapping techniques confirmed a close linkage between the caseins, establishing that the casein loci reside on chromosome 6 within a region of less than 200 kb in the order of α_{s1}-CN, β-CN and κ-CN (Ferretti et al., 1990; Threadgill and Womack, 1990). Therefore, the allelic effects of these loci are confounded in statistical analyses, even when they are included simultaneously in the model (Ojala et al., 1997). Certain alleles at the casein loci may hence appear together either more or less frequently than expected with a random combination. Consequently, the effects of casein genotypes should be estimated using composite casein genotype instead of separate genotypes, as suggested by Ojala et al. (1997) and confirmed by Ikonen et al. (1999b).

The aim of this study was to investigate the effects of β–κ-casein composite genotypes on milk coagulation, milk yield and milk quality traits in Italian Holstein cows reared in Northern Italy.

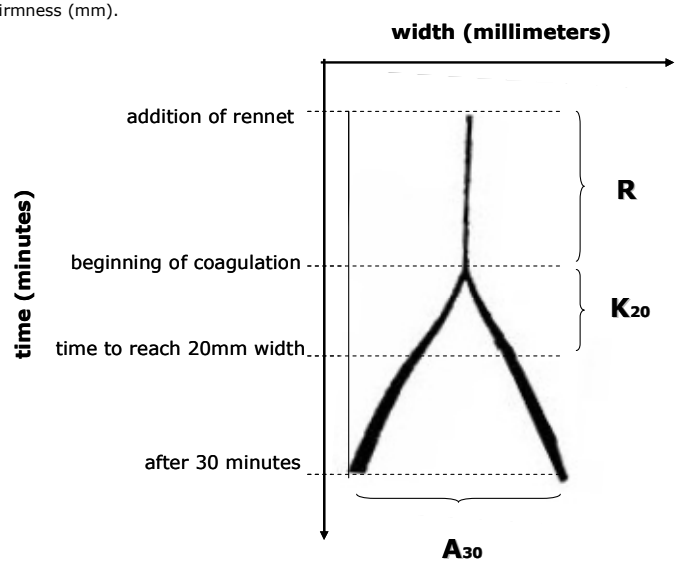
Materials and methods

Data collection. During January to July 2004, we sampled 1071 Italian Holstein cows (progeny of 54 sires) reared in 34 herds in Northern Italy. Individual milk samples were collected during morning milking, concurrently with monthly milk recording. Samples were analyzed for milk coagulation properties, pH and titratable acidity, milk yield, fat, protein and casein content, somatic cell count and major milk protein genotypes.

Background information about cows and herds was provided by a specific dataset created by the Department of Animal Science of University of Padova in collaboration with some Provincial Breeders Association of Veneto region regarding a main project, called MTP, about monitoring and improvement of productive and functional traits (Cassandro et al., 1999). Pedigree information was supplied by the Italian Holstein-Friesian Breeders Association (ANAFI, Cremona, Italy).

Laboratory analyses. The milk coagulation properties (MCP) were determined using a Computerized Renneting Meter (Polo Trade SaS, Monselice, Italy). No preservative was added, so milk was analyzed within a maximum of three hours after collection. An amount of 10 ml per sample were added with 200 µl of rennet (Hansen standard 190, Pacovis Amrein AG, Bern, Switzerland) diluted with distilled water (1,6:100) and measured after 30 minutes. In cheese-making process, in fact, curd is usually cut 30 minutes after the addition of the clotting enzyme. As a result of this analysis, we obtained a diagram for each sample (Figure 1) which supplied the three traits describing milk coagulation properties (MCP): milk coagulation time (R) in minutes, curd-firming time (K_{20}) in minutes, and curd firmness (A_{30}) in millimeters. R measures the time required from the addition of clotting enzyme to the beginning of coagulation; K_{20} represents the time from the beginning of coagulation to the moment the width of the curve reaches the amplitude of 20 mm; A_{30} describes the firmness of the curd, measured as the width of the diagram. The milk samples that did not form a curd in 30 minutes were defined as non-coagulated (NC) samples.

Figure 1. Diagram representing the milk coagulation traits. R = coagulation time (min), K_{20} = curd firming time (min), A_{30} = curd firmness (mm).



The same laboratory provided also composition and quality traits of milk: fat and protein contents (COMBI FOSS 6000 FC, Electric A/S, Hillerød, Denmark), casein content (CELL

FOSSOMATIC 250, Foss Electric A/S, Hillerød, Denmark), pH and titratable acidity expressed in Soxhelet-Henkel degrees (CRISON COMPACT D, Crison Instruments SA, Alella, Spain) and somatic cell count (CELL FOSSOMATIC 250, Foss Electric A/S, Hillerød, Denmark).
The main genetic variants at α_{s1}-CN, β-CN, κ-CN and β-LG loci were detected by isoelectric focusing analysis (Erhardt et al., 1998; Chessa et al. 2005).

Statistical Analyses. The milk samples that did not coagulate in 30 minutes supplied no suitable information about MCP, so they were excluded from some of the statistical analyses. Further data editing aimed to discard records with sampling or recording errors, such as protein content less than 1,5 % or above 5,0 % and fat content out of range 1,5 - 7,0 %. The data were preanalyzed using the GLM-procedure of SAS to test the statistical significance of the fixed effects in the model.
The effects of β-CN and κ-CN genotypes on milk coagulation, production and quality traits were investigated using a single-trait animal model.

$$y_{ijklm} = \mu + Herd_i + Dim_j + Parity(age)_k + \beta\text{–}\kappa\text{–}CN_l\ Anim_m + \varepsilon_{ijklm}$$

where:

y_{ijklm}	= milk coagulation, production or quality traits;
μ	= general mean;
$Herd_i$	= fixed effect of herd (j = 1 to 34);
Dim_j	= fixed effect of days in milk (j = 1 to 14);
$Parity(age)_k$	= fixed effect of age at calving within parity classes (k = 1 to 9);
$\beta\text{–}\kappa\text{–}CN_l$	= fixed effect of composite β–CN and κ–CN genotype (l = 1 to 16);
$Anim_m$	= random additive genetic effect of animal, N(0, Aσ2a)
ε_{ijklm}	= random residual effect, N(0, Iσ2ε)

Days in milk were grouped into 14 classes: 5 to 30, 31 to 60, 61 to 90, 91 to 120, 121 to 150, 151 to 180, 181 to 210, 211 to 240, 241 to 270, 271 to 300, 301 to 360, 361 to 420, 421 to 480, and over 480 days after calving. Parity was divided into three classes: first, second, and third to seventh calving. Within each class of parity, three classes of age at calving were made: 18 to 25, 26 to 27 and > 27 months of age for first parity; 32 to 38, 39 to 41, and > 41 months of age for second parity; 46 to 53, 54 to 63, > 63 months of age for third or more parities. Missing information about parity occurred for seven cows, so these records were discarded. β–κ–CN composite genotypes was grouped into 16 classes. Because of the low frequency of some genotypes (Table 1), we grouped together all the ones with less than 1 % of observations ("rare" class). For 73 samples it has not been possible to determine the exact genotypes; however, they were well distributed among herds and sires, so we grouped them all together ("unknown" class) and kept them in the analyses, to avoid any lack of information. The relationship matrix **A** included cows with observations and their known ancestors until 8 generations back in the pedigree, with a total number of 7387 animals taken in the statistical analyses.
The statistical significance of the effect of β–κ–CN genotypes was tested using the *F-test* of PEST package (Groeneveld, 1990). The hypothesis test was the maximum number of independent estimable contrasts between genotype classes would be equal to zero. When testing the genotypes, the β–κ–CN genotype A_2A_2AA was set to the class of comparison; this both because it was the class with the largest number of observations and because that composite genotype was homozygote for both loci. It follows that the standard errors of the genotype-effects are standard errors of the differences between one genotype and the most frequent β–κ–CN genotype, A_2A_2AA.

Results and discussion

Milk protein polymorphism. BB genotype of α_{s1}-CN was common to almost all the sampled animals (99.3%, data not shown). Because of this lack of variability, it was

impossible to establish its effect upon the studied traits. The observed genotypes of β-LG were AA, AB and BB (26%, 47% and 27% respectively, data not shown), but in preliminary analyses they did not show any significant effect on MCP and milk production traits, both alone or combined with β-CN and κ-CN genotypes. This has been confirmed also by Ikonen et al. (1997 and 1999b), who referred a significant effect of β-LG on protein content and composition but not on MCP or yield traits. For that reason, we decided to investigate primarily the role of the association of β-CN and κ-CN genotypes on milk coagulation properties, milk production and milk quality traits.
The most frequent genotypes of β-CN were A_1A_2 and A_2A_2, whereas AA and AB were the most common ones of κ-CN (Table 1). The composite genotype A_2A_2AA represented the most frequent genotype of β-κ-CN, followed by A_1A_2AA; together they constituted almost a half of the data. A_2A_3 and BB genotypes of β-CN as well as BE and EE genotypes of κ-CN were very rare, and they never occurred together as a combination. There were other significant differences between observed and expected frequencies of certain β-κ-CN genotypes (Table 1); in particular, the observed frequencies of A_1A_1AE, A_1A_2AE, A_1BAB, and A_2BAB genotypes were twice as much as expected, whereas the observed frequencies of A_1A_1AB and A_2A_2AE genotype were one-half of the expected ones. Linkage disequilibrium in the β-CN and κ-CN loci led, therefore, to the extremely unbalanced data.
Linkage disequilibrium was reported also in Finnish Ayrshire cattle by Ikonen et al. (1999a) which could be due to the typical structure of dairy cow populations using artificial insemination, in which there are relatively few sires with a large number of daughters. In addition, sampled cows were the daughters of the most frequently used sires in Italy, and those bulls carried and transmitted only certain casein haplotypes to their offspring.

Table 1. Number of cows for the β-CN and κ-CN genotypes and observed and expected (above and below respectively) frequencies (percentage) for the composite β-κ-CN genotypes in 998 Italian Holstein cows.

		β-CN						
		A_1A_1	A_1A_2	A_1B	A_2A_2	A_2A_3	A_2B	BB
κ-CN	no.	123	444	42	318	5	63	3
AA	451	5.3 5.6	18.9 20.1	0.3 1.9	20.1 14.4	0.4 0.2	0.1 2.9	 0.1
AB	334	2.7 4.1	12.4 14.9	3.0 1.4	10.0 10.7	0.1 0.2	5.2 2.1	 0.1
AE	141	3.4 1.7	10.4 6.3	 0.6	0.3 4.5	 0.1	 0.9	
BB	43	0.1 0.5	1.3 1.9	0.6 0.2	1.0 1.4	 	1.0 0.3	0.3
BE	16	0.3 0.2	1.0 0.7	0.2 0.1	0.1 0.5	 	 0.1	
EE	13	0.5 0.2	0.4 0.6	0.1 0.1	0.3 0.4	 	 0.1	

Differences between observed and expected number of composite β-κ-CN genotypes are statistically significant (P<0.0001in chi-square test)

Effects of casein genotypes. Milk coagulation traits (R and A_{30}), milk yield and protein yield were significantly affected by β-κ-CN genotypes (Table 2). No statistically significant effects were instead found for fat yield, and fat, protein and casein content, SCS and acidity of milk.

The most favorable MCP were associated with the β-κ-CN genotypes containing at least one B allele in both loci: A_1BAB, A_2BBB and A_2BAB (Table 2, Figure 2). The most frequent genotype, A_2A_2AA, and the genotypes A_1A_2BE and A_1A_2AE were instead associated with poorly coagulating milk. The κ-CN E allele rarely occurred in association with β-CN A_2A_2 and A_2B genotypes, and combined with the other β-CN genotypes, it was associated with the worst MCP, as previously assessed by Ikonen et al. (1999a). Considering the other κ-CN alleles, B was the most favorable one (better than A) for milk coagulation properties, in every combination with β-CN genotypes. Moreover, it seems that κ-CN locus affected milk coagulation traits more than did the β-CN locus, even if β-CN B allele turned out to be more favorable for milk coagulation than A_1 and A_2 alleles. The composite β-κ-CN genotypes including the κ-CN B allele were associated with the best MCP also in the Finnish Ayrshire cattle (Ikonen et al.,1999a; Ojala et al., 2005), but the β-CN locus in that population did not include the B allele. The same authors confirmed also the stronger role of κ-CN locus on MCP, but in default of β-CN B allele in their sampled population, other comparisons with their study are not possible.

The highest milk and protein yield were related to the genotypes A_2A_2BB and A_1A_1AB, whereas the lowest ones were associated with genotypes A_1A_2BB and A_1BAB (Table 2, Figure 2). Thus, the most favourable composite genotype for milk and protein yield differs from the least favourable one only by A_2 allele in β-CN locus (instead of A_1). The same occurs between the second most and second least favourable genotypes (A_1 instead of B, in this case). It seems that β-CN locus has a strong effect on production traits, in particular the A_2 allele was more favourable than A_1, and A_1 was more favourable than B. Furthermore, the β-CN alleles tend to have an opposite effect on milk production traits and on milk coagulation properties, meaning that the most favourable ones for milk and protein yield lead to milk less suitable for cheese making. The role of the κ-CN genotypes on production traits was unclear, because their effects depended on the stronger effect of the β-CN genotypes. For example, BB genotype of κ-CN resulted to be favorable for milk and protein yield when associated with A_2A_2 and A_2B genotypes of β-CN, but it became unfavourable when associated with A_1A_2. Similarly, κ-CN genotype AB was favorable for production traits if combined with β-CN genotypes A_1A_1, A_1A_2, and A_2A_2, but not in association with A_1B and A_2B. This confirms the stronger role of β-CN locus than κ-CN locus on milk yield traits, as well as the high relationship between these two loci in affecting milk production traits.

The positive effect of β-κ-CN haplotype A_2B on milk and protein production was already confirmed by previous studies both on Californian Holstein (Ojala et al., 1997) and Finnish Ayrshire cows (Ikonen et al., 1999b and 2001). Also the strong effect of β-CN genotypes on milk and protein yield was confirmed by (Ng-Kwai-Hang et al., 1984; Ikonen et al., 1999a), even though we differently did not find any effect on fat content. In our study, κ-CN genotypes have no clear effect on production traits, in agreement with previous studies (Aleandri et al., 1990; Davoli et al., 1990; Ikonen et al., 1999b), but the finding of no significant effect of κ-CN genotypes on protein percentage was unexpected and nonconforming with references.

The evidence that the most frequent β-κ-CN haplotype (A_2A) was associated with high milk and protein yield but with poor MCP, could be explained as the result of past breeding schemes in Italian Holstein population, which were mainly focused on milk production rather than milk quality and coagulability.

Table 2. Estimates (EST) of the effect of β- κ-CN genotypes on milk coagulation traits1 and yield traits.

β-κ-CN genotype	cows (n°)	R x = 16.86 min		A_{30} = 32.06 mm		Milk yield = 32.26 kg		Protein yield = 1.09 kg	
		EST[2]	SE[3]	EST	SE	EST	SE	EST	SE
A_1A_1AA	53	-0.79	0.70	3.27	0.70	-1.29	0.07	-0.03	0.03
A_1A_1AB	27	-2.23	0.90	5.54	0.17	1.45	0.42	0.06	0.04
A_1A_1AE	34	-1.34	0.82	5.98	0.99	0.94	0.27	0.03	0.04
A_1A_2AA	189	-0.66	0.45	3.03	0.09	0.10	0.69	0.01	0.02
A_1A_2AB	124	-2.11	0.50	7.53	0.22	0.64	0.78	0.06	0.02
A_1A_2AE	104	-0.38	0.55	1.47	0.32	-0.10	0.83	0.01	0.03
A_1A_2BB	13	-2.12	0.25	9.43	0.06	-2.54	0.95	-0.07	0.06
A_1A_2BE	10	-0.11	0.42	-0.59	0.49	-1.09	0.21	-0.02	0.07
A_1B AB	30	-4.88	0.85	10.90	0.05	-1.62	0.35	-0.04	0.04
A_2A_2AA	201	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
A_2A_2AB	100	-1.01	0.55	4.91	0.33	-0.04	0.85	0.08	0.03
A_2A_2BB	10	-1.51	0.42	3.59	0.48	2.98	0.19	0.08	0.07
A_2BAB	52	-3.13	0.68	10.37	0.66	-0.97	0.09	-0.04	0.03
A_2BBB	10	-4.42	0.34	10.80	0.29	-0.98	0.18	0.05	0.07
rare[4]	41	-3.64	0.74	8.83	0.82	0.92	0.18	0.06	0.04
unknown	73	-1.45	0.63	4.60	0.56	-3.51	0.97	-0.11	0.03
F test		<0.001		<0.001		0.017		<0.001	

[1]R=coagulation time (min), A_{30}=curd firmness (mm), A_{30}all = curd firmness including zero-values (mm). [2]EST = the difference between a genotype and the genotype of comparison, A_2A_2AA. A_2A_2AA. [3]SE= Standard Error of estimation. [4]Genotypes with less than 1% of observations were grouped together as "rare": A_1A_1BB (n=1), A_1A_1BE (n=3), A_1A_1EE (n=5), A_1A_2EE (n=4), A_1BBB (n=6), A_1BBE (n=2), A_1BEE (n=1), A_2A_2AE (n=3), A_2A_2BE (n=1), A_2A_2EE (n=3), A_2A_3AA (n=4), A_2A_3AB (n=1), A_2BAA (n=1), BBBB (n=3).

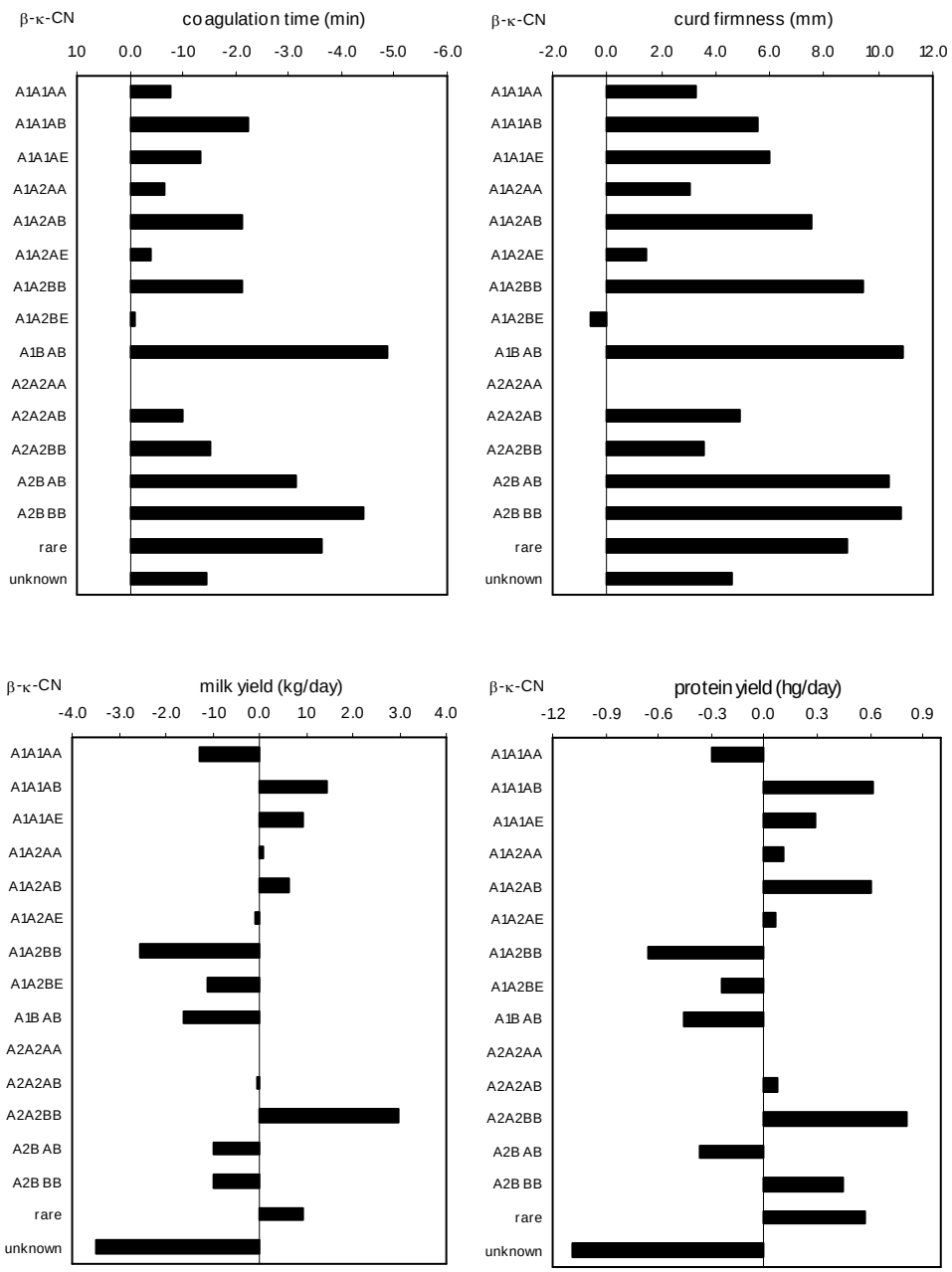
Conclusions

The β-κ-casein composite genotypes showed a strong effect on both milk coagulation and milk production traits, but not on milk quality traits. The κ-CN locus affected more MCP, whereas the β-CN locus affected more milk production, but the tight relationship found between the two loci, makes the composite genotypes more appropriate, when considering their use in selection.

Acknowledgmentsg

This work was funded by a national PRIN project. The authors thank Stefania Chessa and Anna Caroli of Department of Food Science, Technology and Microbiology of University of Milano for their cooperation within the project detecting the genetic variants of the main milk proteins.

Figure 2. Effect of β–κ–CN genotypes on milk coagulation and production traits in Italian Holstein cows.



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