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Milk protein genotypes and milk coagulation properties of Estonian Native cattle

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The genetic variation of α_{s1}-, β- and κ-caseins and b-lactoglobulin was determined and their effects on the rennet coagulation properties were examined using 335 milk samples from 118 Estonian Native (EN) cows. We found 16 aggregate casein genotypes (α_{s1}-, β-, κ-caseins), of which four – namely, BB A^2A^2 AA (21.2%), BB A^1A^2 AB (16.9%), BB A^1A^2 AA (14.4%), and BB A^2A^2 AB (10.2%) – occurred among nearly two-thirds of the analysed cows. Aggregate casein genotype had a significant overall effect on rennet coagulation parameters. Better rennet coagulation properties were found for aggregate casein genotypes CC A^2A^2 AB and BC A^1A^2 BB, among frequent genotypes for BB A^1A^2 AB. Of the cattle breeds raised in Estonia, milk from EN had the best coagulation properties and highest frequency of favourable κ-Cn B allele.

Key-words: Estonian Native cattle, milk protein polymorphism, coagulation properties

Introduction

Up until the 19th century, the Estonian farmers raised indigenous cattle. Breeding of Estonian Native (EN) cattle was started in 1910 when the West-Finnish breed was accepted as a breeding component. In 1956–1961 and 1989–1992, Jersey bulls were used to reduce the level of inbreeding in EN cattle. Swedish Red Polled bulls were employed in the late 1990s to modify the breed composition of the local cattle population (Kalamees 2004).

Estonian Native cattle are typically yellow-whitish red and hornless, with a medium wide chest and strong legs and hooves. Adult males weigh

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on average 700 kg and females 436 kg, and their wither height is 134 cm and 128 cm, respectively. The breed is characterised by good longevity, adaptation to the local conditions, easy calving, and low feed consumption per production unit. Average milk yield per cow per lactation was 4524 kg in 2005, i.e. lower than of other breeds in Estonia, whereas their milk fat (4.59%) and protein (3.44%) content were the highest.

According to the Estonian Agricultural Registers and Information Board, the total EN cattle population at the beginning of 2005 was 1,525 head including crossbreds (752 cows, 554 female calves, 143 young bulls and 76 bulls), of which 538 EN cows (including 420 purebreds) from 167 farms were included in milk recording. The share of native cattle has not decreased but remained at the same level, constituting about 0.5% of the total cattle population in Estonia. Due to its small population size, the Estonian Native cattle was categorised as an endangered breed by FAO in 1993. At present the breed has the risk status of an endangered-maintained breed (World Watch List for Domestic Animal Diversity 2000).

Since the most sustainable conservation strategy is to promote self-supporting, productive populations, it would be beneficial to establish a well-functioning selection programme for the breed. Genetic improvement could concentrate on maintaining or increasing the profitability of production in traits for which the breed still possesses a competitive edge (Toro and Mäki-Tanila 1999). Suitability of milk for cheese production could be one such trait. A preliminary comparison of milk coagulation properties among Estonian dairy breeds in an earlier study showed certain advantages of milk from EN cows, despite the limited number of EN cows in the study (Kübarsepp et al. 2005a). Moreover, once its suitability for cheese production is confirmed, milk from EN cows can be used for the production of Protected Denomination of Origin (PDO) cheeses. The PDO cheese-making process requires milk with good renneting properties from specific (local) breeds. Bertoni et al. (2005) found that the PDO cheeses have gained increasing value, not only in economic but also in cultural terms, particularly in some European countries. Besides showing certain organoleptic characteristics, these cheeses also represent a production system that is traditional and environmentally friendly.

The objective of this study was to examine the genetic variation of different milk proteins in milk from EN cows, and to determine the genotypic distributions and their effects on milk coagulation properties. To this end, we studied the rennet coagulation properties of milk among EN cows to assess its suitability for cheese production, and thereby to increase public interest in the breed and offer EN breeders better opportunities to maintain their EN herds.

Materials and methods

Cows and sampling

Milk samples (n=335) were collected from 112 cows on six farms recommended by the Estonian Native Cattle Breed Society, once every two months from March through November 2004, and from 6 cows on the Põlula Research Farm once a month throughout 2004. The sample represents more than 21% of the total EN cows included in milk recording.

Samples were taken simultaneously with the monthly milk recording using in-line milk meters at two consecutive milkings, and preserved with Bronopol® for an analysis of milk composition and renneting. Samples for determing milk protein genotypes were collected from the cows in March 2004, preserved with sodium azide and transported without delay to Freising, Germany.

Laboratory analyses

Concentrations of fat and protein were measured from each milk sample at the Milk Analysis Laboratory of Estonian Animal Recording Centre using an automated infrared milk analyser (System 4000, Foss Electric).

Milk protein genotypes (α_{s1}-casein, β-casein, κ-casein and β-lactoglobulin) were analysed at the Laboratory of Raw Milk, Munich University of Technology, Freising, Germany, by an isoelectric focusing/electrophoresis technique (Baranyi et al. 1993).

Milk rennet coagulation properties were determined on the day after milking at the Laboratory of Milk Quality, Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, by a Formagraph method (Kübarsepp et al. 2005b). Two milk coagulation parameters were measured: milk coagulation time (RCT = time in minutes from rennet addition to milk until the beginning of coagulation) and curd firmness (E_{30} = diagram width in mm 30 min after rennet addition). If diagram width was less than 20 mm, the samples were classified as milk with poor rennet coagulation properties (NK_{20}). In commercial cheese production such poorly coagulating milk would not reach the firmness needed to properly cut the curd. For samples that did not coagulate at all, it was only possible to record curd firmness (E_{30}=0), and these samples were classified as noncoagulated milk (NCM).

Statistical analysis

The statistical analysis was performed on a dataset of altogether 335 milk samples from 118 Estonian Native cows (Table 1).

Information on the birth, calving and pedigree of the cows was obtained from the Estonian Animal Recording Centre. The pedigree data used for statistical analysis covered two to four generations, amounting to a total of 606 animals in the pedigree file. The cows in the dataset had calved 1 to 12 times, and parity was grouped into four classes: 1, 2, 3 to 4, and ≥ 5 parities. Lactation stage was grouped into 11 classes of 30-day intervals, except for the last class, which covered the days from the 301st day after calving to the end of lactation. Rennet coagulation time was logarithmically transformed to obtain a normal distribution.

Results were evaluated statistically using a general linear mixed model assuming a first-order autoregressive variance structure of repeated measurements from the individual cows (SAS Inst. Inc. 2006). In order to estimate the effects of different factors on the milk coagulation, compositional parameters, the following models were used:

$$y_{ijklmof} = \mu + parity_i + lactmonth_j + \alpha\beta\kappa\_Cn_k + \beta\_Lg_l + a_o + pe_f + \varepsilon_{ijklmof},$$

where: $y_{ijklmnop}$ = milk coagulation (log RCT, E_{30}), production (daily milk yield) or compositional trait (milk fat and protein contents), μ = general mean, $parity_i$ = fixed effect of parity class i (i = 1 to 4), $lactmonth_j$ = fixed effect of month of lactation j (j = 1 to 11), $\alpha\beta\kappa\_Cn_k$ = fixed effect of aggregate α_{s1}-, β- and κ-Cn genotypes k (k = 1 to 15), β_Lg_l = fixed effect of β-Lg genotype l, (l = 1 to 3); a_o = random additive genetic effect of animal o, N(0, $A\sigma^2_a$); pe_f = random permanent environmental effect of farm f, N(0, $I\sigma^2_{pe}$); ε = random residual effect with spatial power covariance structure, N(0, R).

Interaction of genotypes at the α-, β- and κ-Cn

Table 1. Descriptive statistics of daily milk production, compositional and rennet coagulation parameters RCT in 118 Estonian Native cows.

	DMY[a], kg	Fat, %	Protein, %	RCT, min	log RCT	E_{30}, mm
Mean	15.8	4.67	3.57	7.3	0.83	33.0
SD[b]	6.34	0.870	0.422	3.05	0.169	12.96
Min	3.6	2.20	2.50	2.5	0.40	0
Max	39.5	7.25	4.72	23.0	1.36	57.0
Count	335	335	335	316	316	335

[a]Daily milk yield, [b]Standard deviation

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loci were considered because of their close genetic linkage.

The basic genetic variability of milk proteins was analysed by applying the Arlequine software package for population genetics (Excoffier et al. 2005). An estimation of gene diversities – expected (H_E) and observed (H_O) heterozygosity – and a probability test for detecting genotypic deviations from Hardy-Weinberg equilibrium were performed. Genotypic disequilibrium was tested under the null hypothesis (genotypes at one locus are independent from those at another locus). A Markov chain method was used to obtain P-value estimates using the Genepop computer program (Raymond and Russout 1995).

Allele and genotype frequencies were computed by direct counts.

Results

Effects of systematic environmental factors on studied traits

Parity did not have any significant overall effect on the studied milk rennet coagulation parameters, daily milk yield (DMY), and milk protein and fat content (Table 2). However, compared to the later parities, there were more noncoagulated and poorly coagulated milk samples in the first parity when milk protein content was lowest. Milk formed the firmer curd in the second to fourth parity when milk fat and protein contents were higher. Daily milk yield exhibited a tendency to improve with increasing number of lactation.

Lactation month had a significant effect on both the studied rennet coagulation traits ($P<0.001$) as well as on DMY and milk protein and fat content ($P<0.0001$). Milk coagulation properties were at their best in a very early stage and curd firmness also improved in the second half of lactation (Fig. 1). The proportions of noncoagulated and poorly coagulated milk were at their lowest at the beginning of lactation and clearly at their highest during midlactation. Daily milk yield declined during lactation. Also, milk fat and protein content decreased over the first three or four months of lactation and then started to increase again during midlactation when the coagulation properties were at their poorest. Milk fat and protein content rose steeply in the second part of lactation.

Genetic variability of milk proteins

All of the analysed proteins showed genetic polymorphism. Two to three alleles per locus were detected by isoelectrophoretic separation of milk (Table

Table 2. Estimates ±SE of effect of parity on studied traits (zero refers to class of comparison) and percentages of noncoagulated (NCM) and poorly (E_{30}<20 mm) coagulated (NK_{20}) milk samples of all samples in respective parity class.

Trait	Parity				P value
	1	2	3–4	≥5	
Number of samples	125	63	106	40	
Daily milk yield, kg	0^{a}	0.72 ± 1.03^{ab}	1.50 ± 0.88^{b}	2.20 ± 1.06^{b}	0.1605
Fat, %	0^{a}	0.50 ± 0.19^{b}	0.31 ± 0.16^{b}	0.19 ± 0.19^{ab}	0.0665
Protein, %	0^{a}	0.13 ± 0.09^{a}	0.07 ± 0.08^{a}	0.05 ± 0.09^{a}	0.5461
log RCT	0^{a}	-0.02 ± 0.04^{ab}	-0.06 ± 0.03^{b}	-0.02 ± 0.04^{ab}	0.2798
$^{1}E_{30}$, mm	0^{ab}	0.92 ± 2.32^{a}	0.98 ± 1.97^{a}	-3.47 ± 2.31^{b}	0.2321
NCM, %	10.4	6.3	1.9	–	
NK_{20}, %	10.4	6.3	5.6	5.0	

[1]Estimates of curd firmness of coagulating (E_{30}>0 mm) milk samples

[a,b]Estimates within row with differing letters in superscript are significantly different ($P<0.05$)

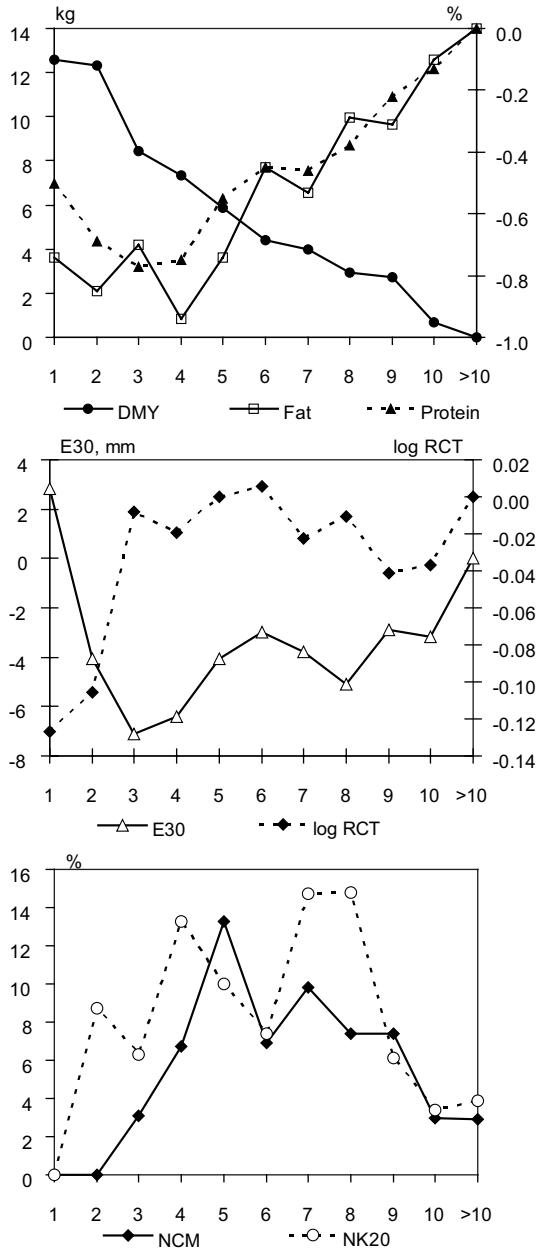

Figure 1. Estimates of effect of lactation month on milk traits (log RCT – logarithmically transformed rennet coagulation time, E30 – curd firmness of coagulating milk samples, DMY – daily milk yield, Fat – milk fat content, Protein – milk protein content) and percentages of noncoagulated (NCM; E_{30}=0 mm) and poorly coagulated (NK20; 0 mm<E_{30}<20 mm) milk samples within respective lactation month. Zero refers to class of comparison.

3). Milk protein gene diversities varied between H_O=0.152 (α_{s1}-Cn) and 0.525 (β-Cn), the average heterozygosity being 0.374. Allele frequencies ranged from 0.038 (β-Cn B allele) to 0.915 (α_{s1}-Cn B allele) as shown in Table 4. The κ-Cn E allele was not detected in EN in current sampling.

In α_{s1}-Cn a single genotype (α_{s1}-Cn BB) was prevalent, found in 83.9% of the studied EN cows (Table 5). Also β-Cn A^2A^2 (42.4%) and κ-Cn AB (52.5%) were frequent genotypes. Of the 16 detected aggregate genotypes, 11 genotypes occurred in more than one individual, and four of these (α_{s1}-β-κ-Cn) BB A^2A^2 AA (21.2%), BB A^1A^2 AB (16.9%), BB A^1A^2 AA (14.4%) and BBA^2A^2AB (10.2%) were found among nearly two thirds of the analysed cows. β-lactoglobulin genotype AA, AB and BB frequencies were 9.9, 42.2 and 47.9%, respectively. The frequencies of heterozygotes were consistent with the observed allele frequencies (Table 3), except for κ-Cn which showed a significant deviation from Hardy-Weinberg equilibrium, with an increased frequency of heterozygotes from both homozygote genotypes.

Among the three possible pairs of casein loci we found disequilibrium between β-Cn and κ-Cn at a significance level of 0.05, where the κ-Cn BB genotype combined with β-Cn genotypes containing A^1 as well as A^2, but was never observed with the homozygote A^2A^2 (Table 5). On the other hand, κ-Cn AA was most frequently found in combination with β-Cn A^2A^2. The α_{s1}-Cn BC genotype combinations occurred only with β-Cn A^1A^2 and A^2A^2. The rare α_{s1}-Cn CC genotype was observed only in one individual, in combination with β-Cn A^2A^2.

Table 3. Number of detected alleles, expected (H_E) and observed (H_O) heterozygosity of milk proteins.

Locus	No. of detected alleles	H_E	H_O
α_{S1}-Cn	2	0.164	0.152
β-Cn	3	0.485	0.449
κ-Cn	2	0.426	0.525*
β-Lg	2	0.441	0.424

*Significant (P=0.0055) heterozygosity excess

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Table 4. Milk protein allele frequencies of Estonian dairy breeds and of breeds used for genetic improvement of Estonian Native cattle (number of cows are given in brackets).

Locus	Allele	Estonian Native (118)	Western Finncattle[a] (41)	Danish Jersey[a] (32)	Estonian Holstein[b] (609)	Estonian Red breed[b] (321)
α_{s1}-Cn	B	0.915	0.939	0.781		
	C	0.085	0.061	0.219		
β-Cn	A^1	0.318	0.293	0.094		
	A^2	0.644	0.671	0.688		
	B	0.038	0.037	0.219		
κ-Cn	A	0.695	0.671	0.512	0.790	0.642
	B	0.305	0.305	0.488	0.138	0.324
	E	–	0.024	–	0.072	0.034
β-Lg	A	0.314	0.098	0.463	0.421	0.254
	B	0.686	0.902	0.537	0.579	0.746

[a]Lien et al. (1999), [b]Kübarsepp et al. (2006).

Table 5. Frequencies of casein genotypes in 118 Estonian Native cows.

		β-Cn					Total
κ-Cn	α_{S1}-Cn	A^1A^1	A^1A^2	A^2A^2	A^2B	A^1B	
AA	BB	0.025	0.144	0.212			0.381
	BC		0.017	0.034			0.051
Total AA		0.025	0.161	0.246			0.432
AB	BB	0.093	0.169	0.102	0.068	0.008	0.441
	BC		0.008	0.068			0.076
	CC			0.008			0.008
Total AB		0.093	0.178	0.178	0.068	0.008	0.525
BB	BB	0.008	0.008				0.017
	BC		0.025				0.025
Total BB		0.008	0.034				0.042
Total		0.127	0.373	0.424	0.068	0.008	1.000

Effect of milk protein genotypes on rennet coagulation properties

Aggregate casein genotype was found to have a significant ($P<0.05$) overall effect on both of the studied rennet coagulation parameters. The β-Lg genotype had a significant effect on curd firmness (Table 6).

Two aggregate casein genotypes, CC A^2A^2 AB and BC A^1A^2 BB, distinctly differed from others in significantly better milk rennet coagulation properties, but they occurred in few animals (one and three cows, respectively). Among the more frequent aggregate genotypes, there was a tendency of firmer curd formed in milk from cows carrying the BB A^1A^2 BB genotype. Among aggregate genotypes possessing the same β- and κ-Cn genotype, there was a tendency for the combinations with α_{s1}-BC or CC genotypes to have shorter milk rennet coagulation time and to form firmer curd than that for combinations with α_{s1}-BB. Within aggregate casein genotypes with α_{s1}-Cn BB and κ-Cn AB, milk from cows possessing β-Cn A^1A^1 or A^1A^2 had significantly longer rennet coagulation time than the cows with β-Cn A^2B, but curd showed the tendency to be firmer and no

noncoagulated milks were observed (Table 6).

Among β-Lg genotypes, β-Lg AB had a significantly softer curd than BB, and the percentages of noncoagulated and poorly coagulated milk samples were the highest (Table 6).

No noncoagulated milk samples were observed in association with β-Lg AA, κ-Cn BB, α_{s1}-Cn CC, or β-Cn A^1B (α_{s1}-Cn CC and β-Cn A^1B were represented only by one animal), whereas 78.9% of all noncoagulated milk samples originated from cows possessing κ-Cn AA genotype (Table 6).

The overall effect of aggregate casein and β-Lg genotypes on daily milk yield, milk fat and protein content was not significant.

Discussion

The results show that stage of lactation had significant effect on the rennet coagulation properties of milk. The variation in coagulation properties with lactation month showed a clear pattern, with the poorest coagulation properties in mid lactation. The results of the current study and our previous studies (Kübarsepp et al. 2005a) are consistent with those reported by Davoli et al. (1990), Tyrisevä et al. (2004), and Ikonen et al. (2004). The present results also showed that milk coagulation characteristics varied between parities, although the overall effect of parity was not significant. Percentages of noncoagulated and poorly coagulated milk samples diminished with increasing parity number. The literature contains contradictory

Table 6. Estimates ±SE of effects of aggregate casein and β-Lg genotypes on milk coagulation parameters (zero refers to class of comparison) and percentages of noncoagulated (NCM) and poorly (NK_{20}) coagulated milk samples within respective genotype

Aggregate (α_{s1}-, β-, κ-) casein genotype	Number of cows/samples	log RCT	$^1E_{30}$, mm	NCM %	NK_{20}, %
BB A^1A^1 AA	3/5	–0.03±0.10^{abc}	0.64±5.95^{ab}	20.0	
BB A^1A^2 AA	17/47	0.01±0.04^{a}	2.33±2.43^{ab}	10.6	10.6
BC A^1A^2 AA	2/8	–0.08±0.09^{abc}	4.48±5.16^{abc}	12.5	12.5
BB A^2A^2 AA	25/94	0^{a}	0^{a}	4.3	8.5
BC A^2A^2 AA	4/14	–0.01±0.08^{ac}	2.24±4.27^{abc}	28.6	14.3
BB A^1A^1 AB	11/20	0.00±0.05^{a}	2.55±3.05^{ab}	–	20.0
BB A^1A^2 AB	20/32	0.01±0.04^{a}	5.26±2.60^{b}	–	6.25
BC A^1A^2 AB	1/4	–0.18±0.12^{abc}	5.17±6.68^{abcd}	–	–
BB A^2A^2 AB	12/34	–0.06±0.05^{abc}	3.21±2.91^{ab}	2.9	2.9
BC A^2A^2 AB	8/27	–0.10±0.05^{abc}	4.98±3.04^{ab}	7.4	3.7
CC A^2A^2 AB	1/10	–0.08±0.04^{b}	17.54±5.98^{cd}	–	–
BB A^1B AB	1/4	–0.28±0.11^{abc}	6.98±7.10^{abcd}	–	–
BB A^2B AB	8/25	–0.15±0.13^{bc}	1.99±2.92^{ab}	4.0	16.0
BB A^1A^2 BB	1/4	–0.08±0.13^{abc}	4.82±6.80^{abcd}	–	–
BC A^1A^2 BB	3/6	–0.27±0.09^{b}	17.21±5.17^{d}	–	–
P value		0.0341	0.0417		
β-Lg AA	12/38	0.05±0.05^{a}	–3.18±2.91^{ab}	–	5.26
AB	49/142	0.01±0.03^{a}	–4.39±1.62^{a}	8.45	11.27
BB	56/154	0^{a}	0^{b}	4.55	4.55
P value		0.5761	0.0293		

[1]Estimates of curd firmness of coagulating milk samples (E_{30}>0 mm)

[a,b,c,d]Estimates within milk coagulation trait and aggregate casein or β-Lg genotype with differing letters in superscript are significantly different (P<0.05)

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results about the influence of parity on milk rennet coagulation properties. Schaar (1984) found a favourable effect of increasing parity number on coagulation properties, but in some other studies parity had either no significant effect (Davoli et al. 1990, Ikonen et al. 2004, Tyrisevä, et al. 2004) or coagulation properties were deteriorating with increasing number of lactation (Tyrisevä et al. 2003).

In genetic terms, the Estonian Native belongs to the Nordic cattle breeds, with a close relationship to Western Finncattle, as revealed by DNA microsatellites in a recent analysis (Tapio et al. 2006).

Our results regarding the casein allele frequencies further support the genetic relationship of EN with Western Finncattle. The observed difference between EN and Finncattle in the frequency of β-Lg variants in the current study probably results from genetic material introduced into EN by Jersey bulls and/or, on a smaller scale, Holstein and/or red breeds. Despite belonging to one genetic cluster with other old indigenous breeds (Tapio et al. 2006), the EN breed showed very similar distribution of milk protein aggregate genotypes with common commercial dairy breeds (Eenennaam and Medrano 1991, Lien et al. 1999).

A comparison of milk protein allele frequencies between EN and the breeds used for improvement (Finncattle, Danish Jersey) revealed similarities between breeds. A predominance of α_{s1}-Cn B (or its monomorphism) has also been observed in the common dairy breeds in Europe (Tervala et al. 1983, Ikonen et al. 1996, Lundén et al. 1997, Erhard et al. 1998, Lien et al. 1999). The same predominant variants in κ- and β-Cn loci have been found in most dairy cattle: κ-Cn A, except for Finncattle, Jersey and Brown Swiss, where B-allele is widespread, and alleles A^1 and A^2 at β-Cn (Tervala et al. 1983, Ikonen et al.1996, Freyer et al. 1999). The frequencies of β-Lg A and B alleles were similar in EN and in Jersey as well as in the dairy breeds of adjacent countries (Bech and Kristiansen 1990, Velmala et al. 1993, Ikonen et al.1996, Lundén et al. 1997). A comparison of milk protein allele frequencies between the EN breed and the other dairy breeds raised in Estonia, namely the Estonian Holstein (EHF) and Estonian Red (EPK), showed that EN's frequency of favourable κ-Cn B allele resembled that of EPK, but was higher than for EHF (Kübarsepp et al. 2005a). Unfavourable κ-Cn E allele (Jakob and Puhan 1992, Buchberger and Dovč 2000) was found both among the commercial breeds EHF and EPK (Kübarsepp et al. 2006), but not among EN cows in the current sampling.

Most of the genotypes in our sample followed the Hardy-Weinberg equilibrium. Only one protein, κ-Cn, displayed significantly higher observed heterozygosity than would have been expected by the allele frequencies. The excess of heterozygote κ-Cn genotypes probably reflects the occurrence of individuals with crossbred ancestors in the study. Freyer et al. (1999) presumed that the heterozygous κ-Cn genotype might have a heterotic effect on the milk yield. The occurrence of linkage disequilibrium between alleles at the different casein loci in our data indicates a relatively recent introduction of genetic material carrying specific casein haplotypes. The most common aggregate genotype was the homozygous combination BB A^2A^2 AA, reflecting a high frequency of the haplotype BA^2A in the breed.

We observed similar effects of casein genotypes on coagulation properties to those reported for other breeds by several research groups (Jakob and Puhan 1992, Van den Berg et al. 1992, Ikonen and Ojala 1995, Lodes et al. 1996, Ng-Kwai-Hang 1998, Buchberger and Dovč 2000).

Due to the close linkage of four Cn genes in chromosome 6 within a region of about 250kb in cattle (Rijnkels 2002) segregation of the α_{sn}-Cn, b-Cn, and κ-Cn variants occurs nonindependently (Aleandri et al. 1990, Eenennaam and Medrano 1991). Because of this close linkage of Cn genes, the use of casein aggregate genotypes is a more appropriate way to estimate the effect of Cn polymorphism on milk production traits than the use of individual Cn genotypes (Ikonen et al. 1999). Aggregate genotypes, similar to those of Estonian Native breed, have been frequent in Swedish Red and White and in Swedish Holstein breed (Lundén et al. 1997). Aggregate casein genotype had statistically significant ($P<0.05$) effect on curd firmness and rennet coagulation time. Most noncoagulated milk samples originated from cows possessing κ-Cn AA genotype.

Although the number of cows sampled was a considerable as a proportion (>21%) of the EN population, the size of the data was statistically speaking

small (Table 6). Although the overall effect of aggregate genotypes on milk rennet coagulation characteristics was significant the differences between genotypes were mostly not significant probably due to the high standard error values resulting from the small number of animals and samples representing each genotype. As the number of animals in the study was relatively small, the statistical analysis described by Hallén et al. (2007) was also carried out (results not shown). The results were no different however, and it was not possible to verify the superiority of any casein locus.

According to Kübarsepp et al. (2005a) milk from EN cows form a stronger curd (E_{30} = 33 mm) than milk from the other Estonian breed, EHF (E_{30} = 27.6 mm) and EPK (E_{30} = 31.1 mm). Also the percentage of poorly coagulated and noncoagulated milk samples (E_{30}<20 mm) was lowest for EN, 13.1%, while the percentages for EHF and EPK were 19.5 and 17.5%, respectively (Kübarsepp et al. 2005a). Several earlier studies (Tervala et al. 1983, Macheboeuf et al. 1993, Auldist et al. 2002) also asserted better renneting properties among native breeds as compared with the Holstein. Differences in milk coagulation properties between breeds may be due to differences in milk composition that is attributable to variation in other parts of genome. The studies mentioned above associated the better milk coagulation properties among native breeds with a higher frequency of κ-Cn B allele. A positive effect of this allele was shown also in the present study on EN cows, which also showed a comparatively high frequency of the allele.

Conclusions

Our present findings confirm previously observed relationships between genetic milk protein variants and milk properties for cheese-making. In contrast to common commercial dairy cattle breeds, Estonian Native cattle breed showed a relatively high frequency of the favourable κ-Cn B allele, although predominantly in heterozygote combination with the A allele, whereas no unfavourable κ-Cn E alleles were detected in EN in current study. On the other hand, favourable aggregate casein genotypes (containing κ-Cn BB, α_{s1}-Cn BC or CC genotype) for improving the conversion of milk protein into cheese were rarely observed in EN. Noncoagulated milk originated mainly from cows possessing κ-Cn AA genotype. If we compare the milk coagulation properties among the cattle breeds raised in Estonia, based on our current and previous results, the best milk for cheese-making comes from Estonian Native cattle.

In order to apply the genetic information obtained from this study in EN breeding programmes, we need to conduct additional determination of milk protein genotypes for all breeding bulls. This is necessary to increase the allele frequencies with a positive effect and to avoid unfavourable alleles in closely linked loci.

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