Agronomy Research **3**(1), 55–64, 2005

Effect of κ-casein and β-lactoglobulin genotypes on the milk rennet coagulation properties

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Abstract. Purpose of this study was to find connections between milk renneting properties of dairy breeds in Estonia and the genetic variants of κ-casein and β-lactoglobulin. Milk (n = 2161) and blood (n = 87) samples were taken from Põlula Research Farm where all dairy cattle breeds are represented: Estonian Holstein (EHF) – 45 cows, Red-and-White Holstein (RHF) – 12 cows, Estonian Red (EPK) – 26 cows and Estonian Native (EN) – 4 cows) raised in Estonia. Milk samples were analysed for fat, protein, calcium, and phosphorus contents, somatic cell count, and rennet coagulation parameters. Rennet coagulation properties of milk from cows of four experimental groups were higher in EK group. No noncoagulated milk samples were observed in this group. Estonian Red breed has the second-best rennet coagulation properties of milk. Percentage of noncoagulated milk samples in the group of EPK (3.6%) was lower than in the groups of EHF and RHF (percentage of noncoagulated milk samples 5.0% and 7.7%, respectively). All measured rennet coagulation parameters were significantly better for the κ-casein BB and worse for the κ-casein AA genotype. κ-Cn BB exhibited also the lowest percentage of noncoagulated milk samples and samples that did not reach K_{20} 30 min after enzyme addition. β-Lg genotypes had no significant effect on milk rennet coagulation parameters, but it was possible to observe tendencies that milk rennet coagulation time was the shortest and the percentages of noncoagulated milk samples and samples with poor coagulation properties (NK_{20}) were lower for the β-Lg AA genotype. Better milk rennet coagulation properties among native breeds are explicable with a higher frequency of κ-Cn B allele. The frequency of κ-Cn B allele has been decreased among Estonian Holstein cows.

Key words: milk coagulation properties, milk proteins, κ-casein, β-lactoglobulin, genetic variants

INTRODUCTION

Since the discovery of genetic polymorphism in β-lactoglobulin by Aschaffenburg and Drewry (1955), genetic variants have been found in all major milk proteins, and many researchers from different countries have demonstrated that milk composition, milk yield and technological properties are connected with milk protein genetic variants (Jakob & Puhan, 1992; Jakob & Puhan, 1995; Ng-Kwai-Hang, 1998). Several studies have demonstrated the influence of genetic variants of milk proteins on the contents of protein and casein in milk (Buchberger & Dovč, 2000). These findings have aroused the interest of many research groups around the world because of the

potential using of milk protein genes as markers to aid in the selection for milk yield and quality.

The coagulation properties of milk are of great importance as they influence cheese yield and quality. Milk with favourable coagulation properties (short coagulation and curd firming times, and a firm curd) is expected to give more cheese with desirable composition than milk with unfavourable properties. In many countries it has been found that, as a result of the breeding of cows, there has been an increase in milk production, however, the coagulation properties of milk have decreased, and the number of cows in the population producing non-coagulated milk has increased (Malossini et al., 1996; Tyriseva et al., 2003). The majority of the reports are based on comparisons between the variants of κ-casein and β-lactoglobulin (Ng-Kwai-Hang, 1998). As the α_{s1}-casein locus is especially monomorphic and variant B occurs in most breeds with a frequency of 95…< 99%, there is practically no report in the literature regarding relationships between genetic variants of these protein and production traits (Jakob & Puhan, 1995; Ng-Kwai-Hang, 1998). Due to a large number of alleles occurring at β-casein locus and a considerable variability of the experimental conditions, the reports on association between β-Cn variant and the composition and technological properties of milk are conflicting (Jakob & Puhan, 1992; Lodes et al., 1996).

About 29% of the milk produced in Estonia is used for cheese production, and for the production of similar cheeses 1 kg more milk than in Europe is needed in Estonia. To improve the efficiency of cheese production, it is necessary to identify technologies and strategies that provide improvement of raw milk rennet coagulation properties. The purpose of this research, carried out on Põlula Research Farm, was to find connections between the milk rennet coagulation properties of dairy breeds in Estonia and the genetic variants of κ-casein and β-lactoglobulin.

MATERIALS AND METHODS

Data. Milk samples (n = 2161) were collected twice a month at the same time as animal recording and preserved with Bronopol (Board Spectrum Microtabs®II, D&F Control Systems, Inc., California, USA) during the years 2001 and 2002 from 87 cows of Põlula Research Farm (RF), divided into four experimental groups, formed on a basis of dairy breeds raised in Estonia: Estonian Holstein (EHF – 45 cows), Red-and-White Holstein (RHF – 12 cows), Estonian Red (EPK – 26 cows), and Estonian Native (EK – 4 cows). Blood samples for detecting the κ-casein and β-lactoglobulin genotypes were taken from all cows (n = 87) during the years 2001–2002.

Keeping and feeding conditions were same for all cows on Põlula RF. The cows were fed identically *ad libitum* on a well-balanced totally mixed rations (during 10–150 day of lactation ration dry matter contained 12 MJ kg^{-1} metabolizable energy (ME), 105 g kg^{-1} metabolizable protein (MP), at least 130 g kg^{-1} crude fibre and from the 151st day of lactation until the end of lactation ration dry matter contained 11 MJ kg^{-1} of ME, 95 g kg^{-1} of MP (at least 150 g kg^{-1} crude fibre).

Laboratory analyses. The pH of milk was determined (MP 220; Mettler Toledo GmbH, Greifensee, Switzerland) at the room temperature before the rennet coagulation analysis. Milk calcium and phosphorus contents were determined once a month during

the year 2001 by using the IDF standard methods (36A:1992, 42B:1990) at the Dairy Laboratory of the Institute of Animal Science. Daily milk performance, milk protein and fat contents, and somatic cell count (SCC) data were received from Estonian Animal Recording Centre.

The milk rennet coagulation properties were determined on the next day after milking at 37°C using a Formagraph (Foss Electric, Hillerød, Denmark) at the Dairy Laboratory of the Institute of Animal Science. Rennet (Maxiren®, DSM, Heerlen, The Netherlands) was diluted 1:100 (v/v), and 0.2 ml rennet solution was added to 10 ml milk. The milk samples were allowed to coagulate for 30 minutes as in dairy industries curd is usually cut 30 min after the addition of rennet to the milk. The three milk coagulation parameters were measured from the diagrams (Fig. 1): milk coagulation time (RCT – time in minutes from the addition of rennet to milk to the beginning of coagulation), curd-firming time (K_{20} – time in minutes from the beginning of coagulation to the moment the width of the diagram was 20 mm) and firmness of the curd (E_{30} – width of the diagram in mm, 30 min after the addition of rennet). If the width of the diagram was less than 20 mm (Fig. 1b) it was impossible to measure the curd firming time and these milk samples were classified as milk with poor rennet coagulation properties (NK_{20}). In cheese production, these poorly coagulating samples would not reach the firmness needed to be able to properly cut the curd. For samples that do not coagulate at all (Fig. 1c), it was possible to record only curd firmness ($E_{30} = 0$), and these samples were classified as noncoagulated milk (NCM).

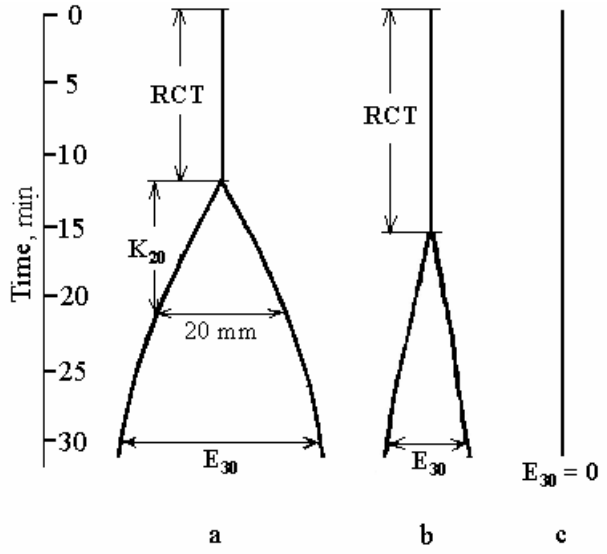

Fig. 1. Diagrams (a – normally coagulated milk, b – poorly coagulated milk and c – noncoagulated milk) produced by a Formagraph, and the three milk rennet coagulation parameters (RCT – rennet coagulation time, K_{20} – curd firming time and E_{30} – curd firmness) measured from the diagrams.

The genetic variants of κ-casein and β-lactoglobulin were determined by PCR-RFLP analysis (Sabre, 2003) at the Laboratory of Genetics of the Institute of Animal Science. The genomic DNA was extracted from blood.

Statistical analysis. Milk samples taken during the first 5 days after calving and samples from cows that were sampled only once were excluded from the statistical analysis. The data used for statistical analysis consisted of 2,161 milk samples collected from 87 cows (Table 1). More than two-thirds of the cows (n = 63) were sampled during two lactations. For somatic cell count, it was impossible to obtain normal distribution and thus, these values were logarithmically transformed and called somatic cell score (SCS). Results were evaluated statistically by using the mixed linear model including both discrete and continuous effects and assuming a first-order autoregressive variance structure of the repeated measurements of an individual cow (SAS INST. Inc., 1991). The autoregressive variance structure took into account that milk samples close in time were more closely correlated than milk samples further apart. In order to estimate the effects of different factors on milk coagulation parameters the following model ($P < 0.001$) was assumed:

$$Y_{ijklmno} = \mu + group_i + parity_j + month_k + Cn_l + Lg_m + b_1 * protein_{ijklmno} + b_2 * Ca_{ijklmno} + b_3 * pH_{ijklmno} + b_4 * SCS_{ijklmno} + e_{ijklmno},$$ where

$Y_{ijklmno}$ – rennet coagulation parameters (RCT, K_{20}, E_{30}),
μ – general mean,
$group_i$ – fixed effect of trial group (breed), $i \in \{EHF, RHF, EPK, EK\}$,
$parity_j$ – fixed effect of parity, $j \in \{1, 2\}$,
$month_k$ – fixed effect of month of lactation $k \in \{1, 2,..., 11\}$ (1…10 months of lactation included 30 days and all days after the 301th day of lactation formed the 11th month of lactation),
Cn_l – fixed effect of κ-casein genotype class, $l \in \{1, 2, 3\}$
Lg_m – fixed effect of β-lactoglobulin genotype class, $m \in \{1, 2, 3\}$
$protein_{ijklmno}$ – milk protein content,
$Ca_{ijklmno}$ – milk calcium content,
$pH_{ijklmno}$ – milk pH,
$SCS_{ijklmno}$ – somatic cell score (logarithmically transformed milk SCC),
b_1, b_2, b_3, b_4 – linear regression coefficients,
$e_{ijklnmno}$ – random residual effect including the effect of repeated measurements of the cows.

Pearson's correlation coefficients were used for describing the linear association between the various traits. To test differences between milk protein genotypes, milk compositional and daily milk yield data were examined for statistical significance by using the two tailed paired *t*-test (Table 4).

RESULTS AND DISCUSSION

Factors affecting milk rennet coagulation properties

All measured milk rennet coagulation parameters (RCT, K_{20}, E_{30}) were significantly influenced by the month of lactation, pH, and milk calcium content (Table 2). Curd-firming time and firmness of the curd were significantly influenced also by parity, somatic cell count and κ-Cn genotype. Milk protein content affected the curd-firming time, β-Lg genotype affected the rennet coagulation time and the experimental group (breed) had an effect on firmness of the curd.

The correlations between different rennet coagulation parameters were clear (Table 3). In the present study, the strongest negative correlation between rennet coagulation parameters and milk production traits was observed between curd-firming time and milk protein content (r = –0.445). The strongest positive correlation was found between firmness of the curd and milk protein content (r = 0.310). The increase of calcium, phosphorus and fat contents in the milk resulted in a shortened time for rennet coagulation and curd firming, whereas the formed curd was firmer at cutting. The rennet coagulation time prolonged since milk pH increased (r = 0.386).

A significant effect of milk protein and calcium content on milk rennet coagulation properties is described also by Tervala et al. (1985) and Kübarsepp et al., (2003). The calcium content of milk had a significant effect on all the rennet coagulation parameters (Table 2) as calcium directly participated in the process of rennet coagulation. During the rennet coagulation process, calcium forms bonds with *para*-casein (product of the action of rennet on casein), resulting in increased aggregation and firmer curd (Lucey, 2002).

An increase of pH in milk contributes to formation of colloidal calcium compounds and a decrease in the dissolved Ca content (Keogh et al., 1982), and increases plasmin activity with a resultant increase in proteolysis (Donnelly & Barry, 1983). The studies on the effect of milk pH on renneting properties are characterised by a large variability. According to the data by Macheboeuf et al. (1993) and Lodes et al. (1996), the milk rennet coagulation properties deteriorate in parallel with rising pH. Grandison et al. (1984) and Ostersen et al. (1997) found that milk pH affects predominantly only the rennet coagulation time. Our data (Tables 2 and 3) indicated that pH influenced statistically significantly all the studied rennet coagulation parameters, most of all the rennet coagulation time of milk (r = 0.386).

Rennet coagulation properties and milk production traits for different milk protein genotypes

All measured rennet coagulation parameters were significantly better for the κ-casein BB and worse for the κ-casein AA genotype (Table 4). κ-Cn BB exhibited also the lowest percentage of noncoagulated milk samples and samples that did not reach K_{20} 30 min after enzyme addition. β-Lg genotypes had no significant effect on milk coagulation parameters: only milk coagulation time was the shortest and the percentages of noncoagulated milk samples and samples with poor coagulation properties (NK_{20}) were lower for the β-Lg AA genotype. Our results are similar to those reported by Ikonen & Ojala (1995) in Finland. Milk coagulation time was the shortest for the β-Lg AA genotype in the Finnish Ayrshire whereas the β-Lg genotypes had no significant effect on any renneting trait in the Finnish Frisian.

Table 1. Means and variations for the studied traits.

Trait	n	Mean	SD	min	max
RCT, min	2,058	8.07	3.19	1	23
K_{20}, min	1,731	8.57	4.47	1	24
E_{30}, mm	2,161	28.98	12.70	0	59
Daily milk yield, kg	2,161	29.8	8.66	4.5	61.8
Fat content, %	2,161	3.79	0.77	1.93	8.87
Protein content, %	2,161	3.53	0.40	2.33	7.13
Log SCC	2,161	2.13	0.59	0.60	4.08
pH	2,161	6.77	0.09	6.38	7.12
Calcium content, %	1,129	0.122	0.017	0.078	0.213
Phosphorus content, %	389	0.098	0.013	0.040	0.141

Table 2. Significance (P) of factors influencing milk renneting properties.

Trait	RCT, min	K_{20}, min	E_{30}, mm
Experimental group (breed)	0.1099	0.0792	0.0026
Parity	0.5585	0.0476	0.0319
Month of lactation	0.0041	<0.0001	<0.0001
κ-Cn genotype	0.2168	0.0284	0.0386
β-Lg genotype	0.0092	0.8456	0.5128
Protein content, %	0.0545	0.0054	0.8801
Calcium content, %	<0.0001	0.0004	<0.0001
pH	<0.0001	<0.0001	<0.0001
SCS	0.6085	0.0320	0.0004

Significant differences between κ-casein and β-lactoglobulin genotypes were found in protein and phosphorus contents (AA<AB<BB; $P < 0.05$). κ-Cn and β-Lg genotypes had no significant effect on milk yield, fat and calcium content, pH and somatic cell count. Only κ-Cn AA contained less fat and κ-Cn BB less calcium, and had lower pH than other genotypes.

The favourable effect of κ-Cn B on the renneting properties of milk has also been confirmed in several studies (Jacob & Puhan, 1992). Reviewing results of different studies, Ng-Kwai-Hang (1998) found that comparing κ-Cn B variant with A variant, the decrease in coagulation time was ranging between 10–40%, and the increase in curd firmness was within a range of 20–140%. The positive effect of κ-Cn B may be partly due to higher fat, and protein, primarily casein, contents in milk having this variant (Ng-Kwai-Hang, 1998; Ikonen et al., 1999).

Table 3. Correlations between the studied coagulation and milk production traits parameters.

Trait	RCT, min	K_{20}, min	E_{30}, mm
Month of lactation	-0.003	-0.227*	0.199*
Daily milk yield, kg	0.123*	0.300*	-0.246*
Fat, %	-0.214*	-0.305*	0.295*
Protein, %	-0.040	-0.445*	0.310*
SCS	0.111*	0.008	-0.037
pH	0.386*	0.144*	-0.146*
Ca, %	-0.206*	-0.291*	0.273*
P, %	-0.072	-0.287*	0.399*
RCT, min	1	0.556*	-0.692*
K_{20}, min		1	-0.803*

$P < 0.001$

Table 4. Least square means of milk coagulation parameters, and mean milk production traits for different κ-casein and β-lactoglobulin genotypes.

	κ-casein			β-lactoglobulin		
	AA	AB	BB	AA	AB	BB
No. of animals	62	21	4	16	42	29
No. of milk samples	1461	580	120	396	1081	684
RCT, min	8.03[a]	7.78[a]	6.77	6.79	7.61	8.18
K_{20}, min	9.12	8.13	6.51	7.92[a]	8.07[a]	7.77[a]
E_{30}, mm	29.5	31.6	37.5	34.1[a]	32.6[a]	31.9[a]
[1]NCM, %	5.54	3.62	0.83	2.77	4.72	5.99
[1]NK_{20}, %	17.73	10.86	4.17	12.59	14.63	17.40
Milk yield, l	30.1[a]	29.4[a]	26.7[a]	29.7[a]	30.1[a]	29.2
Fat, %	3.76	3.81[a]	3.94[a]	3.67[a]	3.80[a,b]	3.82[b]
Protein, %	3.49	3.58	3.70	3.40	3.53	3.60
Log SCC	2.096[a]	2.195[a]	2.205[a]	2.123[a]	2.088[a]	2.191
pH	6.77[a]	6.77[a]	6.76	6.77[a]	6.77[a]	6.77[a]
Ca, %	0.1219[a]	0.1230[a]	0.1180	0.1198[a]	0.1227[b]	0.1223[a,b]
P, %	0.0964[a]	0.1000[a]	0.1009	0.0916	0.0977	0.1001

[1] percentage of noncoagulated milk samples (NCM) and samples that did not reach K_{20} 30 min after enzyme addition (NK_{20}) from samples of respective κ-casein or β-lactoglobulin variant

[a,b] means with the same superscripts in the same row inside of κ-casein or β-lactoglobulin genotypes are not significantly different ($P > 0.05$)

Table 5. Milk rennet coagulation parameters for different breeds in the trial.

		The Whole Farm	EHF	RHF	EPK	EK
Number of cows		87	45	12	26	4
RCT, min	n	2058	974	347	619	118
	$\bar{x}$	8.1	8.2	8.0	8.2	6.9
	SD	3.2	3.1	3.4	3.3	2.1
K_{20}, min	n	1731	804	280	530	117
	$\bar{x}$	8.6	9.4	9.1	7.5	6.3
	SD	4.4	4.5	4.6	4.1	3.5
E_{30}, mm	n	2161	1025	376	642	118
	$\bar{x}$	28.9	27.6	26.0	31.1	38.7
	SD	12.7	11.9	12.9	13.2	9.2

Table 6. Allele frequencies of κ-casein and β-lactoglobulin in earlier and present studies in Estonia.

Year of study	Breed	n (κ-Cn/ β-Lg)	Allele frequencies			
			κ-Cn		β-Lg	
			A	B	A	B
1972*	EHF	114 / 2033	0.69	0.31	0.47	0.53
	EPK	86 / 710	0.71	0.29	0.10	0.90
2000*	EHF	632	0.96	0.04	0.69	0.31
Present	EHF	45	0.92	0.08	0.49	0.51
	RHF	12	0.92	0.08	0.29	0.71
	EPK	26	0.65	0.35	0.37	0.63
	EK	4	0.75	0.25	0.50	0.50
	Farm	87	0.83	0.17	0.43	0.57

* Results of Toome (1972) and Orasson (2000)

Milk rennet coagulation parameters, κ-casein and β-lactoglobulin alleele frequencies for different breeds

From the studied milk samples (n = 2161), 2,058 samples coagulated, and it was possible to record curd firming time in 1,731 cases (Table 5). From the studied milk samples, 103 samples (4.8%) did not coagulate. At least one noncoagulated milk sample appears in 34 cows (39% from cows in the trial).

Rennet coagulation properties of milk from cows of the five experimental groups were higher in EK group (RCT = 6.9 min; K_{20} = 6.3 min; E_{30} = 38.7 mm). No noncoagulated milk samples were observed in this group. Estonian Red breed has the second-best coagulation properties of milk. The percentage of noncoagulated milk

samples in the group of EPK (3.6%) was lower than in the groups of Estonian Holstein and Red-and-White Holstein (percentage of noncoagulated milk samples 5.0% and 7.7%, respectively). It has to be mentioned that with the percentage of cows given, at least one noncoagulated milk sample in the group of Estonian Holstein breed was lower than in the groups of Estonian Red and Red-and-White Holstein. The number of cows with noncoagulated milk samples was, at least once during lactation, 17 (38%) in the group of EHF, 11 (42%) in EPK and 6 (50%) in RHF.

Several earlier studies (Tervala, et al. 1983; Macheboeuf, et al. 1993; Auldist, et al. 2002) asserted better renneting properties among native breeds, compared with the Holstein breed. Differences between breeds in milk coagulation properties may result from differences in milk composition derived from the genotype. Better milk coagulation properties among native breeds have been explained by the mentioned studies with a higher frequency of κ-Cn B allele. On Põlula Research Farm, the frequencies of κ-Cn A and B allele were 0.83 and 0.17, respectively, and these of β-Lg A and B allele 0.43 and 0.57, respectively (Table 6). Results of earlier studies in Estonia (Toome, 1972; Orasson, 2000) about allele frequencies of κ-Cn and β-Lg are presented in Table 6. The results of the present study indicate that the κ-Cn B allele frequency was considerably decreased in the Estonian Holstein cows. The frequency of κ-Cn B allele among local (red) breeds (EPK and EK) has remained at the same level.

CONCLUSIONS

Significant differences were found in renneting properties between κ-casein genotypes (AA<AB<BB). β-lactoglobulin had a significant effect only on milk coagulation time, having the shortest time for the β-Lg AA genotype.

Cows of Estonian Native and Estonian Red breeds giving milk with better coagulation properties have higher frequencies of κ-casein B allele. The frequency of κ-casein B allele, associated with better coagulation properties, has been considerably decreased in the Estonian Holstein cows.

ACKNOWLEDGEMENTS. The studies were carried out with the financial support of the Estonian Science Foundation (grant 4823, grant 5001, and grant 6158).

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