FREQUENCIES OF κ-Cn AND β-Lg GENETIC VARIANTS AMONG ESTONIAN CATTLE BREEDS AND THEIR EFFECT ON THE MILK RENNETING PROPERTIES

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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. 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 majority of the reports are based on comparisons between variants of κ-casein and β-lactoglobulin (Ng-Kwai-Hang 1998). The α_{s1}-casein locus is especially monomorphic and variant B occurs in most breeds with frequency of 95…<99% (Ng-Kwai-Hang 1998). Due to a large number of alleles occurring at β-casein locus the reports on association between β-Cn variant and the composition and technological properties of milk are conflicting (Jakob, Puhan 1992). About 29 % of the milk produced in Estonia is used for cheese production. To improve efficiency of cheese production is necessary to identify strategies provide improvement of raw milk rennet coagulation properties. The purpose of this research was finding the frequencies of the genetic variants of κ-casein and β-lactoglobulin and their connections between milk rennet coagulation properties of dairy breeds in Estonia.

MATERIAL AND METHODS

Milk samples (n=5189) were collected over monthly during the year 2003 from 930 cows (609 Estonian Holstein (EHF), and 321 Estonian Red (ER)). Cows were selected from 7 farms, suggested by the animal breeding organisations.

Genetic variants of κ-casein and β-lactoglobulin were determined by PCR-RFLP analysis. The genomic DNA was extracted from blood.

The milk coagulation properties were determined on the next day after milking at 37°C using a Formagraph (Foss Electric, Hillerød, Denmark). Rennet (Milase MRS 750 IMCU/ml; CSK Food Enrichment B.V., Netherlands) was diluted 1:100 (v/v), and added 0.2 ml to 10 ml milk. The two milk coagulation parameters were measured from the diagrams: milk coagulation time (**RCT** – time in minutes from the addition of rennet into milk up to the beginning of coagulation), and firmness of the curd ($\mathbf{E_{30}}$ – width of the diagram in mm 30 min after the addition of rennet) Milk protein content data were received from Estonian Animal Recording Centre.

Results were evaluated statistically using the following mixed linear model including both discrete and continuous effects and random regression part corresponding to individual cows (SAS Inst. Inc., 2006):

$$Y_{ijklmn} = \mu + breed_i + farm_j + Cn_k + Lg_l + b_1 * protein_{ijklmn} + b_2 * month_{ijklmn} + b_3 * month^2_{ijklmn} + a_m + b_{2m} * month_{ijklmn} + b_{3m} * month^2_{ijklmn} + e_{ijklmn},$$

where Y_{ijklmn} – rennet coagulation parameters (RCT, E_{30}); μ – general mean; $breed_i$ – fixed effect of breed, i ∈ {EHF, EPK}; $farm_j$ – fixed effect of farm, j ∈ {1, 2, …, 7}; Cn_k – fixed effect of κ-casein genotype class, k ∈ {1, 2, …, 6}; Lg_l – fixed effect of β-lactoglobulin genotype class, l ∈ {1, 2, 3}; $protein_{ijklmn}$ – milk protein content; $month_{ijklmn}$ – fixed effect of month of lactation (1…10 months of lactation included 30 days and all days after 301. day of lactation formed 11. month of lactation); b_1, b_2, b_3 – fixed regression coefficients; a_m – random animal effect, m ∈ {1, 2, …, 930}; b_{2m}, b_{3m} – random regression coefficients; $e_{ijklnmn}$ – random residual effect.

RESULTS AND DISCUSSION

Milk protein, κ-casein (κ-Cn) and β-lactoglobulin (β-Lg), genetic variants were detected for 930 cows, which formed according to Estonian Animal Recording Centre data ~1% from dairy cattle population (n = 101 785) in Estonia in year 2003. Most frequent genetic variant for κ-Cn was AA and for β-Lg AB and BB (Table 1). Favourable κ-Cn BB and AB genetic variants were most frequently associated with β-Lg BB variant and unfavourable AE, EE, and BE κ-Cn variants with β-Lg AB variant.

Table 1. Frequencies of κ-casein and β-lactoglobulin genetic variants

b-Lg \ k-Cn	AA	AB	AE	BB	BE	EE	Totally
AA	0.076	0.046	0.010	0.006	0.003	-	0.142
AB	0.247	0.116	0.047	0.020	0.011	0.001	0.443
BB	0.223	0.124	0.042	0.024	0.003	-	0.415
Totally	0.546	0.286	0.099	0.051	0.017	0.001	1.000

Both measured rennet coagulation parameters were significantly ($p<0.0001$) influenced by the κ-casein genetic variants and better for the κ-casein BB and worse for the κ-casein AA, AE, and EE genetic variants (Table 2). κ-Cn BB exhibited also the lowest percentage of noncoagulated milk samples and samples that did not reach 20 mm curd firmness 30 min after enzyme addition. The favourable effect of κ-Cn B on the renneting properties of milk has also been confirmed in several studies (Jacob, Puhan 1995). The positive effect of κ-Cn B may be partly due to higher fat, and protein, primarily casein, contents in milk containing this variant (Ng-Kwai-Hang 1998; Ikonen *et al.* 1999). In our study also milks with κ-Cn AB and BB variants contined more protein (protein content was 3.48 and 3.50%, respectively) than milks on the average (3.46%).

β-Lg genetic variants effect on rennet coagulation parameters was not significant. Rennet coagulation time was shorter and percentages of noncoagulated milk samples was lower for the β-Lg AA genotype. Our results are similar to those reported by Ikonen and 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 2. Milk coagulation parameters (LSM±SE) for different κ-casein and β-lactoglobulin genetic variants

Genetic variant		n	RCT	E_{30}	NCM[1]	NK_{20}[1]
κ-Cn	AA	3070	8.02±0.13^{a,b}	28.4±0.44^{a,b}	4.04	17.10
	AB	1359	7.06±0.15^{a,c}	35.0±0.53^{a,c}	2.57	7.43
	AE	453	7.71±0.25^{c}	28.1±0.87^{c,d,e}	4.42	16.11
	BB	220	5.92±0.34^{a,c}	39.7±1.18^{a,d}	1.36	1.82
	BE	82	6.66±0.54^{b}	35.6±1.89^{b,e}	1.22	6.10
	EE	5	5.78±2.23	30.0±7.77		20.00
β-Lg	AA	887	6.72±0.44	32.4±1.52	1.92	13.53
	AB	2402	6.78±0.39	32.8±1.38	3.79	13.11
	BB	1900	7.06±0.41	33.2±1.41	3.89	14.42

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

^{a,b,c,d,e} means with the same superscripts in the same column inside of κ-casein or β-lactoglobulin genetic variants are significantly different ($p<0.05$)

Table 3. Allele frequencies of κ-casein and β-lactoglobulin in Estonian dairy breeds according to different studies

Year of study	Breed	n (κ-Cn/β-Lg)	κ-Cn			β-Lg	
			A	B	E	A	B
1972	EHF	114 / 2033	0.693	0.307	-	0.465	0.535
	ER	86 / 710	0.709	0.291	-	0.103	0.897
2000	EHF	632	0.956	0.044	-	0.688	0.312
Present	EHF	609	0.790	0.138	0.072	0.421	0.579
	ER	321	0.642	0.324	0.034	0.254	0.746
	All	930	0.739	0.202	0.059	0.363	0.637

Several earlier studies (Tervala, *et al.* 1983; Macheboeuf, *et al.* 1993; Auldist, *et al.* 2002) asserted better renneting properties among native breeds, comparing with Holstein breed. We found that milk from Estonian Red cows has significantly ($P<0.05$) better coagulation properties than milk from Estonian Holstein cows. Differences between breeds in milk coagulation properties may result from the differences in milk composition derived from genotype. Mentioned studies explain better milk coagulation properties among native breeds with higher frequency of κ-Cn B allele.

In our study the frequencies of κ-Cn A, B, and E allele were 0.739, 0.202 and 0.059, respectively and β-Lg A and B allele 0.363 and 0.637, respectively (Table 3) and B allele. Results of earlier studies in Estonia (Toome 1972; Orasson 2000) about allele frequencies of κ-Cn and β-Lg indicate that the κ-Cn B allele frequency was considerably decreased in the Estonian Holstein cows. Frequency of κ-Cn B allele among local red breed (EPK) has remained on the same level. In earlier studies have not been detected presence of κ-Cn unfavorable E allele.

CONCLUSION

Most frequent genetic variant for κ-Cn was AA and for β-Lg AB and BB. All measured rennet coagulation parameters were significantly better for the κ-casein BB and worse for the κ-

casein AA, AE, and EE genotypes. β-Lg genetic variants had no significant effect on rennet coagulation parameters. Percentages of noncoagulated milk samples was lower for the β-Lg AA genotype. Frequency of κ-casein B allele, associated with better coagulation properties, has been considerably decreased in the Estonian Holstein cows. In earlier studies in Estonia have not been detected presence of unfavorable κ-Cn E allele.

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