THE EFFECT OF MILK PROTEINS ON MILK COAGULATION PROPERTIES IN ESTONIAN DAIRY BREEDS

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Summary. Coagulating properties of milk have influence on cheese-making ability, cheese yield, and its quality. The aim of this study was to find the effects of major milk proteins contents and their genetic variants on milk rennet coagulation properties in Estonian dairy breeds. During the years 2001-2005 a total of 1,269 cows were on repeated occasions sampled (n = 8,282). Higher contents of milk protein, total casein, the casein number, and α_{S1}-, β-, and κ-Cn, and β-Lg, reduced the rennet coagulation time and formed a firmer curd. A higher proportion of κ-Cn with respect to α_{S1}-Cn and β-Cn assisted in forming a firmer curd. All measured rennet coagulation parameters were significantly better for the κ-Cn BB, and worse for the κ-Cn AA, AE, and EE genotypes. Noncoagulated milk originated mainly from cows possessing κ-Cn AA genotype. Better milk coagulation properties among ER and EN cows, compared to EHF cows, are explainable by a higher frequency of the κ-casein B allele, associated with better coagulation properties, and ER cows also recorded higher contents of milk proteins, compared to EHF cows.
Key words: milk rennet coagulation, milk proteins, casein, noncoagulated milk.

PIENO BALTYMŲ ĮTAKA ESTIJOS MELŽIAMŲ KARVIŲ PIENO KOAGULIACIJAI

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Santrauka. Nuo pieno koaguliacijos savybių labai priklauso sūrio kiekis ir kokybė. Tyrimo tikslas buvo nustatyti pieno baltymų ir jo genetinės įvairovės įtaką Estijos melžiamų karvių pieno koaguliacijai. 2001–2005 metais paimti pakartotini mėginiai iš 1,269 karvių (n = 8,282). Esant didesniam pieno baltymų, bendro kazeino ir kazeino kiekiui, buvo nustatytas α_{S1}-, β- ir and κ-Cn, bei β-Lg, sutrumpėjęs koaguliacijos bei varškės formavimosi laikas. Padidėjęs κ-Cn kiekis esant α_{S1}-Cn ir β-Cn turėjo teigiamą įtaką varškės formavimuisi. Statistiškai aukštesni koaguliacijos rodikliai nustatyti κ-Cn BB genotipų, o žemesni κ-Cn AA, AE, ir EE genotipų. Pieno koaguliacija nevyko, kai vyravo genotipas κ-Cn AA. Geresni koaguliacijos rodikliai nustatyti ER ir EN karvių nei EHF. Tą galima paaiškinti dažnesne κ-Cn B alele. Taip pat bendras pieno baltymų kiekis ER karvių buvo didesnis už EHF karvių.
Raktažodžiai: pieno koaguliacija, pieno proteinas, kazeinas, nekoaguliuotas pienas.

Introduction. The food industry yields a quarter of the total industrial output of Estonia; the dairy industry is the largest (~⅓ of the total food industry). Dairy farming is the main source of income for agricultural holdings in Estonia. Within the dairy industry, cheese is a product of major importance; about one third of the milk produced in Estonia is converted into cheese and cheese production is increasing.

The coagulation properties of milk are of great importance because 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 (Ng-Kwai-Hang, et al., 1989). Milk used for cheese production, has to have, in addition to good quality parameters, also good rennet coagulation properties to ensure conversion of milk solids to cheese and to prevent losses in profit to the dairy companies. In many countries it has been found that, as a result of the cattle breeding, there has been an increase in milk production, but 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; Tyrisevä, et al., 2003). Previous studies in Estonia (Kübarsepp, et al., 2003) showed, that about 8–9% of milk did not coagulate and additionally 17–20% of milk had poor rennet coagulation properties. To improve the efficiency of cheese production it is necessary to identify strategies to improve raw milk rennet coagulation properties. This study involves a more extensive analysis to ascertain specific markers that could be used to identify milk suitable for cheese-making and thereby provide an economic advantage to the dairy industry. The aim of this study was to find the effects of major milk proteins contents and their genetic variants on milk rennet coagulation properties in Estonian dairy breeds.

Material and methods. *Collection of data.* Milk samples (n = 2,758) were taken during the years 2001–2005 on repeated occasions from Põlula Research Farm, where individuals representing all dairy cattle breeds raised in Estonia were kept under similar conditions. In addition, during the year 2003 milk samples (n = 5,189) were collected every second month from 930 cows (609 Estonian Holstein (EHF) and 321 Estonian Red (ER)) on six more farms, recommended by the breeding organisations. Milk samples (n = 335) were collected from 112 Estonian Native (EN) cows on six farms, recommended by the Estonian Native Cattle Breed Society once every two months from March through November 2004. A total of 1,269 cows were sampled: 765 (60.3%) EHF, 378 (29.8%) ER, and 126 (9.9%) EN. All milk samples were taken simultaneously with milk recording.

Laboratory analyses. The milk coagulation properties (MCP) were determined on the day after milking at 37 °C using a Formagraph (Foss Electric, Hillerød, Denmark). In the Formagraph analysis the milk rennet coagulation data was recorded diagrammatically (Kübarsepp, et al., 2005): milk rennet coagulation time (RCT – time in minutes from the addition of rennet into milk to the beginning of coagulation), and curd firmness (E_{30} – width of the diagram in mm 30 min after the addition of rennet). 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 adequately cut the curd. For samples that did not coagulate at all, it was only possible to record curd firmness (E_{30} = 0 mm), and these samples were classified as non-coagulated milk (NCM).

To separate and quantify the major milk proteins: α_{S1}-, α_{S2}-, β- and κ-Cn, and β-Lg in a single run a modification of the reversed-phase high-performance liquid chromatography (RP-HPLC) methods proposed by Visser et al. (1991) and Trujillo et al. (2000) was used (Jõudu, et al., 2008).

Genetic variants of κ-casein and β-lactoglobulin were determined by PCR-RFLP analysis (Sabre, 2003) in the Laboratory of Genetics of the Institute of Animal Sciences of the Estonian University of Life Sciences. Milk protein (α_{S1}-, β- and κ-Cn, and β-Lg) genotypes from Estonian Native cows were analysed at the Laboratory of Raw Milk, Munich University of Technology, Freising, Germany, by an isoelectric focusing/electrophoresis technique (Baranyi, et al., 1993).

Information on the birth, calving, and pedigree of the cows and daily milk yield (DMY), milk protein, fat, and lactose contents, and somatic cell count (SCC) data were obtained from the Estonian Animal Recording Centre.

Statistical analysis. Mixed linear model incorporating fixed effects of breed, parity, month of lactation, genotype of κ-Cn, and β-Lg, and assuming a first-order autoregressive variance structure of the repeated measurements of the individual cow were used for statistical evaluation of results (SAS INST. Inc., 2006). To study the effect of aggregate casein genotype the fixed effect of κ-Cn genotype was replaced with fixed effect of aggregate casein genotype in the model. The effects of content of α_{s1}-, α_{s2}-, β- and κ-Cn and β- Lg, the relative content of α_{s1}-, α_{s2}-, β- and κ-Cn in total casein, or the casein number (casein:protein), ratio of κ-Cn:β-Cn and ratio of κ-Cn:α_S-Cn were entered into the model to study the effect of milk proteins on rennet coagulation parameters (Jõudu, 2008).

Results and discussion. *Effect of milk protein composition*. Protein, a major component in most cheeses, has a significant influence on the MCP (Guinee, 2003). Rennet coagulation time shortened, and a firmer curd was formed, in parallel with increasing content of milk protein, primarily with the contents of the studied milk protein fractions (α_{S1}-, β- and κ-Cn, and β-Lg) including total casein and casein number. A stronger curd was formed when the proportions of α_{S2}-Cn and β-Cn in total casein were smaller or the κ-Cn:Cn, κ-Cn:β-Cn and κ-Cn:α_S-Cn ratios were higher (Jõudu, et al., 2008).

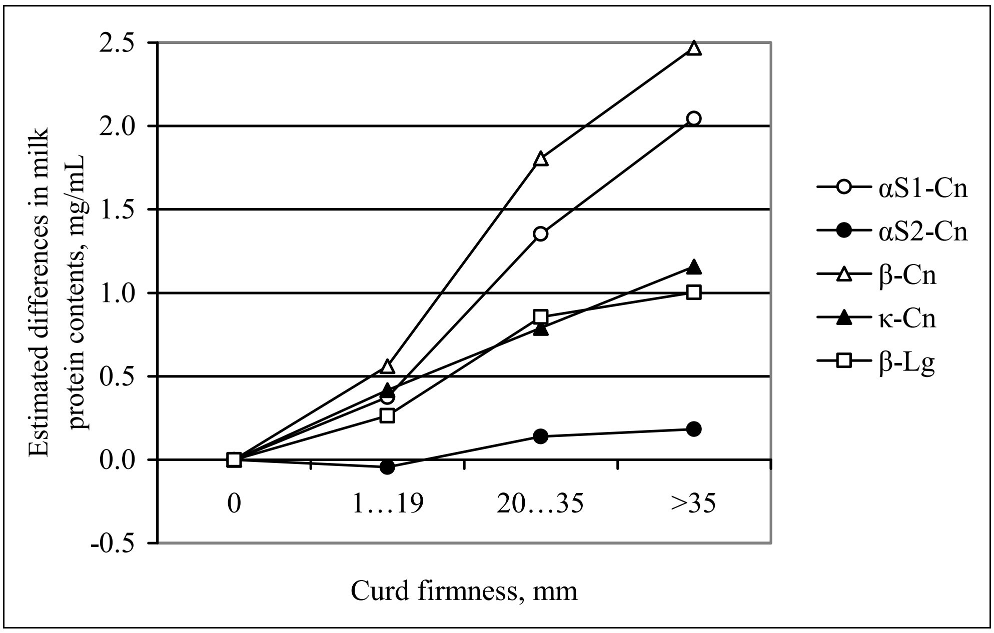

Figure 1. **Estimated differences in milk protein contents for the curd firmness classes** (E_{30} = 0 mm was class of comparison)

The contents of all studied milk proteins were significantly lower for noncoagulated (E_{30} = 0 mm) and poorly coagulated (E_{30} = 1…19 mm) milk as compared to normally and well coagulated milk ($E_{30} \geq 20$ mm; Figure 1). The relative amount of casein in total protein was higher in milk that formed a stronger curd. Noncoagulated milk, compared to well coagulated milk, had a higher content of α_{S2}-Cn and a lower content of κ-Cn in total casein, and the κ-Cn:β-Cn and κ-Cn:α_S-Cn ratios were also lower. The β-Cn ratio in total casein was higher for noncoagulated and poorly coagulated milk than that for well coagulated milk.

The results obtained from this study are similar to the results from Auldist et al. (2002) indicating that the effect of casein and κ-Cn contents on coagulation parameters is larger, but the effect of total milk protein content is smaller. A lower content of κ-Cn and its proportion in relation to α_S-Cn and β-Cn, in poorly coagulated milk, have also been observed by Wedholm et al. (2006). An increased rate of β-Cn in total casein has a negative effect on the rennet coagulation properties of milk (Jõudu, et al., 2008). St-Gelais and Hache (2005) also obtained similar results; they described the poorer milk coagulation properties of milk enriched with β-Cn powder. Dalgleish (1992) proposes a significant effect of κ-Cn content on milk coagulation properties, that an increase in κ-Cn concentration leads to a higher number of smaller casein micelles, which form a firmer curd than would be formed by larger micelles. In contrast, an increase in the ratio of β-Cn in total casein leads to the formation of larger micelles and the deterioration of the rennet coagulation properties of milk.

Effect of milk protein polymorphism. MCP were significantly ($P < 0.0001$) influenced by the κ-Cn genetic variants. MCP were better for the κ-Cn BB and worse for the κ-Cn AA, AE, and EE genetic variants (Figure 2). κ-Cn BB also exhibited the lowest percentage of NCM and samples that did not reach 20 mm curd firmness 30 min after enzyme addition.

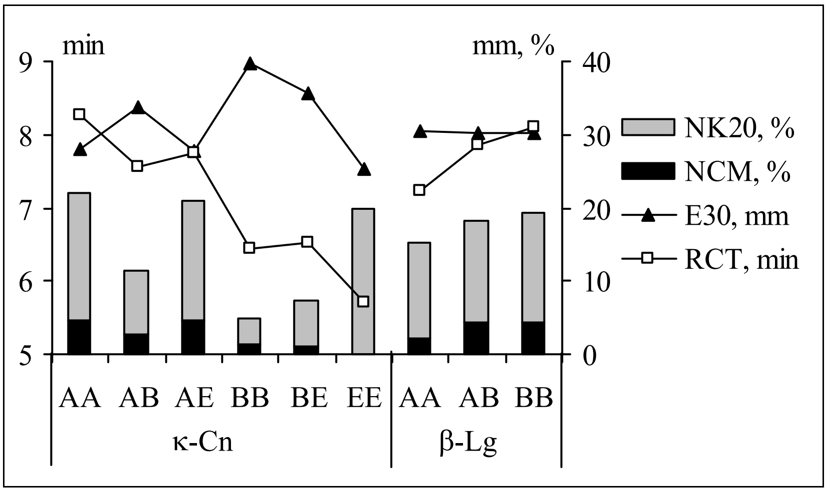

Figure 2. **Milk coagulation parameters for different κ-casein and β-lactoglobulin genotypes**

The favourable effect of κ-Cn B on the renneting properties of milk has been confirmed in several studies, as reviewed Jacob and Puhan (1992) and Buchberger and Dovč (2000). Reviewing the results of different studies, Ng-Kwai-Hang (1998) found that, comparing the κ-Cn B variant with the A variant, the decrease in coagulation time ranged between 10–40%, and the increase in curd firmness was within the range of 20–140%. The positive effect of κ-Cn B may be partly due to the higher fat and protein, primarily casein, contents in milk containing this variant (Ikonen, et al., 1999; Ng-Kwai-Hang, 1998). In our studies (Jõudu, 2008) milk with κ-Cn AB and BB variants contained more protein (3.48 to 3.58% and 3.50 to 3.70%, respectively) than means of all milk sampled (3.46 to 3.53%). Milk from cows having the κ-Cn AB and BB genotypes was, throughout the lactation, more suitable for cheese making than the mean of all milk (Kübarsepp, et al., 2005).

An overall effect of β-Lg genetic variants on the MCP was not clearly identified. Milk rennet coagulation time exhibited a tendency to be shorter, and percentages of noncoagulated milk samples and samples with poor coagulation properties (NK_{20}) were lower for the β-Lg AA genotype (Kübarsepp, et al., 2005). 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 Friesian.

Due to the close linkage of four casein genes in chromosome 6 within a region of about 250kb in cattle (Rijnkels, 2002), segregation of the α_S-Cn, β-Cn, and κ-Cn variants occurs nonindependently. Ikonen et al. (1999) reported that a possible reason for the conflicting results for the effect of single casein genotypes on milk production traits can be the close linkage of casein genes. They suggested the use of casein aggregate genotypes as a more appropriate way to estimate the effect of casein polymorphism than the use of individual casein genotypes. Of the 16 detected aggregate genotypes, 11 genotypes occurred at a frequency above 1%, 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 BB A^2A^2 AB (10.2%), were found among nearly two thirds of the analysed EN cows (Jõudu, et al., 2007). α_{s1}-Cn BC and CC genotype combinations occurred only with β-Cn A^1A^2 and A^2A^2. Similar aggregate genotypes have been to be frequent in the Swedish Red and White and Swedish Holstein breeds (Lunden, et al., 1997). The aggregate α_{S1}-β-κ-Cn genotype was found to have a significant ($P < 0.05$) overall effect on MCP among EN cows (Jõudu, et al., 2007). Two aggregate casein genotypes, CC A^2A^2 AB and BC A^1A^2 BB, significantly differed from others, to a large extent, in terms of better curd firmness (Figure 3), but they occurred at low frequency (0.8 and 2.5%, respectively). Among frequent aggregate genotypes, better rennet coagulation parameters were observed for BB A^1A^2 BB. 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 a shorter milk rennet coagulation time and to form firmer curd than those 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 the curd showed a tendency to be firmer and no noncoagulated milk were observed (Jõudu, et al., 2007). Among EN cows 78.9% of all noncoagulated milk samples originated from cows possessing the κ-Cn AA genotype. No noncoagulated milk samples were observed for κ-Cn BB, α_{S1}-Cn CC, or β-Cn A^1B genotypes (α_{S1}-Cn CC and β-Cn A^1B were represented only by one cow).

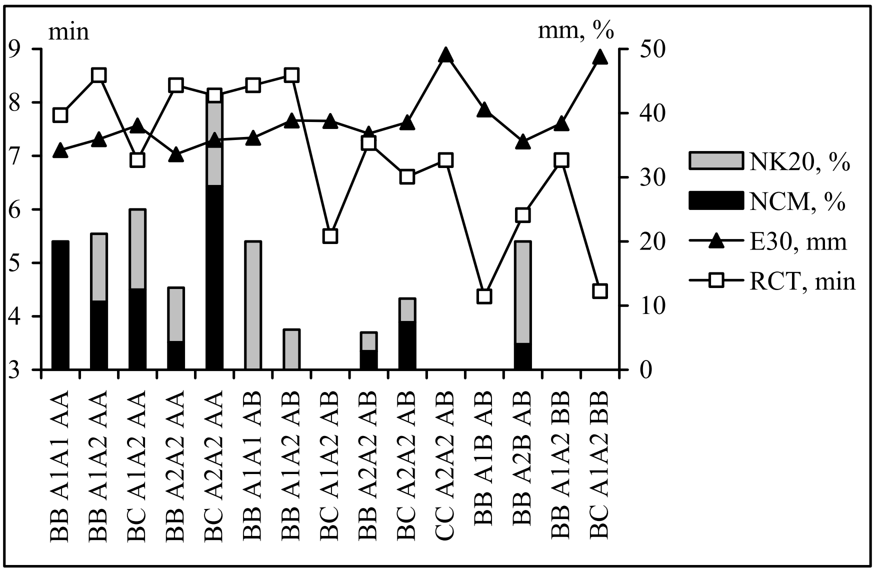

Figure 3. **Effects of aggregate α_{S1}-β-κ-casein genotypes on MCP**

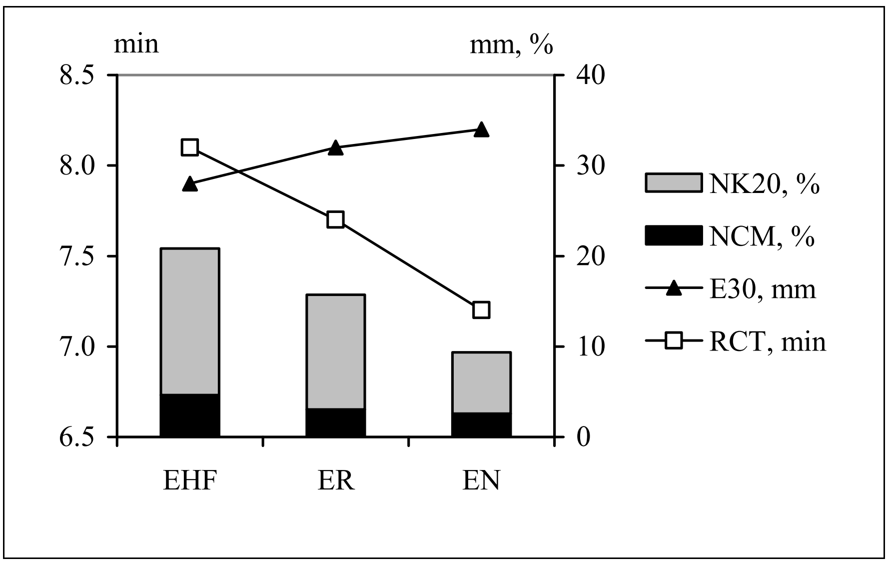

Figure 4. **Milk coagulation parameters for different breeds**

Effect of breed. Breed had a significant effect on MCP (Jõudu, 2008; Kübarsepp, et al., 2005). Improved MCP were recorded for ER and EN cows, compared to EHF cows (Figure 4). The percentages of noncoagulated milk samples and also percentage of milk samples with insufficient curd firmness ($0<E_{30}<20$ mm) were higher among EHF cows, and lower among EN and ER cows. Among all cows (n=1,269) percentages of cows that provided noncoagulated milk samples at least at once during lactation were 42% in EHF, 32% in ER and 22% in EN.

Differences in milk coagulation properties between breeds may be due to differences in milk composition that are attributable to variation in the genome. Several earlier studies (Tervala, et al., 1983; Macheboeuf, et al., 1993; Auldist, et al., 2002) associated better renneting properties among native breeds compared with the Holstein with a higher frequency of κ-Cn B allele. A positive effect of this allele was also shown in our studies on EN cows, which also showed a comparatively high frequency of the allele (Jõudu, 2008). A comparison of milk protein allele frequencies between the EN breed and the other dairy breeds raised in Estonia showed that EN's frequency of the favourable κ-Cn B allele resembled that of ER, but was higher than for EHF. The unfavourable κ-Cn E allele was found both among the commercial breeds EHF and ER, but not among EN cows in the current sampling. Results of earlier studies in Estonia (Тооме, 1972; Orasson, 2000) about allele frequencies of κ-Cn indicate that the κ-Cn B allele frequency was considerably decreased in the Estonian Holstein cows. The frequency of the κ-Cn B allele among ER and EN has remained at the same level Kübarsepp, et al., 2005).

Conclusions

Higher contents of milk protein, casein and all the studied protein fractions reduced the rennet coagulation time and formed a firmer curd. Milk formed a firmer curd when the proportion of α_{S2}-Cn and β-Cn in total casein was smaller, or the proportion of κ-Cn in total casein was higher. In addition, a higher proportion of κ-Cn with respect to α_{S1}-Cn and β-Cn assisted in forming a firmer curd.

All measured rennet coagulation parameters were significantly better for the κ-Cn BB, and worse for the κ-Cn AA, AE, and EE genotypes. κ-Cn BB also exhibited the lowest percentage of poorly and non-coagulated milk samples. Noncoagulated milk originated mainly from cows possessing κ-Cn AA genotype. Aggregate casein genotype had a significant overall effect on rennet coagulation parameters. Better rennet coagulation properties were found for aggregate α_{S1}-β-κ-casein genotypes CC A^2A^2 AB and BC A^1A^2 BB, among frequent genotypes for BB A^1A^2 AB. 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.

Better MCP among ER and EN cows, compared to EHF cows, are probably partly explainable by a higher frequency of the κ-casein B allele, and ER cows also recorded higher contents of milk proteins, compared to EHF cows.

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References

1. Auldist M., Mullins C., O'Brien B., O'Kennedy B.T., Guinee T. Effect of cow breed on milk coagulation properties. Milchwissenschaft. 2002. Vol. 57. P. 140-143.

2. Baranyi M., Bösze Z.S., Buchberger J., Krause I. Genetic Polymorphism of Milk Proteins in Hungarian Spotted and Hungarian Grey Cattle: A Possible New Genetic Variant of ß-Lactoglobulin. Journal of Dairy Science. 1993. Vol. 76. P. 630-636.

3. Buchberger J., Dovč P. Lactoprotein genetic variants in cattle and cheese making ability. Food Technologie and Biotechnologie. 2000. Vol. 38. P. 91-98.

4. Dalgleish D.G. Bovine milk protein properties and the manufacturing quality of milk. Livestock Production Science. 1992. Vol. 35. P. 75-93.

5. Guinee T.P. Role of protein in cheese and cheese products. In P.F. Fox and P.L.H. McSweeney (eds.): Advanced Dairy Chemistry 1: Proteins, Part B, 3rd ed. Kluver Academic/Plenum Publisher, 2003. P. 1083-1174.

6. Ikonen T., Ahlfors K., Kempe R., Ojala M., Ruottinen O. Genetic parameters for the milk coagulation properties and prevalence of noncoagulating milk in Finnish dairy cows. Journal of Dairy Science. 1999. Vol. 82. P. 205-214.

7. Ikonen T., Ojala M. Effect of milk protein genotypes on milk renneting properties assuming alternative models. IDF Bulletin No. 304. 1995. P. 16-17.

8. Ikonen T., Ojala M., Ruottinen O. Associations between milk protein polymorphism and first lactation milk production traits in Finnish Ayrshire cows. Journal of Dairy Science. 1999. Vol. 82. P. 1026-1033.

9. Jakob E., Puhan Z. Technological properties of milk as influenced by genetic polymorphism of milk proteins. A review. International Dairy Journal. 1992. Vol. 2. P. 157-178.

10. Jõudu I. Effect of milk protein composition and genetic polymorphism on milk rennet coagulation properties. PhD Thesis, Estonian University of Life Sciences. 2008. 114 pp.

11. Jõudu I., Henno M., Kaart T., Püssa T., Kärt O. The effect of milk proteins contents on the rennet coagulation properties of milk from individual dairy cows. International Dairy Journal. 2008. Vol. 18. P. 967-970.

12. Jõudu I., Henno M., Värv S., Kaart T., Kalamees K., Kärt O. Milk protein genotypes and milk coagulation properties of Estonian Native cattle. Agricultural

and Food Science. 2007. Vol. 16. P. 222-231.

13. Kübarsepp I., Henno M., Mihhejev K., Kärt O. Factors influencing milk coagulation properties. Proceedings "Research for Rural Development", Jelgava, Latvia, May 21-24, 2003. P. 48-52.

14. Kübarsepp I., Henno M., Viinalass H., Sabre D. Effect of k-casein and ß-lactoglobulin genotypes on the milk rennet coagulation properties. Agronomy Research. 2005. Vol. 3(1). P. 55-64.

15. Lunden A., Nilsson M., Janson L. Marked Effect of β-Lactoglobulin polymorphism on the ratio of casein to total protein in milk. Journal of Dairy Science. 1997. Vol. 80. P. 2996-3005.

16. Macheboeuf D., Coulon J.-B., D`Hour P. Effect of breed, protein genetic variants and feeding on cow's milk coagulation properties. Journal of Dairy Research. 1993. Vol. 60. P. 43-54.

17. Malossini F., Bovolenta S., Piras C., Rosa M.D., Ventura W. Effect of diet and breed on milk composition and rennet coagulation properties. Annales of Zootechniky. 1996. Vol. 45. P. 29-40.

18. Ng-Kwai-Hang K.F. Genetic polymorphism of milk proteins: Relationships with production traits, milk composition and technological properties. Canadian Journal of Animal Science. 1998. Vol. 78. P. 131-147.

19. Ng-Kwai-Hang K.F., Politis I., Cue R.I., Marziali A.S. Correlations between coagulation properties of milk and cheese yielding capacity and cheese composition. Journal of Canadian Institute of Food Science and Technology. 1989. Vol. 22. P. 291-294.

20. Orasson A. Genetic polymorphism of Estonian Holstein milk proteins. Proceedings of the 6th Baltic Animal Breeding Conference, Latvia, Jelgava, 2000. P. 33-37.

21. Rijnkels M. Multispecies comparison of the casein gene loci and evolution of casein gene family. Journal of Mammary Gland Biology and Neoplasia. 2002. Vol. 7. P. 327-345.

22. Sabre D. Molekulaargeneetilise informatsiooni kasutamise võimalusi tõuaretuses. /The possibilities for using molecular genetic information in animal breeding. MSc Thesis. Estonian Agricultural University, 2003. 72 pp. (in Estonian).

23. St-Gelais D., Hache S. Effect of β-casein concentration in cheese milk on rennet coagulation properties, cheese composition and cheese ripening. Food Research International. 2005. Vol. 38. P. 523-531.

24. Tervala H.-L., Antila V., Syväjärvi J., Lindström U.B. Variations in the renneting properties of milk. Meijeritieteellinen Aikakauskirja. 1983. Vol. XLI (2). P. 24-33.

25. Trujillo A.-J., Casals J., Guamis B. Analysis of major ovine milk proteins by reversed-phase high-performance liquid chromatography and flow injection analysis with electrospray ionisation mass spectrometry. Journal of Chromatography A. 2000. Vol. 870. P. 371-380.

26. Tyrisevä A.-M., Ikonen T., Ojala M. Repeatability estimates for milk coagulation traits and non-coagulation of milk in Finnish Ayrshire cows. Journal of Dairy Research. 2003. Vol. 70. 91-98.

27. Visser S., Slangen C.J., Rollema H.S. Phenotyping of bovine milk proteins by reversed-phase high-performance liquid chromatography. Journal of Chromatography A. 1991. Vol. 548. P. 361-370.

28. Wedholm A., Larsen L.B., Lindmark-Månssom H., Karlsson A.H., Andrén A.Effect of protein composition on the cheese-making properties of milk from individual dairy cows. Journal of Dairy Science. 2006. Vol. 89. P. 3296-3305.

29. Тооме А.А. Генетиеский полиморфизм β-лактоглобулинов и казеинов и возможности его использования в селекции пород крупного рогатого скота в Эстонской ССР. Автореф. канд. с.-х. наук, Тарту, 1972. 27 с.

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