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MILK PROTEIN K-CASEIN GENE AND ITS ASSOCIATION TO MILK PRODUCTIVITY TRAITS IN LATVIAN BROWN CATTLE

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Abstract

The objective of this study was to evaluate the k-casein allele frequency in Latvian Brown cows (n=30), Latvian Brown bulls (n=19) which are Latvian genetic resources breed and other red breeds bulls (n=38) which are using for artificial insemination (AI) of the Latvian Brown cows. In Latvian Brown cattle were found A, B allele and in other breeds bulls were found A, B and E allele. The k-casein allele frequencies A and B were about 0.8 and 0.2 in Latvian Brown cows and bull's population, the k-casein E allele was not found in LB population. The k-casein A allele (genotypes AA, AB and AE) were associated with highest average breeding values of Red breeds bulls in milk yield and low breeding values in protein content.

Key words: *Latvian Brown breed, genetic resources, k-casein gene*

1. INTRODUCTION

In Latvia, the dairy industry is the most important branch of the agriculture sector. Two dairy cattle breed Latvian Brown (LB) and Holstein Black and White are selected for milk productions. The LB is Latvian native breeds; with population size about 90 thousand cattle. Average milk production in 2008 was 5103 kg milk yield per lactation with fat content 4.44% and protein content 3.36% (http://www.ldc.gov.lv/?u=lv/ciltsdarbs/parraudziba/piena/govis/govis). The breed is characterized by longevity and by resistance to the diseases.

Cows with brown hair at first time were mentioned in 18th century. In the end of 19th century native cattle was improved with Angler breed. In the beginning of 20th century Danish Red breed was accepted to improve the native breed. In 1922 Latvian brown was confirmed as a separate dairy breed. During the last century, animals and semen of Danish Red, Angler, and Danish Red with bloodness of Swiss Brown and Holstein Red, and Swedish Red-and-White breeds have been used in order to improve LB production traits (Strautmanis, Lidaks, 2004). In the last time is very popular to use Europe breeds, such as Danish Red, Holstein Red-and-White, and Swedish Red for artificial insemination (AI) of the Latvian brown cows. Now we should work not only in direction to improve LB breed production traits, but us well to preserve Latvian brown cattle breeds. According to Latvian Agriculture Data Processing centre data the LB cows under recording in 2008 were 63 163, and only 99 LB cows were registered as genetic resources animals.

In the next time for Latvia milk yield traits are economically more important. Most selection of dairy cattle has been based on quantitative traits such as milk yield, fat yield and in the last time selection

for protein yield too. The main objective of genetic improvement is to obtain the true criteria for the selection and matching for mating of animals, determine the most essential factors affecting the productivity and evaluate additive genetic values of animals. Genetic improvement of quantitative traits is relatively slow, productivity traits are affected by many genes and depend on the environmental factors, can only be measured in one sex during productivity time.

The molecular techniques applied to genetic in conjunction with animal breeding techniques could give greater genetic gain by determining the potential of an animal, even before it is expressed phenotipically. A genetic marker serves to relate favourably alleles for quantitative characteristics with information about the individual mode of action and their interaction of gene, helping to understanding of the quantitative variations and their practical use in animal husbandry.

2. GENETIC POLIMORPHISMS AT THE K-CASEIN LOCUS

In cattle, four genetic variants (A, B, C and E) have been detected at the k-casein locus. The kappa-casein gene (CSN3) presents two common genetic variants, A and B, and these alleles differ by two amino acids substitutions, Thr136/Ile and Asp148/Ala (Lin et al., 1992). A and B alleles present in all breeds with variable frequency. A variant prevails in Holstein Friesian, Ayrshire, Danish Red and its frequency is near 60-85% (Ikonen et.al, 1996; Zaton-Dobrovolska et al., 2006). B variant is predominant in Jersey breed (McLean et al., 1987).

K-casein C is less common, but was found in many breeds. Besides Grey Alpine and Italian Brown, it was detected in German Simmental (Seibert et al., 1987), German Fleckvieh (Erhardt, 1989) and Red Holstein - Friesian (Bovenhuis and Arendonk, 1991). K-casein E, nevertheless is considered a not very common variant, in Finnish Ayrshire was found also with high frequency (~30%) (Ikonen et al., 1996) recently this variant has been detected also in Czech Fleckvieh (Kucerova et al., 2006), Czech Red and German Red breeds (Zaton-Dobrovolska et al., 2007).

The polymorphism of the milk k-casein gene has been associated to differences in milk productivity traits and milk coagulation properties (MCP). Several studies have reported results concerning the effect of k-casein polymorphism on milk-production traits are inconsistent.

Among European dairy breeds, the k-casein A allele is associated with a higher milk production. (Bovenhuis et. al., 1991, Ikonen T. et al., 1999). Allele B is associated with higher milk protein content and milk quality (Boettcher et al., 2004; Caroli et al., 2004).

In Finish Ayrshire cows investigation by Ikonen et al. (1999), k-casein EE, BE, and AE genotypes is associated with the highest milk production and low protein content, BB and AB genotypes is associated with the highest protein content and low milk production.

Results of Sabour et al. (1996) study (n=566) showed that k-casein loci had no significant effects on milk, fat, and protein ETA of the Canadian Holstein bulls. The contrast between the k-casein genotypes were not significant for productivity traits within 371 dairy cows of the Swedish Red and White breed and 204 cows of the Swedish Holstein breed (Lunden et al., 1997).

3. MATERIAL AND METHODS

Genotyping of k-casein gene were analysed in Latvian Brown breed genetic resources - 30 lactating Latvian Brown cows and 19 Latvian Brown bulls by Polymerase Chain Reaction and Restriction Fragment Length Polymorphism methods (PCR-PFLP). DNS was obtained from blood sample of

cows and semen of sires. 38 bulls of 4 red breed German Red (GR), Danish Red (DR), Swedish Red (SR) and Holstein Red and White (HRW) from one AI station were included in the study.

The laboratory investigation was done for cows in Genetic laboratory of Latvia University of Agriculture (LUA) for LB cows and for bulls in Genetic Laboratory of Estonian University of Life Science.

PCR reaction and DNA amplification for cow's population

The primers used for amplification of the k-casein gene were described by Alexander LJ, at al (1998), with the following nucleotide sequence: K1- 5`- CAC GTC ACC CAC ACC CAC ATT TAT C -3`; K2- 5`- ATT AGC CCA TTT CGC CTT CTC TGT -3`; KY- 5`- CGT TGT CTT CTT TGA TGT CTC C -3`. The primers were used in pairs such as K1 with K2, K1 with KY.

Amplification reactions were done in a final volume of 25 µL, containing 50 ng DNA, 10 pMol of each primer, 0.5 U Taq polymerase, 1X PCR buffer [10 mM Tris-HCl (pH 9.0), 1.5 mM MgCl2 and 50 mM KCl], 100 µM dNTPs.

The reaction followed the sequence:

95◦C 60 s

95◦C 60 s

56◦C 60 s

72◦C 60 s

30 cycles

72◦C 7 min

After amplificationed gene fragments, they were subjected to digestion by Hind III restriction enzymes in a total volume of 20 µL (2 µL enzyme buffer, 0.2 µL enzyme, 7.8 µL H2O and 10 µL amplification fragments). After digestion, the fragments were subjected to electrophoresis in an (3.5%) agarose gel, TBE 1X buffer (Tris-HCl 1MpH 7.4; EDTA 0.5MpH8.0 and 10.8 g boric acid) with ethydium bromide, at 60 V for approximately 2.5 h. Visualization of the bands was done under ultraviolet lighting.

Digestion of fragment K2 generated two fragments of 280 and 120 bp, respectively. For fragment KY generated two fragments, however, these were of 180 bp and 100 bp, respectively.

4. RESULTS AND DISCUSSION

In the LB breed presented the presence of two alleles was observed at the κ-casein locus: A and B, of which allele A was more frequent. The LB population has a structure that is typical for red dairy cattle population. Similar studies, covering the Red Cattle breeds showed the frequency of alleles of κ-casein to range between 0.69 and 0.81 for allele A, 0.081 and 0.307 for allele B and 0.012 and 0.307 for allele E (table1). The k-casein E allele was not found in LB cow's population.

Table 1. Allele frequency at the k-casein locus in Latvian brown and other red breed cows

κ-casein allele	Latvian Brown	Polosh red	Finish Ayrshire	Lithuanian Red	German Red	Danish Red
	n = 30	n = 313	n = 20 990	n = 181	n = 28	n = 169
A	0.833	0.690	0.612	0.714	0.814	0.810
B	0.167	0.310	0.081	0.265	0.174	0.190
E	-	-	0.307	0.021	0.012	-
Reference	-	Erhardt et al, 1997	Ikonen et.al, 1996	Pečiulaitiene, 2005	Zaton-Dobrovolska et al., 2007	Zaton-Dobrovolska et al., 2006

The k-casein E allele was not found in LB cow's population.

The frequencies of k-casein genotypes AA and AB were 0.733 and 0.200, respectively. Only some cows had preferable genotype BB, genotype EE was found not at all. In LB cow's population the k-casein AB and AA genotypes were associated with the higher fat and protein content and low milk yield (table 2).

Table 2. Milk productivity traits 1st lactation LB cows

Genotype	Genotype frequency	Milk productivity traits $\bar{x} \pm s_{\bar{x}}$		
		Milk yield, kg	Protein content, %	Fat content, %
AA	0.733	4029.8±733.19	3.25±0.285	4.48*±0.264
AB	0.200	4371.8±649.94	3.39*±0.113	4.29±0.212
BB	0.067	4633.0±179.61	3.02*±0.198	3.90*±0.467

* $p<0.05$

Several study have reported that K-casein BB genotypes related to the higher protein content (Ikonen et.al, 1999) and milk coagulation properties (RCT and / curd firmness) (Patil M.R. et al., 2003; Kübarsepp I., et al., 2005). Marziali and Ng-Kwai-Hang (1986) reported a cheese production can be increased by 10 percent if milk is from cows of the k-casein genotype BB, when compared with milk from AA animals.

Table3. Allele and genotype frequency at the k-casein locus in Latvian brown bulls breed and breeds, which is using for AI of LB breed

κ-casein allele and genotype frequency	Latvian brown n=19	LB with different n=6	German Red n=4	Danish Red n=15	Swedish Red n=4	Holstein Red and White n=9
A	0.816	0.583	0.875	0.567	0.750	0.611
B	0.184	0.167	-	0.300	-	0.222
E	-	0.250	0.125	0.133	0.250	0.167
AA	0.632	0.333	0.750	0.267	0.500	0.333
AB	0.368	0.167	-	0.467	-	0.333
BB	-	-	-	0.067	-	-
AE	-	0.333	0.250	0.133	0.500	0.223
BE	-	0.167	-	-	-	0.111
EE	-	-	-	0.067	-	-

According to research results in the LB bulls population were found two k-casein A and B allele, in the other investigated breeds were found A, B and E allele (Table 3). The frequency of E allele was between 0.133 for Danish Red bulls and 0.25 for Swedish Red. K-casein AA genotype was found in all six red breeds and at relatively high frequency 0.75-0.50 for German Red, Latvian brown, and Swedish Red and at low frequency 0.267-0.333 for Danish Red and Holstein Red and White. The preferable BB genotype was found only in Danish Red bull's population.

Average breeding values of LB genetic resources bulls was positive in content parameters but negative in milk, protein and fat yield parameters. In the LB improve the average breeding values for milk yield was higher and positive while fat and protein content breeding values were negative. The negative LB sires breeding values of milk yield could be due the bull's evaluation were done previous then for improved bulls (Table 4).

Table 4. The relation between genotypes of k-casein gene and LB breed bulls breeding values of milk productivity traits

Genotype	Milk yield, kg	Fat		Protein	
		kg	%	kg	%
Latvian Brown, genetic recourses					
AA	-257.5	-7.75	0,09	-6	0,06
AB	-256.3	-13	0,03	-6,6	0,05
Latvian Brown improved by German Red, Danish Red, Holstein Red and White					
AA	492,5	8,25	-0,25	12,0	-0,09
AB	419,0	44,8	0,49	17,1	-0,06
AE	910,0	19,0	-0,37	23,5	-0,12
BE	293,0	9,9	-0,15	18,8	0,10

Two genotypes AA and AB were found in LB breed there are no significant differences between breeding values for milk productivity traits between genotypes. Four genotypes AA, AB, AE and BE were found in LB improve breed. A significant effect of AA genotype was found on milk yield breeding values, the breeding values were higher for AA genotype than for other. The k-casein BE genotype was associated with higher protein yield and protein content genetic values (Table 4).

Kučerová et al. (2006) found a lover breeding values for milk yield associated with genotype AA and high breeding values for protein content associated with genotype BB in Czech Fleckvieh breed. Ikonen et al. (1999) reported the associations between the k-casein EE, BE and AB genotypes and high milk production were true only in certain genotype combinations between k-casein and β-casein.

Only two genotypes AA and AE were analysed in SR and HRW breeds and genotypes AA and AB in DR breed because the HRW and DR young bulls with other genotypes have not yet breeding values estimation. Average breeding values of SR sires was positive for milk productivity traits except protein content breeding values for AA and AE genotypes. HRW sires the average breeding values for yield traits was positive while fat and protein content breeding value were negative (Table 5).

Table 5. The relation between genotypes of k-casein gene and red breed bulls breeding values of milk productivity traits

Genotype	Milk yield, kg	Fat		Protein	
		kg	%	kg	%
Swedish Red (SR)					
AA	495,5	17,8	0,08	23,9	-0,14
AE	599	12,1	0,27	12,7	-0,13
Holstein Red and White (HRW)					
AA	507	4,7	-0,28	9,4	-0,13
AE	772	13,6	-0,32	24	-0,02
Danish Red (DR)					
AA	825,5	13,8	-0,41	16,1	-0,21
AB	449,8	15,5	-0,09	12,2	-0,06
Average breeding value of Red breeds bulls					
AA	608,7	11,8	-0,26	15,6	-0,08
AB	449,8	15,5	-0,09	12,2	-0,06
AE	632,0	14,6	-0,23	17,0	-0,07

The k-casein AA, AB and AE genotypes were associated with highest average milk yield breeding values of Red breed's bulls and low protein content breeding values. The breeding values for milk yield were in range from 4449.8 kg to 632.0 kg.

5. CONCLUSIONS

In Latvian Brown cows and bulls genetic resourses populations were found k-casein A and B alleles and genotipes AA and AB. There are no significant differences in milk productivity traits between genotypes AA and AB. The preferable BB genotype was not found in LB population. The k-casein locus had effect on protein yield and protein content, the k-casein BE genotype was associated with higher protein content and protein yield breeding values in LB improve and investigated Red breeds bulls. The k-casein A allele (genotypes A, AB and AE) were associated with highest average milk yield breeding values of Red breeds bulls and low protein content breeding values.

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REFERENCES

Bovenhuis H., Van Arendonk J. A. M., Korver S. (1992) Associations between milk protein polymorphisms and milk production traits. J. Dairy Sci. 75, pp.2549–2559.

Bovenhuis H., van Arendonk J.A.M. (1991) – Estimation of milk protein gene frequencies in crossbred cattle by maximum likelihood. Journal of Dairy Science, 74, pp. 2728-2736.

Erhardt G. (1989) k-Kaseine in Rindermilch – Nachweis eines weiteren Allels (k-CnE) in verschiedenen Rassen. Journal of Animal Breeding and Genetics, 106, pp. 225-231.

Ikonen T., Ojala M., Ruottinen O. (1999) Associations between milk protein polymorphism and first lactation milk production traits in Finnish Ayrshire cows. Journal of Dairy Science Vol. 82 No. 5 pp.1026-1033

Ikonen T., Ruottinen O., Erhardt G., Ojala M. (1996) – Allele frequencies of the major milk proteins in the Finnish Ayrshire and detection of a new k-casein variant. Animal Genetics, 27, 179-181.

Kübarsepp, I., Henno M., Viinalass H., Sabre D. (2005) Effect of κ-casein and ß-lactogobulin genotypes on the milk rennet coagulation properties. Agric. Res. 3, pp. 55–64.

Kučerová J., Matějíček A., Jandurová O.M., Sorensen P., Němcová E., Štípková M., Kott T. Bouška J., Frelich J. (2006) Milk protein gene CSN1N1 CSN1N1 CSN2 CSN3, LGB and their relation to genetic value of milk production parameters in Czech Fleckvieh. Czech J. Anim. Sci., 51, 6, pp. 241-247.

Lin, C.Y., M.P. Sabour, A.J. Lee. 1992. Direct typing to milk proteins as an aid for genetic improvement of dairy bulls and cows: A review. Animal Breeding Abstracts, 60: 1-10

Lundén A., Nilsson M., Janson L. (1997) Merked effect of β-lactoglobulin polymorphism on the ratio of casein to total protein in milk. Journal of Dairy Science Vol. 80, pp. 2996-3005

Marziali AS., Ng-Kwai-Hang K.F. (1986) Effects of milk composition and genetic

polymorphism on cheese composition. J.Dairy Sci., 69, pp. 2533-2542 .

Patil M.R., Borkhatriya V.N., Boghra V.R., Sharma R.S. (2003) Effect of bovine milk κ-casein genetic polymorphs on curd characteristics during cheddar cheese manufacture. Journal of food science and technology Vol. 40, no 6, pp. 582-586.

Sabour M. P., Lin C. Y. , Lee A. J., Mcallister A. J. (1996) Associations between milk protein genetic variants and genetic values of Canadian Holstein bulls for milk yield traits. Journal of Dairy Science 79, pp. 1050-1056.

Seibert B., Erhardt G., Senft B. (1987) – Detection of a new k-casein variant in cow's milk. "Animal Genetics", 18, 269-272.

Strautmanis D., Lidaks M. (2004) Possibilieties of Maintenance and Reproduction of local Latvian Brown dairy breed. "Animal Breeding in the Baltic conference", Tartu, Estonia, pp.91-95.

Zatoń-Dobrovolska M., Čitek J., Filistowicz A., Řehout V., Szulc T. (2007) Genetic distance between the Polish Red, Czech Red and German Red cattle estimated based on selection loci of protein coding genes and DNA microsatellite sequences. Animal Science Papers and Reports vol. 25 no.1, Institute of Animal Genetic and Animal Breeding, Jastrzebiec, Poland, pp. 45-54.

Zatoń-Dobrowolska M., Čitek J., Filistowicz A., Řehout V., Szulc T. (2006) An estimation of the genetic distance between Polish Red and other Red Cattle breeds on the basis of selected milk protein loci. EJPAU 9(2), #10. Available Online: http://www.ejpau.media.pl/volume9/issue2/art-10.html