Short communication

Kappa-casein genotypic frequencies in Russian breeds Black and Red Pied cattle

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Abstract
Casein is a family of milk proteins that exists in several molecular forms and is the main protein present in the bovine milk. The B variant of bovine k-casein is reported to be favorable for quality and quantity of cheese derived from milk and considered to be included in breeding strategies of dairy cattle. Genotypes of 72 Russian Black Pied and 80 Red Pied cows were determined for kappa casein locus by restriction fragment length polymorphism analysis (PCR-RFLP) of amplified DNA. A 530 bp. fragment of the genomic bovine kappa casein gene was amplified by PCR. Digests by Hind III thus genotypes AA, AB and BB, were recognized by agarose gel electrophoresis. This technique was used to determine the kappa casein allelic frequency in Black and Red Pied dairy herds. Estimated gene frequencies were 0.83, 0.69 for A, and 0.17, 0.31 for B alleles, for Black and Red Pied breeds, respectively. The heterozygosis of 0.28, 0.50 for Black and Red Pied breeds, respectively were observed. This molecular genetic technique based on molecular markers allows direct genotyping for milk Kappa casein with certainty and accuracy in bulls and females an can be used in programs of dairy cattle improvement. Therefore, an early and precise identification of milk protein genotypes should have a direct impact on dairy cattle breeding strategies.
Keywords: *Kappa casein; Polymorphism; Cattle; PCR-RFLP; Milk production*

Milk protein polymorphisms have received considerable interest because of their potential use as an aid to genetic selection and to genetic characterization of bovine breeds (Del Lama and Zago, 1996; Golijow *et al.*, 1996; 1999; Kemenes *et al.*, 1999). The k-casein variants *A and B* differ in amino acid 136 and 148 (Lin *et al.*, 1992). In position 136, Thr (ACC) is changed for Ile (ATC) and in position 148, Asp (GTA) is changed for Ala (GCT). Several studies have reported that some of these bovine protein variants, particularly k-casein are associated with lactation performance and have a major influence on milk composition and its processing properties, including cheese yield (Kastonina *et al.*, 2004; Denisenko and Kalashnikova, 2004; Konovalova *et al.*, 2004; Romonasova, 1999; Marziali and Ng-Kwai-Hang, 1986; Aleandri *et al.*, 1990). Relationships between genotypes for different milk proteins and yield traits have been reported by several authors (Lin *et al.*, 1992). Although reports on the association between k-casein genetic variants and milk yield are somewhat conflicting. Whereas Strzalkowska *et al*., 2002, Aleandri *et al.* (1990) found no significant associations; results from other group (Ikonen *et al.*, 1999) indicated that there is indeed a relationship. However, because of economic interests, it has been suggested that favourable milk protein genotypes, k-casein BB, should be included into the criteria for selection of dairy cattle. In a study by Bovenhuis *et al.* (1992), k-casein genotype had a significant effect on milk production ($p<0.001$), with cows of the BB genotype producing 173 Kg less milk than AA cows. Furthermore, k-casein genotypes had a highly significant effect on protein content ($p<0.001$), such that k-casein BB cows produced milk with a 0.08% higher protein content than that of AA cows. The same authors found that, the effect of k-casein genotype was not significant on fat content, while for fat yield, the *B* allele was associated with a significantly lower production of fat when compared with the *A* allele. Genotyping of milk proteins, such as k-casein,

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can be performed by electrophoresis, directly from milk samples, as the expression of caseins occurs only during the lactation phase in mammary gland cells. Therefore, the use of electrophoresis for genotyping of milk proteins is strongly limited because it can only be used in cows in the lactation stage. With newly developed techniques based on DNA analysis, which include polymerase chain reaction and restriction fragment length polymorphisms (PCR-RFLP) methods (Medrano and Aguilar- Cordova, 1990; Denicourt *et al.*, 1990), it is now possible to determine the k-casein genotype of all individuals in a given population under selection, regardless of sex, age or physiological status. As a result, it is now possible to include information on milk protein genotypes into selection programs, which should result in more accurate predictions of breeding values of animals to be selected, and thus improve response to selection. Genetic variability in the k-casein locus has been reported for several breeds, with allelic frequencies incorporated into studies on genetic diversity among breeds (Golijow *et al.*, 1996; Del lama and Zago, 1996; Kemenes *et al.*, 1999).

The objectives of this work was to study gene frequencies at the k-casein locus in Black and Red Pied cattle, and compare them with those reported for different commercial cattle breeds.

For this purpose since 1977 began the work on the creation of new breed Red Pied in Russia. The Red Pied breed cattles were created via the crossing of Simmental cows with the bulls of the Red Pied Holstein. Russian Red Pied with a population of approximately 100,000 animals, mainly raised in the Krasnodar, Stavropoul, Omsk, Rostov Stats and other western states of Russia.

The Black Pied breed developed from crossing the local cattle in various areas with the Dutch Black Pied and East Friesian breeds. Some animals were imported from Germany, the Netherlands, Estonia and Lithuania during 1930-40 and distributed in various parts of the Russia. Russian Black Pied with a population of about 10,000,000 animals, have a broad geographical distribution, raised in various parts of the Russia.

The of Black Pied breed is noted for high milk production (the highest among the dairy breeds), good conformation and good beef qualities. Due to the high productivity, adjustment to machine milking, well-defined beef features and the ability to acclimatize, the population of this breed is increasing year by year.

72 Black Pied and 80 Red Pied cattle were included in this analysis. Blood samples for DNA genotyping were collected from the jugular vein.

DNA was isolsted from whole blood done with a method described by Denicourt *et al*. (1990) and DNA samples were typed using the PCR-RFLP method. To analyze the k-casein (k-CN) locus, a 530-bp fragment covering the sequence containing the mutation site was amplified according to the procedure proposed by Medrano and Aguilar-Cordova (1990). The primer sequences used for the amplification of K-CN were as follows: Cas A 5´-ATA GCC AAA TAT ATC CCA ATT CAG T-3´, Cas B 5´- TTT ATT AAT AAG TCC ATG AAT CTT G 3´. Samples were amplified for 35 cycles under the following conditions: 95°C-1 min (denaturation); 58°C- 1 min (primer annealing); 72°C- 1 min (primer extension). The amplification product was digested with *Hind*III restriction endonuclease (MBI Fermentase, Lithuania) at 37°C for 3h, to distinguish between *A* and *B* alleles. The restriction fragments were separated in agarose gel (3%) and stained with ethidium bromid. Allele frequencies were determined by gene counting. A Chi-square test was performed to evaluate if the population was in Hardy-Weinberg equilibrium.

Identification of *A* and *B* alleles of k-casein was performed by amplification of a DNA fragment of 530 bp, by the PCR-RFLP method. The DNA fragment amplified from allele *B* shows only one restriction site, resulting in two fragments of 400 and 130 bp. Allele *A* was characterized by the presence of one fragment, corresponding to 530-bp. Genotypic frequencies for Black Pied were 68.89, 28.22 and 2.89 for AA, AB and BB, respectively; and were 44.12, 50.00 and 5.88 for AA, AB and BB, respectively for Red Pied cattle.

Table 1. Polymorphism at the K-casein locus in Russian breeds Black and Red pied cattle.

Breed	Genotype	Frequency	Allele (frequency)	χ^2
Black Pied	AA	68.89	A-0.83	0.7ns
	AB	28.22		
	BB	2.89	B-0.17	
Red Pied	AA	44.12	A-0.69	2.57ns
	AB	50.00		
	BB	5.88	B-0.31	

ns= not significant

Table 2. Quantity and quality of milk production in cows with different k-casein genotype.

Breeds	Genotype	Milk yield (Kg)	Fat		Protein	
			Kg	%	Kg	%
Black Pied	AA	7807.04 (725.53)	343.45 (32.92)	4.42 [a] (0.20)	243.58 (20.59)	3.12 [a] (0.09)
	AB	6996.77 (719.35)	296.20 (32.64)	4.30 [a] (0.19)	218.19 (20.42)	3.12 [a] (0.09)
	BB	6821.83 (859.41)	324.13 (38.99)	4.79 [b] (0.23)	226.27 (24.39)	3.31 [b] (0.10)
Red Pied	AA	6709.24[a] (185.62)	247.29[a] (17.35)	3.58[a] (0.04)	219.39 (20.59)	3.27 (0.09)
	AB	6182.38[a] (163.09)	236.54[b] (16.46)	3.71[b] (0.04)	200.31 (20.42)	3.24 (0.09)
	BB	7239.00[b] (287.99)	260.03[c] (21.40)	3.63[a] (0.07)	237.4 (24.39)	3.28 (0.10)

[a] within columns frequencies bearing the same superscripts differ significantly at P≤0.05. Figures in the brackets are standard deviation.

Frequencies of alleles *A* and *B* for two breeds estimated from genotypic frequencies were 0.83 and 0.17 for Black Pied and for Red Pied breeds, 0.69 and 0.31, respectively (Table 1). Contrary to other breeds, the frequency of desirable casein B allele in the Red Pied breeds was high.

Nearly identical results of B variant of k-casein (0.13) for Black Pied cattle were obtained by Zinovieva and Gladir (2003). However, results of Solimova *et al*. (1992), Tinaev *et al*. (2003) and Iolchiev (1993) showed frequency of B allele k-casein 0.53, 0.40 and 0.42, respectively. Until now, the k-casein allele frequency has not been studied for Red Pied cattle.

The (A) allele was more frequent in both breeds than the k-casein B allele. The frequency of k-casein B was higher in Red Pied (0.31) than in Black Pied cows (0.17). The genotypic frequencies of k-casein AA and AB were significantly different in the two breeds examined. No significant departure from Hardy-Weinberg equilibrium ($p<0.05$) was observed in the two breeds.

In studies of genetic characterization of cattle breeds, it has been found that the B allele of k-casein occurs at higher frequencies in breeds originating from *Bos taurus* than in those of *Bos indicus* origin (Backer and Manwell, 1980; Golijow *et al.*, 1996; Del lama and Zago, 1996; Kemenes *et al.*, 1999). Black and Red Pied breed showed a high degree of genetic variability for the k-casein *locus*, with a frequency of the *B* allele of 0.17 and 0.31, respectively.

Table 2 shows the effect of the k-casein gene on the milk production traits in cows studied. In Blach Pied breed the effect of polymorphism of k-casein was significant (P≤0.05) for fat and protein percentage.The milk from cows with the BB genotype showed the highest percentage of fat and protein. In Red Pied cows, statistically significant difference for milk yield, fat and fat content were found.

According to Denisenko (2004), cheese production can be increased by 5% if milk is from cows of the BB genotype for k-casein, when compared with milk from AA animals. In the Holstein and Jersey breeds it has been shown that the *B* allele is associated with higher protein content in milk (Denisenko, 2004), and it has been suggested that appropriate weights could be given to genotypic information and polygenic breeding value in order to improve selection response (Van Arendonk and Bovenhuis, 1996).

This allele has been shown to be favorably related to milk composition in dairy cattle breeds. Therefore, studies aimed at establishing this possible relationship are of crucial importance, as selection could be enhanced by the inclusion of genetic markers in selection decisions.

References

Aleandri R, Butazzoni LG, Schneider JC, Caroli A, Davali R (1990). The effects of milk protein polymorphisms on milk components and cheese-producing ability. *J Dairy Sci*. 73: 241-255.

Backer ACM, Manwell C (1980). Chemical classification of cattle. I. Breed groups. Anim Blood Groups Biochem. *Genet*. 11: 127-150.

Bovenhuis H, Van Arendonk JAM, Korver S (1992). Associations between milk protein polymorphisms and milk production

traits. *J Dairy Sci*. 75: 2549-2559.

Del Lama SN, Zago MA (1996). Identification of kappa-casein and beta-lactoglobulin genotypes in Brazilian Bos indicus and Bubalus bubalis population. *Braz J Genet*. 19: 73-77.

Denicourt D, Sabour MP, Mcallister AJ (1990). Detection of bovine k-Casein genomic variants by the polymerase chain reaction method. *Anim Genet*. 21: 215-216.

Denisenko EA (2004). Milk production and technological property milk of Black Pied breeds with difference genotypes of kappa casein in region Siberia. *Ph.D. Thesis*. Lesni poliani. Moscow.

Denisenko EA, Kalashnikova LA (2004). Milk production of Black Pied breeds with difference genotypes of kappa casein. *4th conference problems biotechnology in farm animal. VIJ. Dobrovitsy*. Russia. 24-25 November. 47-48.

Golijow CD, Giovambattista G, Poli M, Dulout FN, Lojo MM (1996). k-casein gene frequencies support subdivision and historical origin of Argentine Creole cattle. *Braz J Genet*. 19: 583-586.

Golijow CD, Giovambattista G, Ripoli MV, Dulout FN, Lojo MM (1999). Genetic variability and population structure in loci related to milk production traits in native Argentine Creole and commercial Argentine Holstein cattle. *Braz J Genet*. 22: 395-398.

Ikonen T, Ojala M, Routtinen O (1999). Associations between milk protein polymorphism and first lactation milk production traits in finish ayrshire cows. *J Dairy Sci*. 82:1206-1033.

Iolchiev BS (1993). Frequency the bovine milk protein genes in dugther's bulls. New in selection of farm animals. *VIJ Dobrovitsy*. 56: 129-132.

kastonina OV, Khripiakov EN, Strikozov NI, Zinoveva NA (2004). Effects differences genotype kappa-casein, Beta-lactogolubin and alpha-lactoalbumin on technological effects of milk. *4th conference problems biotechnology in farm animal*. VIJ Dobrovitsy. Russia. 24-25 November. 54-60.

Kemenes PA, Reginato LCA, Rosa AJM, Parker IU, Razook GA, Figueiredo LA, Silva NA, Etchegaray MAL, Coutinho LL (1999). k-casein, b-lactoglobulin, growth hormone allele frequencies and genetic distances in Nelore, Gyr, guzera, Caracu, Charolais, Canchin and Santa Gertrudis cattle. *Genet Mol Biol*. 22: 539-541.

Konovalova EN, Seltcov VI, Zinoveva NA (2004). Polymorphism gene kappa casein and its effects on production traits cows Simmental breeds. *4th conference problems biotechnology in farm animal*. Dobrovitsy Russia. 24-25. November. 49-54.

Lin CY, Sabour MP, Lee AJ (1992). Direct typing to milk proteins as an aid for genetic improvement of dairy bulls and cows: A review. *Anim Breeding Abstracts*, 60: 1-10.

Marziali AS, Ng-Kwai-Hang KF (1986). Effects of milk composition and genetic polymorphism on cheese composition. *J Dairy Sci*. 69: 2533-2542.

Medrano JF, Aguilar-Cordova E (1990). Genotyping of bovine k-casein loci following DNA sequence amplification. *Biotechnology*, 8: 144-146.

Nei M (1987). *Molecular evolutionary genetics*. Columbia University Press, New York.

Romonasova EG (1999). Effect of genotypes and environment on milk traits and quality technology of milk Swiss breeds. *Ph.D. Thesis* . Dobrovitsy, Russia.

Solimova GE, Sokolova CC, Smikozova OP, Ngot LM, Berberov EM (1992). Analysis of polymorphism DNA Gene's clusters of cattle: Gene's k-casein and BoLA. *Cytol Genet*. 26:18-26

Strzalkowska N, Krzyzewski J, Zwierzchowski L, Ryniewicz Z (2002). Effects of k-casein and b-lactoglobulin loci polymorphism, cows' age, stage of lactation and somatic cell count on daily milk yield and milk composition in polish Black-and-White cattle. *Anim Sci Pap Rep*. 20: 21-35.

Tinaev AS (2003). Effects of polymorphic milk protein genes on milk yield at heifer Black Pied breed. In: Book (Selection, nutriation, composition farm animals) Vol. 15, All-Russian Research Institute of animal breeding, Moscow, Russia. 52-54.

Van Arendonk JAM, Bovenhuis H (1996). The application of genetic markers in dairy cow selection programs. p. 105. In "*Progress in Dairy Science*", Phillips, C.J.C. (Ed.). CAB International.

Zinovieva NA, Gladir EA (2003). *Introduction to molecular genetics detection for Farm animal*. VIJ Press, Dobrovitsy Russia.