ANALYSIS OF KAPPA-CASEIN (*CSN3*) ALLELES IN LATVIAN BROWN AND LATVIAN BLUE BREED POPULATIONS

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Abstract. Genotypes of *CSN3* gene were detected in 71 individuals of Latvian Blue breed and in 30 individuals of Latvian Brown breed using a PCR-RFLP method. Animals were chosen at random from each heard. This study investigated the alleles A and B of *CSN3* gene, while determining the allele and genotype frequencies and Hardy-Weinberg equilibrium proportions in both populations. The results showed that in our analyzed samples from 71 Latvian Blue breed only 7 animals (10%) had the genotype BB, and in analyzed samples from 30 Latvian Brown breed only 3 had the genotype BB (10%). Frequencies of *CSN3* genotypes AA, AB, and BB correspond to Hardy-Weinberg equilibrium proportions and populations in genetic equilibrium. A wide variation in the B allele frequency among Latvian Blue and Latvian Brown breeds was found suggesting that molecular selection for animals carrying the allele B of *CSN3* could impact breeding programs for dairy production in Latvia.
Key words: kappa-casein alleles, cows, genetic structure of population.

Introduction

Caseins are milk proteins secreted by mammary gland cells. They constitute about 78-82% of bovine milk proteins and are subdivided into four main groups: αS1-casein, αS2-casein, β-casein, and κ-casein (Eigel et al., 1984; Rohallah et al., 2007). These proteins and their genetic variants have been extensively studied and reported as an important factor associated with lactation performance, milk composition, and cheese yield efficiency (Aleandri et al., 1990). Bovine casein is encoded by a 200 kb DNA fragment located at chromosome (Chr) 6 q31-33 (Ferretti et al., 1990) arranged in the order of αS1, αS2, β, and κ. *CSN3* fragment spans the 13 kb DNA sequence divided into five exons and intervening sequences, and constitutes about 25% of the casein fraction (Alexander et al., 1988; Lien and Rogne, 1993; Martin et al., 2002). The αS1-, β-, and αS2-casein genes are the most closely linked and form an evolutionarily related family, whereas the *CSN3* gene is at least 70 kb away from them (Ferretti et al., 1990).

CSN3 has been extensively studied for its role in stabilizing the casein micelles and its influence on the manufacturing properties of milk. For several breeds, the genetic variability in the *CSN3* locus has been reported each with a different allelic frequency based on genetic diversity among breeds. Various allelic variants have been described for *CSN3* gene in different cattle breeds, which include A, B, C, E, F, G, H, I, and AI (see review by Soria et al., 2003). Among these, variants A and B are most commonly found and variant B is predominantly concerned with processing properties of milk and has better lactodynographic properties (Lin et al., 1992). In variant B, due to a single base mutation in the *CSN3* locus, isoleucine substitutes threonine and aspartic acid is substituted by alanine (Pinder et al., 1991).

Selection for the allele B of *CSN3* gene is integrated into cattle breeding programs in many countries, and it also should be done in Latvia too. The research of the milk protein genes is needed to obtain the information of the GAS program development in Latvia and to promote the cows' milk protein breeding in Latvia. This research helps to create conditions for single gene or QTL assisted breeding methodologies for dairy cattle breeding of milk protein, their use of other economically important traits in the breeding perfection.

Materials and Methods

Animals were chosen at random from each heard. The blood was taken from the jugular vein and was collected in K3-EDTA coated sterile vaccutainers and stored at -200 °C until used for DNA extraction. DNA was extracted using the Fermentas Genomic DNA Purification Kit # KO512. The *CSN3* alleles were identified using the PCR-RFLP (Polymerase Chain Reaction and Restriction Fragment Length Polymorphism) method in accordance with methodology provided by J.F. Medrano and E. Aguilar-Cordova (1990) and G.E. Sulimova (2007). We used primers: *SGE* 5'-TAT CAT TTA TGG CCA TTG GAC CA-3' and *SGO* 5'- CTT CTT TGA TGT CTC CTT AGA GTT-3'from methodology of G.E. Sulimova (2007). The amplification was carried out in Applied Biosystems 2720 Thermal Cycler with the following amplification conditions: 95 °C for 2 min (initial denaturation), then followed 35 cycles with denaturation at 95 °C for 30 sec, annealing at 55 °C for 40 sec, and extension at 72 °C for 30 sec with a final extension step of 72 °C for 7 min. Samples of PCR products (25 µl) were digested with *HindIII* and *HinfI* endonucleases according to the manufacturer's recommendations (Fermentas). Restrictive fragments that were obtained this manner were separated in a 2% agarose gel with ethidium bromide (10 µl EtBr 100 ml^{-1} of 2% agarose gel). Electrophoresis on 2% agarose gel was used for visualisation of the restricted DNA bands (60V, 150 min) in 0.5X TBE buffer. Research was done in the Laboratory of the Molecular Genetic Researches of the Faculty of Agriculture of LLU.

The data on cows milk performance were acquired from the LDC (Latvia data centre) system database.

Table 1

Bovine *CSN3* genotype frequencies in Latvian Blue and Latvian Brown breed populations

Genotype	Latvian Blue breed		Latvian Brown breed	
	Number of genotype	Frequency of genotype	Number of genotypes	Frequency of genotype
AA	33	0.465	14	0.467
AB	31	0.437	13	0.433
BB	7	0.099	3	0.100

Allele frequencies were calculated by using the appropriate diallele locus expressions, where allele A relative frequency was designated as p, and the relative frequency of B allele as q. We obtained the p and q expressions:

$$p = \frac{2D + H}{2N} \quad (1) \quad \text{and} \quad q = \frac{2R + H}{2N} \quad (2),$$

where:

D, H, R - the number of individuals with genotypes AA, AB, and BB;
N - total number of animals in the analysis;
2N - total number of alleles in the analysis.

Calculations were made with Microsoft Office Excel 2007 standard package assistance, but computer program package TFPGA was used as a population genetic basis of the accuracy of testing (Miller, 1997).

Results and Discussion

In the analysis we found that *CSN3* (κ-casein gene) genotype distributions are very similar in both of the tested populations (Table 1).

In both analyzed populations we found practically similar allele frequencies: 0.683 for allele A, and 0.317 for allele B. It was seen that the frequency of k-casein allele A in both populations was two times higher than the frequency of k-casein allele B. Examining the population genetic equilibrium in these alleles, it was found that in both populations frequencies of genotypes correspond to Hardy-Weinberg equilibrium proportions:

$$(0.683 + 0.317)^2 = 0.466 + 0.433 + 0.100.$$

Comparing κ-casein A and B allele frequency of Latvian Brown breed cows (Table 2) in the samples of our analysis (n = 30) and recently published data (L. Paura et al., 2009), we found that in the population could have been a change and increased desirable allele B frequency from 0.167- 0.184 to 0.316, i.e. about 0.132 or 0.149.

The fact that the alleles A and B frequencies in both analyzed populations (Latvian Blue and Latvian Brown breed) turned out to be virtually identical, and could indicate that the factors which affect the frequency of both populations are identical, and the favourable allele is allele A. This factor should be put on the clearance of subsequent studies, particularly because of the selection process would be desirable to reduce the frequency of allele A and increase frequency of desirable allele B and frequency of the genotype BB. In our analyzed samples from 71 Latvian Blue breed only 7 animals (10%) had the genotype BB, and in analyzed samples from 30 Latvian Brown breed only 3 had the genotype BB (10%). Our sample data is analyzed within the framework we tried to determine the genotypes AA, AB and BB, as influence factors on cow productivity in the first standard lactation. However, our sample volume proved insufficient to speak of statistically significant results. We can only point to possible trends.

Table 2

***CSN3* alleles A and B and the corresponding genotype frequency analysis of Latvian Brown breed**

Genotypes and genes	Our results Cows, n=30			(Paura et al., 2009) Bulls, n=19			(Paura et al., 2009) Cows, n=30		
	De facto	HW	±	De facto	HW	±	De facto	HW	±
AA	0.467	0.468	0.001	0.632	0.666	0.034	0.733	0.694	0.039
AB	0.433	0.432	0.001	0.368	0.300	0.068	0.200	0.278	0.078
BB	0.100	0.100	0	-	0.034	0.034	-	0.028	0.028
A	0.684	-	-	0.816	-	-	0.833	-	-
B	0.316	-	-	0.184	-	-	0.167	-	-

HW – Hardy - Weinberg equilibrium

Table 3

Analysis of milk characteristics estimated for the 1st lactation depending on genetic variant of *CSN3* in the investigated population

Genotype	Number of genotype	Milk yield, kg	Fat content, %	Protein content, %
Latvian Blue breed				
AA	28	4225.2±211.73	4.24±0.108	3.22±0.046
AB	21	3712.6±217.56	4.33±0.111	3.29±0.047
BB	5	4592.8±430.42	3.98±0.220	3.29±0.093
Latvian Brown breed				
AA	12	4129.2±305.46	4.23±0.103	3.09±0.076
AB	14	4489.5±371.79	4.55±0.126	3.27±0.093
BB	3	3801.2±981.52	4.00±0.332	3.56±0.244

The study of animals used for yield data in the 1st standard lactation (Table 3) had the following limits: milk yield ranging from 3713 kg to 4592 kg, fat content 3.98-4.50%, and milk protein content 3.09-3.56%.

By the analysis of *CSN3* different genotype effects on cow productivity characteristics, we obtained that sample volumes were still insufficient. However, the genotype BB probably tended to prevent the milk fat content expression. As a test of possible effects of genotypes on milk protein content we can note that the Latvian Brown breed where the difference between AA and BB genotype cows average protein content of the 0.47 and $t_{emp.} = 1.839 < t_{teor.\ (0.05,13)} = 2.16$, which is already close to $\alpha = 0.05$ requirements.

We found references in the literature about the positive effect of κ-casein BB genotype on dairy cow milk dietary and technological properties. If such a relationship could be found in our breed populations, probably it would be purposeful to increase the frequency of B allele in our cattle populations. If we view that so far these allele frequencies are not selective controlled and the results identified a gene drift, then the question remains, however, which resulted in A allele frequency prevalence, which we found in the data.

Conclusions

1. DNA samples (n = 101) of Latvian Blue and Latvian Brown breeds, the examinatition found that *CSN3* A and B allele frequencies are 0.683 and 0.317, and both populations are practically same.
2. Frequency of k-casein allele A more than twice times higher than the frequency of *CSN3* allele B and the reason which caused the prevalence of k-casein allele A is not known yet.
3. Frequencies of *CSN3* genotypes AA, AB, and BB (Latvian Blue breed, n=71, 0.465, 0.437, 0.099, and Latvian Brown breed, n = 30, 0.467, 0.433, 0.100) correspond to Hardy-Weinberg equilibrium proportions: $(0.683+0.317)^2=0.466+0.433+0.100$ and populations in genetic equilibrium.

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