



## Polymorphism and allelic frequency of CSN1S1 in different cattle breeds in Bulgaria

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### ABSTRACT

*The present study aims at determining the genetic polymorphism at the milk protein genes CSN1S1 and their allelic frequency in widespread and endangered cattle breeds raised in Bulgaria. Analyzed were 390 tissue samples of two widespread cattle breeds, i.e. 129 of the Bulgarian Brown Cattle and 129 of the Bulgarian Black and White Cattle, as well as of two endangered cattle breeds- 23 of the Rhodopean Short - Horned Cattle and 109 of the Iskar Cattle. Determined are significant differences in the frequency of the heterozygous genotypes, i.e. higher frequency in the local breeds. The Iskar Cattle stands out with the highest percentage of the BC heterozygous genotype, i.e.- 66.972% compared to the other breeds included in our study.*

(Keywords: polymorphism, allelic frequency, milk proteins, indigenous cows)

### INTRODUCTION

The polymorphism of the milk proteins found in milk enjoys continuous scientific interest. Established is its relation with the milk production /Bech et al., 1990/, milk composition (Robitaille et al., 2002) as well as the process parameters in the manufacture of various cheese types (Hill et al., 1997; Ng-Kwai and Hang, 1998; Mclean et al., 1987; Schaar et al., 1985; Van den Berg et al., 1992). The studies carried out in this connection are the basis giving faith and hope that it is possible to effectively use the genetic polymorphism of the milk proteins as a genetic marker in the genomic selection in dairy cattle breeding.

The use of the polymorphic loci and their use as microsatellites for mapping and selection based on markers (Simianer et al., 2003) have a growing importance for preserving the specific genetic diversity of the local and endangered cattle breeds.

Dalvit et al. (2009) study the genetic diversity in endangered Bulgarian cattle breeds. Study of the polymorphism at the milk proteins in the local breeds has not been carried out.

The present study aims at determining the genetic polymorphism at the milk protein genes CSN1S1 and their allelic frequency in widespread and endangered cattle breeds raised in Bulgaria.

## MATERIALS AND METHODS

### Facts

Analyzed were 390 tissue samples of two widespread cattle breeds-129 of the Bulgarian Brown Cattle and 129 of the Bulgarian Black and White Cattle as well as of two endangered cattle breeds, i.e. 23 of the Rhodopean Short - Horned Cattle and 109 of the Iskar Cattle. Tissue samples were taken by an innovative marking technology according to which special containers with drying agents were put aside upon marking of each animal, object of the study.

### Laboratory Analysis

DNA was purified from ear tags using the Maxwell®16 Tissue DNA Purification Kit (Promega) according to manufacturer's instruction. Twenty nanograms of purified DNA were used for the amplification of portion of the CSN1S1 gene using primers CSN1S1-10-F 5'-TGC CTA TCC ATC TGG TGC CTG G-3' and CSN1S1-10-R 5'-GCT CCA CAT GTT CCT GAG TAA TGG-3' and standard PCR condition. 5 µl of PCR products were purified using ExoI and SAP (ThermoScientific) for 15 min at 37 °C followed by an inactivation step at 85 °C for 15 min. Allelic discrimination was performed using the Genome™Lab SNP-Primer Extension Kit (BeckmanCoulter) following manufacturer's instructions and interrogation primer CSN1S1\_SBE: 5'-TAT TAA TC CAT TGG CTC TGA GAA CAG TG-3'. Electropherograms produced by CEQ8000 automatic capillary sequencer (BeckmanCoulter) were analyzed using Genetic Analysis Software v9.0 (BeckmanCoulter). The genetic polymorphism of the milk proteins has been determined using PCR-RFLP analysis in the Padova University Laboratory. For data analysis the software product Systat 13 was used.

## RESULTS AND COMMENTS

*Table 1* shows the results in the allelic frequencies of the CSN1S1 genes in widespread and endangered cattle breeds in Bulgaria. Two alleles-B and C were detected in CSN1S1 gene. As seen from the *Table* in all breeds included in our study, allele B features higher frequency than allele C.

**Table 1**

### Allelic frequency of CSN1S1 in different cattle breeds in Bulgaria

Allelic frequency	B	C
Bulgarian Brown Cattle	0.814	0.186
Bulgarian Black and White Cattle	0.748	0.252
Rhodopean Short - Horned Cattle	0.565	0.435
Iskar Cattle	0.619	0.381

In the animals of the Bulgarian Brown Cattle breed, determined is the highest allelic frequency of the B allele, i.e. 0.814 and the lowest allelic frequency of the C allele, i.e. 0.186. The allelic frequencies determined by us for the Bulgarian Brown Cattle, i.e. for the B allele-0.748 and for the C allele-0.252, are similar to those determined for the a.m. breed. These similarities in the frequencies in two of the most widespread cattle breeds in our country most probably is attributable to the focused selection on particular

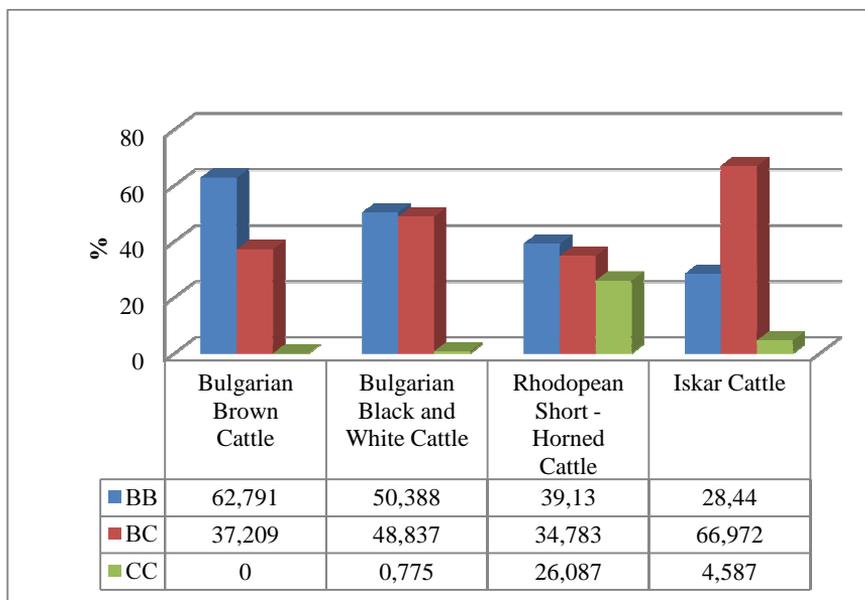
qualities. The results determined by us correspond to those published by *Smiltina et al.*, (2009), studying the population of the Latvian Brown Cattle. Other authors determine a higher frequency for the B allele, i.e. 0.86 and a lower frequency for the C allele, i.e. 0.14 in the Holstein cattle breed (*Yasemin et al.*, 2006; *Zakizadeh et al.* 2013; *Miciński et al.* 2008).

In the Rhodopean Short - Horned Cattle the lowest frequency of the B allele-0.565 and the highest frequency of the C allele-0.435 is determined. Similar values are also determined for the Bulgarian Rhodopean Cattle-the B allele being 0.503 and the C allele being 0.497 which most probably is attributable to the fact that this breed has been produced on the basis of the Rhodopean Short - Horned Cattle (*Yordanova et al.*, 2013). The Iskar Cattle features the following allelic frequencies: for the B allele – 0.619 and for the C allele – 0.381. According to *Boettcher et al.* (2004), the B allele is wider spread than the C allele.

*Figure 1* shows the polymorphism of CSN1S1 in different cattle breeds raised in Bulgaria. *Caroli et al.* (2009) state that CSN1S1 features 9 genetic variants (A, B, C, D, E, F, G, H, I) as most common are the B and C alleles. The results determined by us correspond to those determined by the author.

**Figure 1**

**Polymorphism of CSN1S1 in different cattle breeds in Bulgaria**



The above figure shows that CSN1S1 features 3 genotypes, i.e. BB, BC and CC. Difference in the percentage of the afore-mentioned genotypes is significant and it may be claimed that there is a significant difference between the widespread and the endangered cattle breeds. The Bulgarian Brown Cattle stands out with the highest percentage in the BB homozygous genotype – 62.791% and the BC heterozygous genotype has a much lesser value, i.e. – 37.209% while for the CC homozygous

genotype the percentage is not determined. The results determined by us confirm the results published by *Smiltina et al.* (2009) showing the non-existence of homozygous animals of the CC genotype in cows from the Latvian Brown Cattle. The frequencies of the homozygous BB and heterozygous BC genotype determined in the Bulgarian Black and White Cattle have similar values.

Determined are significant differences in the polymorphism of the Rhodopean Short - Horned Cattle and the Iskar Cattle. Determined are similar values of the percentage frequency of the genotypes of CSN1S1 in the Rhodopean Short - Horned Cattle as the results we determined are as follows: BB- 39.13%, BC- 34.783% and CC- 26.087%. The Iskar Cattle features the highest percentage of the heterozygous genotype, i.e. BC- 66.972%, while the homozygous genotypes BB and CC have lower values, i.e. 28,44% and CC- 4,587% respectively. *Zakizadeh et al.* (2013) determine similar results in studying the genetic frequency of CSN1S1 in Holstein and indigenous breeds in Iran.

### COMMENTS

The differences in the B and C allele frequencies in widespread and endangered cattle breeds in Bulgaria most probably result from the various selection objectives and approaches-intensified import of genetic plasma in the widespread breeds and natural breeding in the case of the Iskar Cattle. We need to mention also the limitations resulting from the population number. In the Rhodopean Short - Horned Cattle there are also specific factors such as prevailing artificial insemination in the breeding area resulting from the limited genetic diversity offered by the male cattle in the population. This might be the reason for the damaged genetic structure and balance in this breed determined by *Dalvit et al.* (2009).

### CONCLUSIONS

1. Two alleles-B and C were detected in the CSN1S1 gene as the B allele features a higher frequency than the C allele in the widespread and endangered local breeds. Determined are significant differences in the frequency of the two alleles in the breeds studied by us.
2. Determined are significant differences in the frequency of the heterozygous genotypes as their frequency in the local breeds is higher. The Iskar Cattle features the highest percentage of the BC heterozygous genetic type - 66.972%, compared to the other breeds included in our study.

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