Genetic Polymorphism of Main Lactoproteins of Romanian Grey Steppe Breed in Preservation

Șt. Creangă, V. Maciuc, A.V. Bâlteanu, S.S. Chelmu

Abstract—The paper presents a part of the results obtained in a complex research project on Romanian Grey Steppe breed, owner of some remarkable qualities such as hardiness, longevity, adaptability, special resistance to bad weather and diseases and included in the genetic fund (G.D. no. 822/2008.) from Romania.

Following the researches effectuated, we identified alleles of six loci, codifying the six types of major milk proteins: alpha-casein S₁ (α S1-cz); beta-casein (β-cz); kappa-casein (K-cz); beta-lactoglobulin (β-lg); alpha-lactalbumin (α-la) and alpha-casein S₂ (α S2-cz). In system αS1-cz allele αS1-Cn B has the highest frequency (0.700), in system β-cz allele β-Cn A₂ (0.550), in system K-cz allele k-CnA₂ (0.583) and heterozygote genotype AB (0.416) and BB (0.375), in system β-lg allele β-lgA₂ has the highest frequency (0.542) and heterozygote genotype AB (0.500), in system α-la there is monomorphism for allele α-la B and similarly in system αS2-cz for allele αS2-Cn A.

The milk analysis by the isoelectric foci-alization technique (I.E.F.) allowed the identification of a new allele for locus αS1-casein, for two of the individuals under analysis, namely allele called αS1-casein αS1-Cn. When experiments were repeated, we noticed that this is not a proteolysis band and it really was a new allele that has not been registered in the specialized literature so far. We identified two heterozygote individuals, carriers of this allele, namely: BIRV and CIRV. This discovery is extremely important if focus is laid on the national genetic patrimony. A thing that allowed the presence of this accentuated polymorphism of lactoproteins [6, 7].

In a first stage of our researches, we established the average value and the variability of the milk yield indices and lactoprotein indices under study. On this occasion, we made a full analysis of locus of kappa-casein (K-cz), establishing the gene frequency, genotype frequency, the standard error of the gene frequency and the state of genetic balance according to Hardy-Weinberg law.

II. MATERIAL AND METHOD

Researches were effectuated on 30 Romanian Grey Steppe cows raised semi-intensively, tie-up stalling, at the Research-Development Station for Bovine Growing Dancu, Iași (S.C.D.C.B. Dancu, Iași). Due to the strictly genetic determinism of lactoproteins, what makes genotype be identical to phenotype, lactoprotein frequency is very different from one breed to another. Hence, the need to run these researches that might establish the genotypic and allelic frequencies of lactoproteins for Romanian Grey Steppe breed, the Moldavian variety from the North-Eastern part of Romania. The study of polymorphism of milk proteins was made by PCR-RFLP technique, and for the study of polymorphism of all bovine lactoproteins we also used the isoelectric foci-alization technique (I.E.F.) [8, 9].

The milk samples were collected individually in 15 ml Falcon tubes, transported at 4°C and then frozen at -20°C until tests were run. Defrosting occurred slowly at room temperature and subsequently, samples were centrifuged at 8,000 rotations/minute, for 5 minutes for milk separation. They were stored for 30 minutes at 4°C for fat solidification and then it was removed from each tube by means of a spatula.

For an optimal protein concentration, samples were diluted with a urea and β-mercaptoethanol solution. Samples were migrated in a polyacrylamide gel with 4% concentration. After migration, the gel was immersed in a solution 10% of trichloroacetic acid. Colouring occurred for 2 hours by means of a solution 0.025% Coomassie Brilliant Blue R-250 in 40% ethanol and 7% glacial acetic acid.
III. RESULTS AND DISCUSSIONS

We suspected the presence of some ancestral alleles undiscovered so far for the loci codifying the milk proteins due to the lack of an improvement programme, what made Romanian Grey Steppe breed keep a high variability for a long time. Unfortunately, the drastic reduction of the number of individuals and the replacement of this breed with other more productive breeds has led to the loss of this variability and this is why the breed has been introduced in a preservation programme of the animal genetic resources from Romania.

In figure 1 we may see the alleles identifies for the six loci codifying the six types of major proteins of milk ($\alpha$ S1-cz; $\beta$-cz; K-cz; $\beta$-lg; $\alpha$-la; $\alpha$ S2-cz).

The genetic structure for polymorph systems of milk proteins: alpha-casein S1 ($\alpha$S1-cz), beta-casein ($\beta$-cz), kappa-casein (K-cz), beta-lactoglobulin ($\beta$-lg), alpha-lactalbumin ($\alpha$-la) and alpha-casein S2 ($\alpha$S2-cz) is presented in table 1.

All researches have undoubtedly showed the favorable influence of variant k-Cn B on milk quality, cheese output and quality. Consequently, in the study of bovine lactoproteins, most researches focused on the determination of the frequency of kappa-casein alleles at different breeds and the possibility of “limited” promotion by selection of kappa casein B.

Variants k-Cn A and k-Cn B are universally discovered at bovines and zebu. In recent years, 3 more variants have been identified: k-Cn C, k-Cn D and k-Cn E, all having frequencies lower than 0.1 and being identified only in some local breeds.

For Romanian Grey Steppe breed from S.C.D.C.B. Dancu Iaşi, K-cz system has a high frequency for allele k-CnA 2 (0.583) and the heterozygote genotype AB (0.416) and BB (0.375). As we already mentioned, ancestral allele B is associated to diverse breeds and a better quality of milk.

Variant k-Cn B has a higher frequency in the breeds from Brună group, of different origins raging between 0.4 and 0.6. The failure to promote k-Cn B by selection triggers in time a reduction of its frequency. In the crossbreeds of different breeds, the frequency of k-Cn B is intermediate between the frequencies.

The higher frequency of allele $A_2$ has a special significance since this allele is the ancestral one from which all the others derived phylogenetically.

### TABLE I

<table>
<thead>
<tr>
<th>Registratio n no.</th>
<th>$\alpha$ S1-cz</th>
<th>$\beta$-cz</th>
<th>K-cz</th>
<th>$\beta$-lg</th>
<th>$\alpha$-la</th>
<th>$\alpha$ S2-cz</th>
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<td>$A_1A_2$</td>
<td>AB</td>
<td>AB</td>
<td>BB</td>
<td>AA</td>
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<td>AB</td>
<td>BB</td>
<td>BB</td>
<td>AA</td>
</tr>
</tbody>
</table>

Genotype frequency:

- $BB = 0.5$
- $BC = 0.3$
- $CIRV = 0.1$
- $BIRV = 0.1$
- $A_1A_1 = 0.2$
- $A_1A_2 = 0.5$
- $A_2A_2 = 0.3$
- $AA = 0.292$
- $AB = 0.50$
- $BB = 0.209$
- $PA1 = 0.417$
- $PA2 = 0.542$
- $PB = 1$
- $pA1 = 0.45$
- $qA2 = 0.55$
- $PA = 1$
- $PA = 0.417$
- $PB = 0.542$
- $PA = 1$
- $PB = 1$
- $PA = 0.55$
- $qA2 = 0.583$
- $PA = 1$
- $PA = 0.458$

Allele frequency:

- $pB = 0.7$
- $qC = 0.2$
- $rIRV = 0.1$
- $qA1 = 0.45$
- $qA2 = 0.55$
- $PA1 = 0.417$
- $PB = 1$
- $PA2 = 0.542$
- $PB = 1$
- $PA1 = 0.458$
- $PB = 1$
- $PA1 = 0.55$

The IEF Profile belonging to some individuals from Romanian Grey Steppe breed highlighting alleles of milk major proteins.
of pure breeds showing the strong influence of crossbreeding in the transmission of the wanted type of kappa-casein.

In system $\beta$-lactoglobulin, variants $\beta$-Lg A and $\beta$-Lg B are universally encountered in bovines and zebu. The distribution of the two variants in most breeds is quite balanced. In our case, $\beta$-LgA$_1$ has the highest frequency (0.542) and the heterozygote genotype AB (0.500).

For $\alpha$-lactalbumin, variants $\alpha$-La A and $\alpha$-La B apparently exist in most zebu populations. In almost all breeds of bovines we encounter only variant $\alpha$-La B. $\alpha$-La A, encountered in zebu, is less rare in the countries from Central and Meridional Europe being discovered in 11 Italian breeds and some Russian and Romanian local ones. In our researches too on Romanian Grey Steppe nucleus, we have encountered a monomorphism for allele $\alpha$-la B.

The milk analysis by IEF and PCR-RFLP allowed the identification of a new allele for locus $\alpha$S$_1$-casein, for two of the individuals analysed from S.C.D.C.B. Dancu, Iași, namely allele called $\alpha$S$_1$-casein I$_{RV}$ [8, 9]. It appears under the shape of a band with isoelectric point situated between alleles B and C, closer to that of allele C as one may see in figures 2, 3, 4, 5, 6 and 7.

When repeating the experiments, we noticed that this is not a proteolysis band but it really is a new allele that has not been registered so fat in the specialized literature: BI$_{RV}$ and CI$_{RV}$ [8, 9].

![Fig. 2 IEF profile for bovine lactoproteins of Romanian Grey Steppe (lanes 1, 4, 5, 6, 8), as against Bâltăța Românească breed (lanes 2, 3, 7) for locus of $\alpha$S$_1$ casein. Genotypes of $\alpha$S$_1$ casein are: 1- BB; 2- BC; 3- CC; 4- CI$_{RV}$; 5- BI$_{RV}$; 6- BB; 7- BC; 8- BB.](image)

![Fig. 3 Comparison between $\alpha$S$_1$-CN B, C and I$_{RV}$ cDNA's restriction map with BseGI enzyme. Note the presence of 240 and 220 by fragments specific to I$_{RV}$ allele and 400 bp product specific to B and C alleles respectively. Lane 1: Ampli Size™ Molecular Ruler, BioRad, Lane 2: Uncut cDNA; Lane 3 and 5: $\alpha$S$_1$-CN BB and CC samples, respectively; Lane 4: BI$_{RV}$](image)

![Fig. 4 Comparison between protein sequences features of B, C and I$_{RV}$ variants (Romanian Grey Steppe breed). The mutations, which are making the difference between this 3 protein variants, are marked with rectangles. Note the differences between isoelectric points (pl) of the 3 proteins, which explain the observed IEF profiles (gel image from the right side). Signal peptide is highlighted with bold italic letters.](image)

![Fig. 5 Phylogenetic origin of alpha S$_1$ I$_{RV}$ allele](image)

![Fig. 6 Comparison between B, C and I$_{RV}$ cDNA theoretical restriction maps of BseGI enzyme. Note 1 restriction sites for BseG in B and C allele and 2 in I$_{RV}$](image)
IV. CONCLUSION

1. For Romanian Grey Steppe breed, we identified alleles for the six loci codifying the six major proteins of milk (α S1-cz; β-cz; K-cz; β-lg; α-lα; α S2-cz). In system K-cz, we noticed a high frequency for allele k-CNκ (0.583) and the heterozygote genotype AB (0.416) and BB (0.375).

2. We have identified new alleles for locus αS1-casein, namely the allele called αS1-casein IRV. Two of all individuals under study are heterozygote carriers of this allele, namely BIIRV and CIIRV. This discovery is extremely important if access is laid on the preservation of the national genetic patrimony.

3. The breeding bulls of Romanian Grey Steppe breed should be checked in terms of milk quality by genomic testing and use of genetic markers as modern methods recently introduced in the genetic improvement of the members of the Bovidae family.

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REFERENCES


