Genetic Polymorphisms of β-Lactoglobulin and α-s1-Casein Genes in Romanian Racka Sheep

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Abstract
The increasingly use of genetic markers in artificial selection shows to be a successful strategy for the animal production systems, the outcome of applying such methods being a higher animal productivity and better quality of the products. The aim of our study was to analyse the polymorphism of β-lactoglobulin and α-s1-casein loci in a population of Racka sheep from Romania. In order to identify the specific alleles we used the PCR-RFLP technique. It is known that the different genotypes observed at these loci are correlated to milk quality, either in terms of the composition, or in terms of the technological properties. We identified three genotypes (AA, BB and AB), at β-lactoglobulin locus, while at α-s1-casein locus, all the analysed animals had nonA homozygous genotype. These molecular markers are useful for the characterisation of the sheep breeds, and their genotyping can contribute to a high quality artificial selection.

Keywords: Racka breed, milk proteins, genetic polymorphism, PCR-RFLP.

1. Introduction

In the recent years, genome studies in farm animals tried to clarify aspects related to the molecular structure, expression and regulation of genes involved in the improvement of animal performance and of the reproductive performance.
Racka sheep are reared in northern Serbia, in south-eastern Hungary and in south-west of Romania, being a transboundary breed. Racka breed is in a proper state of conservation in Hungary, in critical state in Serbia, and lost interest in favour of other breeds in Romania [1]. Actually it is reared in just a few places in the area of Banat, at the border with Serbia. The reason for this situation is that Racka breed is not economically competitive, it does not accommodate to the harsh winters in open field, while the wool, milk and meat productions are lower than those of other breeds that underwent selection [1].
β-lactoglobulin is protein present in the milk of many animal species, particularly in ruminants. Three genetic variants have been identified in sheep: β-LG A, β-LG B [2] and β-LG C [3]. Variants A and B differ just by the substitution of one amino acid (histidine into tyrosine) at position 20 of the polypeptide chain. Several studies have been conducted recently regarding the genotypes of the β-lactoglobulin encoding gene in different sheep breeds. According to these findings the frequency of A allele is in the majority of cases higher than the frequency of B allele, while the frequency of the genotypes varies from one breed to another [4-7].
Caseins form a heterogeneous group of phosphoproteins, which account for about 80% of the total milk proteins in ruminants. The existence

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of 4 casein fractions is generally accepted: α_{s1}-, α_{s2}-, β- and κ- casein. In sheep, 8 allelic variants have been identified so far at the locus of α_{s1}-casein locus [8-10]. Studies regarding the polymorphism of the gene encoding this protein have been conducted on several breeds [11, 12], revealing the presence, with variable frequency of the different alleles depending on the breed.
In Romania, the studies of polymorphism at the two loci have been rather few and focused on Botoşani Karakul and Palas Merino breeds [7, 11, 13]. There were also other studies that have analysed the polymorphisms of encoding genes for milk proteins in other species, such cattle [14, 15]. The purpose of our study was to determine the polymorphism at the loci of β-lactoglobulin and α-s1-casein in a population of Racka sheep from Romania.

2. Materials and methods

The blood samples were collected from 40 Racka sheep belonging to private farmers from Caraş-Severin County. DNA extraction was done with the Wizard Genomic DNA Extraction kit (Promega).
Genotyping the locus for β-lactoglobulin was done according to the protocol described by Feligni et al. 1998 [16]. The amplification reaction were carried out in 25μL final volume and consisted of 1X PCR Buffer, 15 mM $MgCl_2$, 200μM dNTPs, 50 ng of DNA template, 0.5 units of AmpliTaq Gold DNA Polymerase, 0.5μL of each primer and nuclease free water. PCR amplifications were performed using a program with 45 cycles included denaturation at 95°C, 30 seconds, annealing at 58°C, 30 seconds and extension at 72°C, 1 minute. The first denaturation step was of 10 minutes at 95˚C and the final extension was of 10 minutes at 72˚C. The amplification products were digested with *Rsa I* endonuclease, for 3 hours, at 37°C. The restriction fragments were directly analyzed by electrophoresis in 3% agarose gels, stained with ethidium bromide, and visualized under UV light.
Genotyping for the locus of α-s1-casein was done according to the protocol described by Pilla et al., 1998 [17]. The amplification reaction were carried out in 25μL final volume and consisted of 1X PCR Buffer, 15 mM $MgCl_2$, 200μM dNTPs, 50 ng of DNA template, 0.5 units of AmpliTaq Gold DNA Polymerase, 0.5μL of each primer and nuclease free water for 45 cycles. Denaturation was performed at 95˚C for 30 seconds, annealing at 57˚C for 30 seconds and extension at 72˚C for 1 minute. The first denaturation step was of 10 minutes at 95˚C and the final extension was of 10 minutes at 72˚C. The amplification products were digested with *Mbo II* endonuclease, for 3 hours, at 37°C. The restriction fragments have been separated in 3% agarose gels and visualized using ethidium bromide and UV transilluminator.

3. Results and discussion

The genotypes of the analyzed individuals were determined by analysing of the electrophoretic gels in which the restriction fragments have been separated, both for β-lactoglobulin alleles, and for α-s1-casein alleles.
The amplification products were digested with *Rsa I* restriction endonuclease in order to evaluate the genomic distribution of the allele for β-lactoglobulin. The polymorphism (C replace T) change the restriction site of the enzyme, which allowed the identification of the homozygous AA or BB and heterozygous AB animals. Following the digestion of PCR products, three fragments of 66, 37 and 17 were inferred for AA genotypes and two fragments of 103 and 17 bp for BB genotype (Figure 1). The heterozygous individuals present all four fragments (103, 66, 37 and 17 bp).

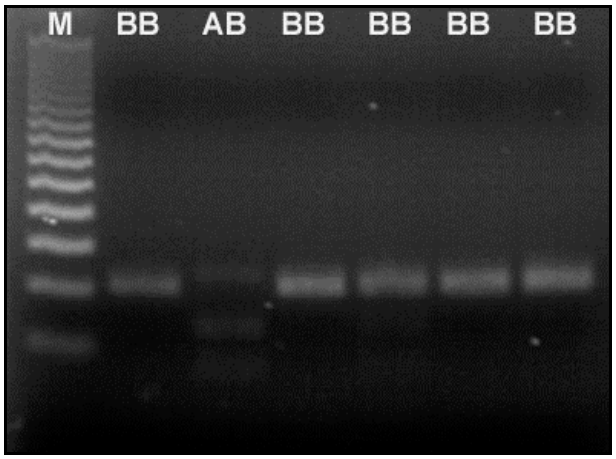

Figure 1. Restriction profile for β-lactoglobulin locus: BB and AB genotypes. M – Molecular Weight Marker 50bp (Promega).

In order to distinguish the individuals which have A allele from those which have nonA allele for

αs1-casein locus, the amplification products were digested with *Mbo II* restriction enzyme. The polymorphism will modify the restriction site, which allows identifying the A/A homozygous, nonA/nonA homozygous and A/nonA heterozygous individuals. The enzymatic digestion produce two fragments of 306 and 66 bp, for the nonA/nonA homozygous individuals, three fragments of 160, 146 and 66 bp, for the A/A homozygous individuals, and four fragments for the A/nonA heterozygous animals (Figure 2).

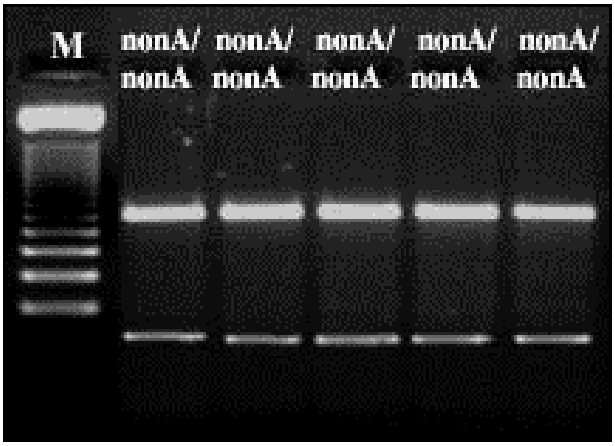

Figure 2. Restriction profile for α-S1-casein locus: nonA/nonA genotypes. M – Molecular Weight Marker 50bp (Promega).

The frequency of alleles and genotypes identified, and χ^2 test results are shown in table 1. The results for χ^2 test were not significant for AA and AB genotypes, which suggest that in this breed, β-lactoglobulin gene has not been affected by the selection process. However, one may notice the high frequency of BB genotype, which is very low or even absent in other sheep breeds [5, 6, 7, 8, 16]. We may also notice that B allele has a noticeably higher frequency than A allele in the studied population.

Genotypes AA and BB of β-lactoglobulin are correlated with a higher milk quality, either in terms of milk composition (protein and fat), or in terms of the technological properties (coagulating time and curd firmness). Genotype AA has higher fat and dry matter content than the other two genotypes. During cheese making, the dry matter loss is lowest for AB genotype, while the loss of fat is higher for genotype AA, compared to genotype BB [18, 19]. Thus, the very high frequency of BB genotype recommends the milk of Racka sheep for cheese making.

Table 1. Allelic frequencies and identified genotypes for Romanian Racka sheep.

	Number of individuals			**Number of individuals**		
Racka breed	**AA**	**AB**	**BB**	**AA**	**A/nonA**	**nonA/nonA**
	3	14	23	0	0	40
	Frequency of β-lactoglobulin genotypes			**Frequency of α-s1-casein genotypes**		
Racka breed	**AA**	**AB**	**BB**	**AA**	**A/nonA**	**nonA/nonA**
	7.5%	35%	57.5%	0	0	100%
χ^2 test	p≥0.5 (NS)	p≥0.066 (NS)	p≤0.05	-	-	-
	Frequency of β-lactoglobulin alleles			**Frequency of α-s1-casein alleles**		
Racka breed	**A**		**B**	**A**		**nonA**
	25%		75%	0	100%	

*NS – Non Significant; differ significantly at p≤0.05

4. Conclusions

The genetic polymorphism of the milk proteins is of great interest for animal producers, because of its influence on milk composition and quality and on the productivity parameters.

The screening of β–lactoglobulin gene polymorphisms in Racka sheep revealed the presence of the all three genotypes (AA, AB and BB), with the genotype BB displaying the highest frequency. Also, the only pattern obtained for α-s1-casein locus was the one for the nonA homozygous animals.

The results suggest the possibility of a future selection process to improve milk quality.

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