**GENOTYPING OF β-LACTOGLOBULIN GENE IN KARAKUL SHEEP BREED**

**GENOTIPIZAREA GENEI PENTRU β-LACTOGLOBULINĂ LA RASA DE OI KARAKUL**

KEVORKIAN STELIANA, MANEA MARIA ADINA, GEORGESCU S.E DINISCHIOTU ANCA, COSTACHE MARIETA

*University of Bucharest, Molecular Biology Center*

β-lactoglobulin is the major milk whey protein in the ruminants. Studies have indicated that this protein is polymorphic in the many breeds of sheep. This is the result of a single base pair substitution in the β-lactoglobulin gene. The genetic variants, A (tyrosine) and B (histidine), differ at the amino acid position 20. The nucleotide mutation disrupts an Rsal site and can be detected by PCR-RFLP analyses. We included in this study 52 animals from Karakul breed. The Karakul may be the oldest breed of domesticated sheep. In Romania it is raised especially for pelts. The aim of this work was to analyze the genotype distribution of β-lactoglobulin in Karakul sheep.

**Key words:** β-lactoglobulin, sheep, PCR-RFLP, genotyping.

**Introduction**

In modern programmes of animal breeding, the polymorphisms of the milk proteins can be used as marker systems. The attention surrounding sheep milk and its cheese production characteristics, has spurred considerable research into the genetic structure of native sheep populations.

The Karakul may be the oldest breed of domesticated sheep. In Romania this breed was obtained from Tsurcana black or Tsurcana gray with rams Karakul black and Karakul gray from Germany and represents about 4% of the total livestock.

β-lactoglobulin is the major milk whey protein in the ruminants. The β-lactoglobulin coding gene is located on ovine chromosome 3. This protein is synthesized in the mammary glands during pregnancy and the lactation stages. Apart from its ability to bind and transport small hydrophobic molecules in milk (e.g. retinol and small fatty acids) its biological function is still unclear.

The genetic variants A (tyrosine) and B (histidine) differ at the amino acid position 20 (Ali *et al.*, 1990). Erhardt (1989) identified a new type of β-lactoglobulin named C, and determined its complete primary sequence. Variant C has a single change Arg-Glu at position 148. Studies have indicated that this protein is polymorphic in the many breeds of sheep (Lourdes Amigo *et al.*, 2000). The nucleotide mutation between variants A and B disrupts a Rsal site and can be...
detected by PCR-RFLP analyses (Cengiz Elmaci et al., 2006). The mutation consists in the transformation of Timine (genotype AA) in Cytosine (genotype BB). The aim of this work was to analyze the genotype distribution of β-lactoglobulin in Karakul sheep.

**Materials and Methods**

56 blood samples collected from Karakul breed of different colors (white, black, gray, brown, chestnut and pink) were analyzed. The isolation of genomic DNA from fresh blood was performed with Wizard Genomic DNA Extraction Kit (Promega).

The PCR conditions were optimized for the primer by varying the annealing temperature between 51-60°C on a gradient thermocycler IQCycler (BioRad).

After determining the optimum temperature of 58°C, the amplification reactions were carried out in 25 µL final volume and consisted of 1X PCR Buffer, MgCl2, 200µM dNTPs, DNA template, 0.5 units of AmpliTaq Gold DNA Polymerase, 0.5µL of each primer (F-CAACTCAAGGTCCCTCTCCA and R-CTTCAGCTCCTCCACGTACA) and nuclease free water. PCR amplifications were performed in 0.2 ml tubes using a program with 45 cycles. Denaturation was performed at 95°C for 30 seconds, annealing at 58°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 PCR products obtained were digested with 1U of RsaI restriction endonuclease (Promega) for 3 hours at 37°C. The restriction fragments were directly analyzed by electrophoresis in 3% agarose gels in 1X TAE buffer, stained with ethidium bromide, and visualized under UV light. The genotypes of the analyzed individuals at the β-lactoglobulin locus were established using the restriction fragments observed in the gel.

In case of the sequencing method, we performed the PCR reactions in the same conditions as above. The amplified fragments were sequenced by ABI Prism 310 Genetic Analyzer, using the ABI Prism ® BigDye Terminator Cycle Sequencing Ready Reaction Kit after a purification step using the Wizard System Kit (Promega). The sequences were processed using DNA Sequencing Analysis 5.1 Software (AppliedBiosytems) and the nucleotide sequences were aligned with the BioEdit program.

**Results and Discussions**

In order to evaluate the genotype distribution of β-lactoglobulin in Karakul sheep we used the PCR-RFLP method. A 120bp fragment of the ovine β-lactoglobulin gene from exon II was amplified (Figure 1). After PCR amplification, enzymatic digestion with RsaI and agarose gel electrophoresis, the β-lactoglobulin A allele yields three bands of 66, 37, and 17 bp. The β-lactoglobulin B allele gives only two fragments of 103 and 17bp, and heterozygote have all four fragments.
The 17 bp fragment result from an *Rsa I* site present in both alleles and is useful as a control procedure.

![Figure 1: Results after digestion with *Rsa I*. Lanes 1, 2, 3, 5: genotype AB, lanes 4, 6: genotype AA, lane 8: molecular weight marker (50 bp).](image)

In breed Karakul the most frequent genotype detected in about 57% of studied animals is AB. The genotype AA represents 43% (Table 1).

**Table 1: Frequency of alleles A and B for the samples of Karakul.**

<table>
<thead>
<tr>
<th></th>
<th>AA</th>
<th></th>
<th>AB</th>
<th></th>
<th>BB</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>42.85</td>
<td>32</td>
<td>57.15</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

In order to confirm our findings we sequenced the 120bp fragment from the β-lactoglobulin gene. In Figures 2 and 3 we present the profiles of the region from the PCR product that contains the point mutation and BioEdit fragment alignment of a region from β-lactoglobulin gene and our PCR product in the case of a homozygous sheep (genotype AA).

![Figure 2: The sequence of the region from the PCR product that contains the single point mutation inside the recognition site for *RsaI* for a homozygous sheep (genotype AA).](image)
Figure 3. BioEdit fragment alignment of GenBank exon II from β-lactoglobulin gene (genotype BB) and our PCR product for a homozygous sheep (genotype AA).

Conclusions

So far in Romania there are few studies concerning β-lactoglobulin gene. Considering the importance of this gene in milk composition it is very important to determine the possible genotypes in sheep and develop different breeding programs and selection programs especially marker assisted selection between different genotypes of milk and cheese characteristics.

Our study provided information on the polymorphism of ovine β-lactoglobulin in Karakul breed. The frequency of AB genotype in the studied breed was highest than other genotypes and we observed no BB genotype. In other studies the BB genotype is associated with milk quality (Giaccone et al., 1997, 2000), while others point to a positive effect of the AA genotype on the fat and protein content (Garzon and Martinez, 1992; Kukovics et al., 1998). Other studies did not reveal any influence on milk traits (Barillet et al., 1993; Recio et al., 1997) thus confirming the conflicting relationships regarding LGB polymorphism and milk composition.

For this reason, in the future, we propose to establish a correlation between our results and the milk productivity for the individuals of Karakul breed.

Bibliography


