Polymorphism and Genetic Structure of LGB Gene (*Rsa*i) in Valachian Sheep Population

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Abstract
The work was oriented to identification of beta-lactoglobulin gene (LGB) polymorphism and analysis of genotype structure in population of Valachian sheep. LGB is the major milk whey protein in the ruminants. AA and AB genotypes are associated with protein and casein content and curd yield. BB LGB genotype sheep were characterized by a significantly higher protein content of milk than sheep with the other two LGB genotypes — AA and AB. LGB variant AB is associated with higher body weight, while genotype AA could be linked with sheep wool density. The material involved 34 Valachian sheep. Ovine genomic DNA was isolated by salting out method and used in order to estimate LGB genotypes by PCR-RFLP method. The PCR products were digested with *Rsa*I restriction enzyme. In the population included in the study, we detected all genotypes: homozygote genotype AA (6 animals), heterozygote genotype AB (12 animals) and homozygote genotype BB (16 animals). In the population of sheep homozygotes BB – 0.4706 were the most frequent, while homozygotes AA – 0.1765 were the least frequent ones. This suggests a slight superiority of allele B – 0.6471.

Keywords: β-lactoglobulin, LGB, PCR-RFLP, Valachian sheep.

1. Introduction

LGB is the major milk whey protein in the sheep, consists of 162 amino acids and forms stable dimers in milk. This protein, synthesis in the mammary glands during pregnancy and the lactation stages [1] and is important in the evaluation of the milk production potential and milk fat and protein percentage [2].

One of the most extensively studied milk protein polymorphisms is the substitution of the aminoacid Tyr^{20} with His in the β-lactoglobulin polypeptide [3] resulting from a single base pair substitution (C→T) in the β-lactoglobulin gene, located on ovine chromosome 3.

AA and AB genotypes are associated with protein and casein content and curd yield. BB LGB genotype sheep were characterized by a significantly higher protein content of milk than sheep with the other two LGB genotypes — AA and AB [4]. Bocharev (1998) [5] found associations between LGB variant AB with higher body weight, while genotype AA could be linked with sheep wool density.

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2. Materials and methods

The material involved 34 Valachian sheep. Ovine genomic DNA was isolated by salting out method [6] and used in order to estimate LGB genotypes by PCR-RFLP method.

DNA primers described by Kučinskiene et al. (2005) [4] were used to PCR amplification: forward primer 5´- AAA AGC CCT GGG TGG GCA GC - 3´ and reverse primer 5´ - TTG GGT TCA GTG TGA GTC TGG - 3´.

The PCR reaction elaborated by Kučinskiene et al. (2005) [4] was modificated.

The reaction mixture in the total volume 25 µl containing 50 ng DNA, 2 U Taq polymerase (INVITROGEN), 2.5 mM $MgCl_2$, 200 µM dNTP, 10 pM of each primer, 20 mg/ml BSA

(PROMEGA). The following amplification parameters were applied: 95 °C for 5 minutes followed by 33 cycles: 95 °C for 50 seconds, 65 °C for 40 seconds, 72 °C for 40 seconds. The reaction was completed by the final synthesis: 72 °C for 5 minutes.
The PCR products of 452 bp were digested with 5 units of the *Rsa*I restriction enzyme (PROMEGA). Restriction digestion fragments were loaded on 3 % agarose gel (Invitrogen) containing ethidium bromide and the gel were analyzed in the UV rays.

3. Results and discussion

*Rsa*I digestion of the PCR produkt, analyzed by 3% agarose-gel electrophoresis. Allele A produced 175, 170, 66 and 41 bp fragments, and allele B produced 236, 175 and 41 bp fragments as the PCR-RFLP.

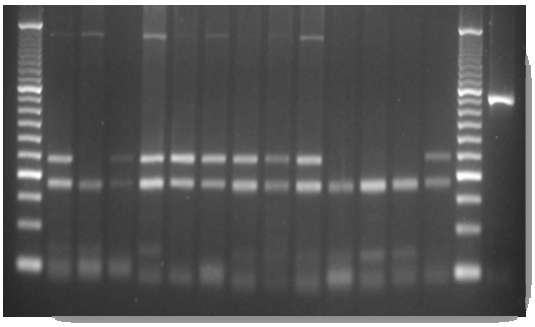

Figure 1: Representatively results of analysis PCR RFLP for *LGB* gene by *Rsa*I on 3 % agarose gel.
1, 15 –DNA Ladder 50 bp (Fermentas); 2, 5, 8, 9, 10, 14 – genotype AB (236 bp, 175 bp, 170 bp, 66 bp, 41 bp); 3, 11, 12, 13 – genotype AA (175 bp, 170 bp, 66 bp, 41 bp); 4, 6, 7 – genotype BB (236 bp, 175 bp, 41 bp); 16 – PCR product (452 bp)

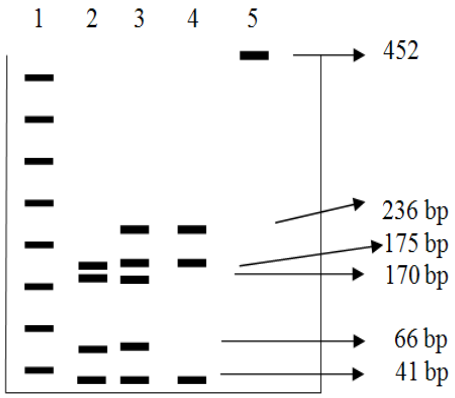

Figure 2. Schematic image of PCR-RFLP products of *LGB* gene.

1 – DNA ladder 50 bp, 2 – genotype AA ((175 bp, 170 bp, 66 bp, 41 bp), 3 – genotype AB (236 bp, 175 bp, 170 bp, 66 bp, 41 bp), 4 – genotype BB (236 bp, 175 bp, 41 bp), 5 – PCR product (452 bp)

In the population of Valachian sheep we detected all genotypes: homozygote genotype AA (175 bp, 170 bp, 66 bp and 41 bp) 6 animals, heterozygote genotype AB (236 bp, 175 bp, 170 bp, 66 bp and 41 bp) 12 animals and homozygote genotype BB (236 bp, 175 bp and 41 bp) 16 animals.
Out of the PCR-RFLP analyse results was calculated genetic structure and by selected parameters was evaluated efectivity of the alleles activity. Genetic structure LGB gene of sheep is presented in table 1. In the total population of sheep homozygotes BB – 0.4706 were the most frequent, while homozygotes AA – 0.1765 were the least frequent ones. This suggests a slight superiority of allele B – 0.6471.
Genetic equilibrium of analysed population was evaluated on the base χ^2-test. In the population included in the study non-significant diffrences in frequencies of genotypes were found.
Effectivity of alleles function of the gene LGB in sheep are presented in table 2. Observed heterozigosity is lower as expected. The values PIC and ENA are closely to limited values. Frequencies of alleles in our population were similar to those of LGB gene as reported by Bolla et al. (1989) [7], who observed superiority of allele B (0.82) in Sarda sheep. In opposite, Recio et al. (1997) [8] present higer frequency of the allele A (0.58) in population of Merino sheep. The higher frequency of the allele A (0.63) in population of Lacaune sheep reported by Barillet et al. (1993) [9].

Table 1. Frequency of genotypes and alleles of *LGB* gene in the population of sheep

FREQUENCY	GENOTYPES (n = 34)			ALLELS		χ^2
	AA	AB	BB	A	B	*df = 2*
ABSOLUTE	*observed*			24	44	1.754
	6	12	16			
	expected					
	4.2364	15.5278	14.2358			
RELATIVE	*observed*			0.3529 ±0.058	0.6471 ±0.058	
	0.1765	0.3529	0.4706			
	expected					
	0.1246	0.4567	0.4187			

Table 2. Effectivity of alleles LGB gene function in population Valachian sheep

Locus	Allels	H_e	PIC	C_a	ENA	V%
CSN2	*A; B*	0.4567	0.3524	0.5433	1.8406	47.05

4. Conclusions

It may be concluded that LGB is a polymorphic gene with a slight superiority of genotype BB and a superiority of allele B. Genetic structure examined in population of Valaska sheep remained within the range quoted in literature for other sheep breeds.

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