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THE B-LG GENE POLYMORPHISM LEVEL OF ASCANIAN KARAKUL AND ASCANIAN FINE-FLEECE SHEEP BREEDS

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A comprehensive study of the farm animals' genome has been and remains the subject of numerous studies. These studies are aimed at identifying the features of the genetic structure, studying the genes expression, which, in turn, play a key role in the formation or regulation the biochemical and physiological processes, and then directly affect to the manifestation of economically useful traits in animals. The beta-lactoglobulin gene is considered as a marker of sheep productivity and is one of the promising candidate genes. The β -LG locus is the most researched of all the specific genes that can influence various sheep economic characteristics. This gene has an increased polymorphism for most sheep breeds [1, p. 45; 2, p. 479–480; 3, p. 8]. Many studies of various sheep breeds indicate that the β -LG gene is promising in terms of influencing the quantity and quality of milk [1, p. 46; 4, p. 256; 5, p. 383; 6, p. 5124].

At the same time, this gene polymorphism in sheep of the Ascanian selection has not been studied before. To establish the peculiarities of the β -LG gene genetic structure in the Ascanian Fine-Fleeced breed of Tavrian Type and the Ascanian Prolific Karakul Breed Type sheep was the main aim of our scientific work.

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The study of the β -LG gene polymorphism was carried out in the Molecular Genetics Laboratory of the IABSR «Ascania Nova» – NSSGCSB on the Tavian Type of Ascanian Fine-Fleece breed ewes ($n = 16$) and the Ascanian Prolific Karakul Breed Type sheep ($n = 26$).

Determination of the animals' genotype was carried out by PCR-RFLP. Genomic DNA was isolated from whole blood according to a standard technique using a Sorbo-B DNA reagent kit (Amplisens).

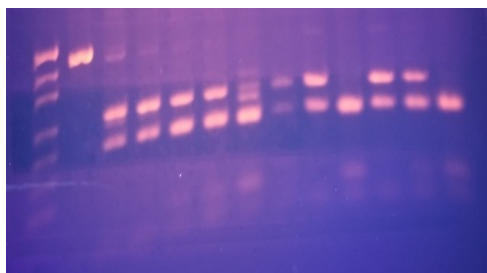
The following primers were used to amplify the β -LG gene fragment:

F: 5'TTG GGT TCA GTG TGA GTC TGG -3'

R: 5'- AAA AGC CCT GGG TGG GCA GC -3'.

PCR was carried out using a Libe Line programmable amplifier according to the following temperature conditions: hot start – 2 min at 74 °C, initial denaturation – 5 min at 95 °C, then 33 cycles: denaturation – 40 s at 95 °C, primer annealing – 40 s at 67 °C and synthesis – 40 s at 72 °C. The reaction is completed by terminal elongation – 5 min at 72 °C. The length of the β -LG gene amplification site was 452 bp.

For the restriction of the β -LG gene, the *RsaI* restriction enzyme (GT / AC) was used [4, p. 266; 5, p. 385; 7, p. 8; 3, p. 7]. The results of the β -LG gene restriction products separation with the *RsaI* restriction enzyme in a 3% agarose gel are shown in Figure 1. Fragments of 236, 175, 170, 66, and 41 bp in length were identified, which indicates the presence of this gene polymorphism.



Lanes: 1 DNA marker (Sibenzyme pUC19 DNA / *MspI* (*HpaII*) Marker 23 (501, 404, 331, 242, 190, 147, 111); 2 – PCR product (452 bp); 3, 4, 8, 9, 11 – genotype BB (236 bp, 175 bp, 41 bp); 5, 6, 7, 12 – genotype AB (236 bp, 175 bp, 170 bp, 66 bp, 41 bp); 10,13 – AA genotype (175 bp, 170 bp, 66 bp and 41 bp)

Figure 1. Electrophoregrams of the β -LG gene restriction products separation by *RsaI* restriction enzyme

Based on the results of PCR-RFLP analysis, the genetic structure of the studied sheep populations was established. At the same time, three genotypes were identified (β -LG AA, β -LG AB, β -LG BB) formed by two alleles (Table 1). Thus, it was determined that in the both breeds environment, the largest proportion were animals – carriers of the heterozygous genotype β -LG AB, this is 56.25% in the environment of the Ascanian Fine-Fleeced breed and

61.54% in the Ascanian Karakul breed. The next in distribution in both breeds was the homozygous β -LG BB genotype – 31.25% in the AFF and 23.08% in the AK. The homozygous β -LG AA variant was the least common (12.5% and 15.38%, respectively).

Table 1

Concentration the β -LG locus genotypes and alleles in the studied breeds of sheep

Genotype / allele	AFF		AK	
	n	%	n	%
AA	2	12,5	4	15,38
AB	9	56,25	16	61,54
BB	5	31,25	6	23,08
Total	16	100	26	100
A	0,406		0,462	
B	0,594		0,538	

In terms of allelic composition, β -LG B turned out to be the most common, both among the animals AFF (0.594) and in the environment of AK (0.538). The alternative β -LGA occurred with a slightly lower frequency: 0.406 and 0.462, respectively.

A more accurate idea of the populations' structure peculiarities by genetic markers is provided by a complex study using several methods, each of which has its own specifics in elucidating the genetic variability of a population [8, p. 72, 191]. The results of the genetic structures comprehensive assessment the studied groups at the β -LG locus are presented in Table 2.

Table 2

Population-genetic parameters of the studied populations by the β -LG locus

Breed	Distribution	Zygoteness Indexes			Population-genetic parameters				
		Homo-zygote, n	Hetero-zygote, n	H	Ca	V	Na	D	χ^2
AFF	ϕ	7	9	0,48	0,52	51,4	1,93	+0,37	0,78
	τ	8,3	7,7	0,35					
AAK	ϕ	10	16	0,50	0,50	51,7	1,99	+0,71	1,77
	τ	13	13	0,29					

Thus, the coefficient of heterozygosity in both studied breeds was 0.48 (AFF) and 0.5 (AK), which indicates a sufficient number of heterozygous genotypes in the populations. Hence, the coefficient of homozygosity (C_a) in both groups was in the range of 0.5-0.52.

The locus polymorphism level (N_a) is the number of active effective alleles in the population. An increase in the degree of homozygosity is accompanied by a decrease in the number of effective alleles, a decrease in genetic and phenotypic diversity, and leads to an increase in population homogeneity. The maximum theoretically possible number of active alleles in the case of a biallelic state of the locus is two. In both groups studied, N_a was 1.93-1.99, which indicates an equal effect of the β -LG gene alleles.

The kurtosis coefficient (D) quantifies the shortage or excess of the actual heterozygosity of the population compared to the theoretically calculated ones. The nature of the value of this indicator in both studied groups has the same – right-sided deviation, indicating a certain excess of actually heterozygous genotypes.

When comparing the theoretically expected and actual distribution of genotypes of the β -LG locus according to Hardy-Weinberg in both populations, no disturbance of the genetic equilibrium was found, as evidenced by low χ^2 indices.

The β -LG gene in the populations of the Tavrian Type of Ascanian Fine-Fleece breed and the Ascanian Prolific Karakul Breed Type sheep turned out to be polymorphic. Two alleles and three genotypes formed by these alleles have been identified. In both populations, the β -LG B allele is found with a slightly higher frequency (0.594 in AFF and 0.538 in AK). The most common genotype was the heterozygous β -LG AB, which was found with a concentration of 56.25% in AFF and 61.54% in AK.

When determining the main population genetic parameters of the two studied sheep breeds, it was found that both populations for the β -LG gene have sufficient heterozygosity with a small actual excess of heterozygous genotypes, the maximum possible number of active alleles, and, in general, are in a state of genetic equilibrium.

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