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Article

On the Results of Genotyping of Sheep of Jaidar, Karakul, Gissar Breeds by Locus of Calpastatin Gene

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Abstract: The article presents the results of genotyping of three local breeds of the Republic of Uzbekistan, jaidary (n-14), karakul (n-17) and hissar (n-18) barracks by the locus of the meat productivity gene, calpastatin (CAST). According to the results of the study, genetic polymorphism was detected in all three breeds, the homozygous MM genotype and the heterozygous MN genotype were determined, but another homozygous NN was not detected in the animals under study, a violation of gene balance is observed, and the M allele (0.82) is predominantly found. The authors of the work used the PCR-RFLP method for genotyping, identification of the CAST gene alleles was carried out using the MspI restriction enzyme, the length of the PCR product was 565 bp, after restriction of the amplification, depending on the genotype of the animals, three fragments were found: 565 bp, 306 bp, 259 bp.

Keywords: breeds of sheep jaidary, karakul, gissra, sheep genotyping, PCR-RFLP analysis, calpastatin gene, CAST, gene equilibrium

1. Introduction

Nowadays, advances in molecular genetics have made it possible to identify genes that can improve animal performance. Scientists have conducted studies to examine possible links between polymorphisms of the calpastatin gene (CAST) Hha1 and growth performance, carcass characteristics and meat quality in sheep. As a result of amplification of the CAST/Hha1 gene, a gene fragment of 622 bp in size was obtained. Three CAST genotypes were found in the studied sheep of the breed: homozygous genotype MM (fragments 385 bp, 281 bp), heterozygous genotype MN (622 bp, 385 bp and 281 bp), homozygous genotype NN (622 bp). The frequencies of the M and N alleles of the CAST/Hha1 gene were: 0.765 and 0.235, respectively, while the genotypic variants MM, MN and NN had a prevalence of 0.586, 0.356 and 0.057, respectively. To study the effect of calpastite gene alleles on the growth of lambs, three groups of animals were formed: with the MM genotype: n = 8; MN: n = 6; and NN: n = 3 lambs that were fed for 70 days to study growth performance. Only final body weight and longissimus muscle width varied significantly between the three genotypes, while no significant differences were found in any other carcass characteristics or meat quality parameters [1].

Livestock breeders pay great attention to increasing the milk productivity of ewes, as this is an effective tool for increasing the gain in live weight of lambs. Selection of ewes according to milk productivity parameters helps to increase the economic efficiency of farms. Therefore, in our opinion, experiments aimed at studying DNA markers associated with meat productivity in local breeds of sheep, including those adapted to the natural and climatic conditions of the Republic of Uzbekistan, are of great scientific and practical interest [2].

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The purpose of the study is to carry out genotyping of rams of local breeds, Jaidara, Karakul, Gissar breeds at the CAST gene locus, to study the prevalence of genetic variants in the studied group of animals, to determine gene balance at the studied gene locus.

2. Materials and Methods

The experiments were carried out on 49 rams of three local breeds of sheep, Jaidara, Karakul, Gissar breeds, bred in the conditions of the Surkhandarya region. In our work, cryopreserved peripheral blood was used as the test material; blood for genotyping was taken from the jugular vein into a vacuum tube with the anticoagulant EDTA. DNA from blood samples was isolated using the classical phenolic method; testing of DNA samples was carried out in the laboratory of "Green Biotechnology and Cell Engineering" of the Kazakh-Japanese Innovation Center of KazNAIU. Amplification of the gene region for detection of calpastatin gene alleles (CAST) was carried out using the following primers: F - 5'-CCTTGTCATCAGACTTCACC-3' and R - 5' -ACTGAGCTTTTAAAGCCTCT-3', the length of the PCR product of the calpastatin gene was 565 bp (Figure 1). The number of cycles was 35 in the following mode: initial denaturation at 920C for 2 minutes, for 35 cycles of amplification conditions - 920°C denaturation, 650°C primer annealing and 720°C elongation, 60:60:120 seconds.

Identification of alleles of the CAST gene was carried out by restriction of the amplifier with the MspI restriction enzyme with the C/CGG recognition site at a temperature of 370°C for 3 hours. Depending on the genotype of the animals, the following fragments are formed after restriction: in homozygous individuals with the NN genotype, one fragment is 565 bp; in heterozygous individuals with the MN genotype, three fragments are 565 bp, 306 bp, 259 bp, in homozygous sheep with the MM genotype, fragments are 306 bp, 259 bp, which are clearly visualized on the electropherogram [2].

3. Results and Discussion

The use of selected primers made it possible to obtain a fairly good quality electropherogram of the calpastatin gene amplification (Fig. 1).

The electropherogram revealed two genetic variants, homozygous MM and heterozygous MN genotype (Figure 2).

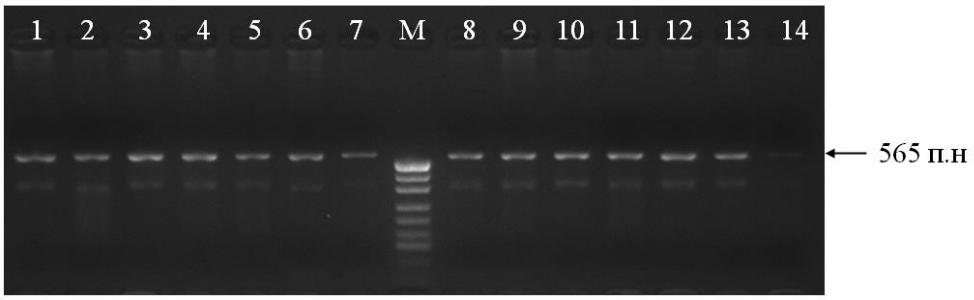

Figure 1. Electropherogram of the amplification, 3% agarose, 1-7, 8-14 – wells amplification of the CAST gene 565 bp long, M-DNA marker pUC19/MspI

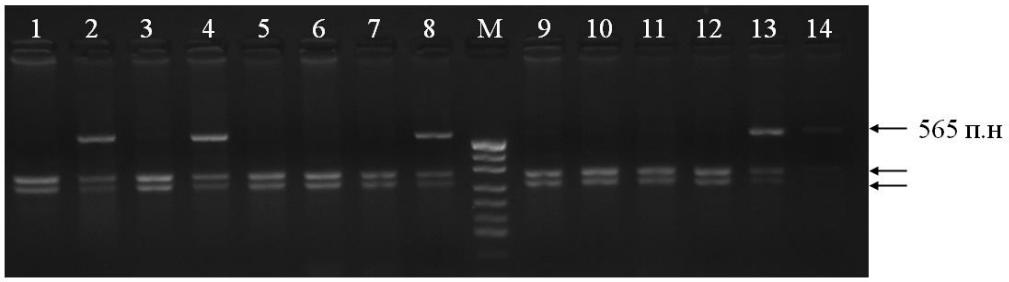

Figure 2. Electropherogram of the CAST gene amplification, after restriction endonuclease MspI, 3% agarose, 1,3,5,6,7,9-12 wells - homozygous MM genotype, fragments 306 bp, 259 bp, 2 ,4,8,13,14 wells – heterozygous MN genotype, fragments 565 bp, 306 bp, 259 bp, M-DNA marker pUC19/MspI

Table 1. Occurrence of genetic variants of calpastatin and frequency of CAST alleles in rams of the Jaidara, Karakul, and Gissra breeds

Number of animals and breed	Genotype at the calpastatin gene locus (CAST)			Allele frequency
	MM	MN	NN	M/N
Karakul sheep (n-17)	10/58,8%	7/41,2%	0	0,79/0,21
Gissar sheep (n-18)	13/72,2%	5/27,8%	0	0,86/0,14
Local sheep (n-14)	8/57,1%	6/42,9%	0	0,78/0,22
Total (n-49)	31/63,3%	18/36,7%	0	0,82/0,18

Analysis of the data in Table 1 shows that in the studied population of rams of the Jaidara, Karakul, Gissar breeds, genetic polymorphism was identified at the calpastatin gene locus (CAST), i.e. of the 49 individuals tested, 31 were found to have the homozygous MM genotype, 17 samples were carriers of the heterozygous MN genotype, the frequencies of the M and N alleles were 0.82 and 0.18, respectively. In all three studied breeds of sheep, animals with the homozygous MM genotype predominate, among the sheep of the Karkul breed 58.8%, the Gissra breed 72.2%, the Jaidars 57.1%, the occurrence of the heterozygous MN genotype was 41.2%, 27.8 %, 42.9%, respectively. In the studied lambs, individuals with the homozygous NN genotype were not identified, which indicates a violation of the gene balance at the calpastatin gene locus.

4. Conclusion and Suggestion

In developed countries, genomic selection, SNP genotyping, and the search for new DNA markers of meat and dairy productivity have recently been successfully used in breeding work. In our work, we conducted a study of one SNP polymorphism in the coding part of the CAST gene in three breeds of the Republic of Uzbekistan, Jaidara, Karakul and Gissarkaya breeds. Genetic polymorphism was discovered in the animals under study; in the future, it is planned to study the effect of calpastatin gene alleles on the meat productivity of rams. It has now been established that alleles of the calpastatin gene have an associative effect on the morphological indicators of meat productivity (growth rate, formation of meat mass, tenderness of meat, deposition of adipose tissue). It should be noted that these local breeds have not been studied in terms of determining the genotype of animals and the influence of gene alleles on meat productivity and on adaptability to harsh climatic conditions.

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