Features of polymorphism of calpastatin and somatotropin genes in young sheep, obtained from crossing ewes of Kalmyk fat-rumped sheeps and dorper rams

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Abstract. The article presents the study of polymorphism of the CAST and GH genes, which determine the features of the manifestation of the productive and biological characteristics of sheep with ½ Kalmyk + ½ Dorper blood system. Calpastatin gene polymorphism represented by alleles M and N, whose frequency was 0,65 and 0,35; genotypes MM, MN – 30 and 70% accordingly. The desired NN genotype has not been identified. A relatively uniform incidence of allele N (0,35) CAST gene and B (0,40) GH was established, what contributed to an almost equal distribution of allele frequencies M (0,65) and A (0,60) genes of calpastatin and somatotropin accordingly. The frequency of heterozygous genotypes occurrence by CAST gene composed 0,7. By GH gene the following distribution of genotype frequencies is observed. Frequency of occurrence homozygous AA and heterozygous AB genotypes was equal to and is 0,4, wherein the frequency of occurrence advised homozygous BB genotype was 0,2. Among the animals studied, sheep with a complex genotype are most common CAST^{MN} GH^{AB} (40 %). The amount of percent CAST^{MM} GH^{AA} and CAST^{MN} GH^{AA} is in 22,2%. 10% is for genotypes CAST^{MM} GH^{BB} and CAST^{MN} GH^{BB}.

1 Introduction

Scientists and major producers are engaged in improving productive qualities using marker-associated selection, since this is the shortest and most promising way to increase the efficiency of livestock in all sectors: cattle breeding pig breeding, poultry breeding and sheep breeding is no exception [1-3].

In modern conditions of economic agricultural production development, the level of competitive sheep breeding ability is determined, mainly, by lamb production. Sheep farming is still an important, traditional livestock industry in Kalmykia. Given the fact that

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until now in our country there exists a problem of animal protein population security – meat, then role of sheep breeding in its solution increases significantly.

The priority vector in sheep breeding became the meat industry development. In recent years, there has been a steady increase in lamb production, amount of specialized meat breeds is increasing [4-5]. Increased production and improved quality of lamb largely determined by introduction of new areas of use based on combination of classical selection methods with molecular genetics, DNA markers in particular, with the help of which significant results have been achieved in sheep breeding.

Currently, the proven connection establishment problem between meat productivity genetic markers and phenotypic traits still completely unresolved, because full manifestation of the animal organism genetic potential depends on a number of factors: both physiological and paratypical [6].

Today marker selection is the leading direction of practical genetics (Marker Assisted Selection – MAS), which involves the use of DNA markers, associated with high level of productivity, which is of significant practical importance for the development of Russian sheep breeding.

The use of new methods based on DNA technology, along with other innovations allows to make sheep farming modern industry competitive and cost-effective [7-8].

The practical significance of such studies lies in solving a number of applied selection problems, one of which is the identification of genetic markers, influencing the sheep meat productivity. In perspective, these studies will allow to identify evaluation criteria for predicting breeding animals' genetic potential. [9].

Promising genes whose allelic variants are associated with the phenotypic manifestation of economically important animal traits are considered as DNA markers, exactly the calpastatin gene (CAST), responsible for meat productivity and tenderness of sheep meat, growth hormone gene (GH), which regulates growth and development.

If abroad the research boom can already be said to have passed, then in Russia calpastatin and somatotropin genes polymorphism is at the stage of study [10-11].

The nucleotide sequences of calpastatin gene were detected and established the connection between polymorphism gene CAST and sheep meat productivity indicators [12-13].

Calpastatin considered as weight gaining and meat quality performance marker, since it encodes polypeptide, which determines tenderness of meat when ripening after slaughter [14-15].

Growth hormone gene (somatotropin, somatotrophic hormone, GH) – one of the promising candidate genes, controlling protein synthesis. somatotropin – the main hypophyseic hormone, has a wide range of biological effects and significantly affects all the body cells [16].

Somatotropic hormone coordinates and regulates the metabolic rate due to deposition of calcium and phosphorus in bones, thanks to it, anabolic processes are activated, accompanied by body size increase and skeletal growth stimulation [17-18]. It is one of the main animals' somatic growth regulators, since GH gene overexpression promotes accelerated growth and development of the body [19-21].

The aim of the work was to determine CAST and GH gene polymorphism and study of the alleles and genotypes frequency distribution in hybrid young sheep.

2 Material and methods of the study

DNA was the biological material for calpastatin and somatotropin genes polymorphism studying, picked out from young 10 cross-breed sheep blood samples ($\frac{1}{2}$ Kalmyk + $\frac{1}{2}$ Dorper) using reagent kits Diatom™ DNA Prep 200 (IsoGeneLab, Russia). Genotyping

studies were carried out in licensed laboratory of immunogenetics and DNA technologies of the North Caucasus Federal Scientific Agricultural Center (№ accreditation POCC RL /001/21PD29).

For carrying out PCR-RFLP (restriction fragment length polymorphism) the kits «GenPak^R PCR Core» were applied, (IsoGeneLab, Moscow). Amplification of genes CAST and GH was carried out on a four-channel programmable thermal cycler "Tertsik" by "DNA-technology" company (Russia). Amplification of CAST gene fragment was carried out using oligonucleotide primers manufactured by Syntol LLC (Russia):

F: 5'-TGGGGCCCAATGACGCCATCGATG-3'.

R: 5'-GGTGGAGCAGCACTTCTGATCACC-3' in the next mode (pre denaturation at 95°C – 4 minutes; cycling – 35 cycles: 94°C – 45 seconds, 62°C – 45 seconds, 72°C – 45 seconds; final synthesis at 72°C – 7 minutes).

The length of the amplified CAST gene fragment composed 622 nucleotide pairs. For restriction and amplified region calpastatin gene endonuclease was used MspI in accordance with manufacturer recommendations (SibEnzyme LLC). Genotype MM represented by fragments: 336 and 286 bps, NN genotype – 622 bps and MN genotype – 622, 336, 286 bps

Amplification of GH gene fragment was carried out using primers:

F: 5'-GGAGGCAGGAAGGGATGAA-3'.

R: 5'-CCAAGGGAGGGAGAGACAGA-3' in the next mode (pre denaturation at 95°C – 5 minutes; cycling – 33 cycles: 95°C – 45 seconds, 60°C – 45 cseconds, 72°C – 45 seconds; final synthesis at 72°C – 10 minutes).

The length of amplified GH gene fragment made up 934 nucleotide pairs. Endonuclease was used to restrict the amplified region of the somatotropin gene HaeIII. The presence of 10 restriction sites corresponded to A allele, and the presence of 11th site – to allele B.

Genotype AA represented by fragments: 277-, 202-, 110-, 100-, 94-, 68-, 49-, 22-, 8- and 4 bps; BB – 256-, 202-, 110-, 100-, 94-, 68-, 49-, 22-, 21-, 8- and 4 bps and AB – 277-, 256-, 202-, 110-, 100-, 94-, 68-, 49-, 22-, 21-, 8- and 4 bps.

To conduct obtained data genetic-statistical analysis, the occurrence frequency of alleles and genotypes was calculated.

According to Hardy-Weinberg's law, the expected distribution of genotypes in the studied sample was calculated. The magnitude χ^2 (pearson conformance criterion) determined by the formula:

$$\chi^2 = \sum \frac{(H_0 - H_E)^2}{H_E}, \quad (1)$$

where H_0 – actually observed number of genotypes,
 H_E – theoretically expected number of genotypes.

3 Results of the study

Analysis of the results of crossbreeding young sheep genotyping ($\frac{1}{2}$ Kalmyk + $\frac{1}{2}$ Dorper) is established, that calpastatin and samatotropin gene polymorphism is represented by two alleles and three genotypes: CAST gene – by alleles M and N, genotypes MM, MN, NN; GH gene – by alleles A and B, genotypes AA, AB, BB with different frequency of occurrence, which is presented in the table 1.

Table 1. Frequency of occurrence of calpastatin and samototropin alleles and genotypes in young sheep crossbreeds (½ Kalmyk fat tail + ½ Dorper).

Calpastin (CAST) genotype/allele	MM	MN	NN	M	N
Frequency of occurrence	0.3	0.7	–	0.65	0.35
Growth hormon (GH) genotype/allele	AA	AB	BB	A	B
Frequency of occurrence	0.4	0.4	0.2	0.6	0.4

A relatively identical N (0,35) gene CAST and B (0,40) GH allele frequency was established, what contributed to almost equal frequency occurrences distribution of alleles M (0,65) and A (0,60) of calpastatin and samototropin genes, respectively.

Significant differences were revealed in the frequency occurrences distribution of homozygous and heterozygous genotypes in the calpastatin and somatotropin genes. The desired genotype NN by calpastine gene in the analyzed sample of animals was not found. The frequency of homozygous genotype MM composed 0,3, and heterozygous genotype MN by CAST gene – 0,7.

A slightly different distribution of genotype frequencies by GH gene is observed. Frequency of occurrence of homozygous AA and heterozygous AB genotypes was equal and composed 0,4, wherein frequency of occurrence of desired homozygous genotype BB composed 0,2.

Value of χ^2 depends on the number of degrees of freedom (table 2).

Table 2. Pearson criterion values, χ^2 by CAST and GH genes.

Actually observed distribution of genotypes			The theoretically expected distribution of genotypes			Pearson criterion, χ^2
Calpastatin (CAST)						
MM	NN	MN	MM	NN	MN	2.90
3	0	7	4.2	1.2	4.6	
Growth gormon (GH)						
AA	BB	AB	AA	BB	AB	0.27
4	2	4	3.6	1.6	4.8	

Criterion calculation χ^2 was carried out at significance level $P \geq 0,99$. Value of χ^2 for this sample amounted to 2,90 by calpastine gene and 0,27 – by growth hormon, which is much less, than standard value $\chi^2 = 9,2$ at a given significance level. Therefore, the gene balance in these samples is not disturbed.

Using the Pennet Grid, of nine theoretically possible complex genotypes, experimental animals were identified five (table 3).

Table 3. Frequency distribution of complex genotypes.

Complex genotypes	Livestock	Frequency of occurrence, %
CAST ^{NN} GH ^{BB}	–	–
CAST ^{NN} GH ^{AB}	–	–
CAST ^{NN} GH ^{AA}	–	–
CAST ^{MM} GH ^{BB}	1	10
CAST ^{MM} GH ^{AB}	–	–
CAST ^{MM} GH ^{AA}	2	20
CAST ^{MN} GH ^{BB}	1	10
CAST ^{MN} GH ^{AB}	4	40
CAST ^{MN} GH ^{AA}	2	20

Animals having the desired complex genotype including combinations of 4 marker alleles CAST^{NN} GH^{BB}, two genes were not identified. В исследуемой выборке отсутствуют животные с генотипами CAST^{NN} GH^{AB}, CAST^{NN} GH^{AA}, CAST^{MM} GH^{AB}. Genotyping analysis indicates, that the most common animals are with CAST^{MN} GH^{AB} complex genotype (40 %). 22,2% accounts for animals, having CAST^{MM} GH^{AA}, CAST^{MN} GH^{AA} genotypes and 10% having CAST^{MM} GH^{BB} and CAST^{MN} GH^{BB}.

4 Conclusion

The polymorphism of the calpastatin and somatotropin genes in the studied sample of animals was determined. Calpastatin gene polymorphism represented by alleles M and N with frequency 0,65 and 0,35. The desired genotype NN was not identified, frequency of MM, MN genotype – 0,3 and 0,7 accordingly. Somatotropin gene polymorphism represented by alleles A and B with frequency 0,60 and 0,40. Genotypes AA, BB and AB founded with frequency 0,4; 0,2 and 0,4 accordingly.

Thus, genotyping analysis results indicates, that in the studied sample of animals, having the desired complex genotype, including combinations of 4 marker alleles CAST^{NN} GH^{BB}, was not identified. In crossbreed young sheep (½ Kalmyk + ½ Dorper) there were no animals of the desired NN genotype by calpastatin gene, but wherein the proportion of heterozygous MN individuals composed 70%. According to growth hormone, the number of desired BB genotypes composed 20% and heterozygotes AB – 40%.

The obtained data can be considered as studying population genetic characteristics and based on its draw a preliminary conclusion about received crossbreeding genetic identity (½ Kalmyk + ½ Dorper). Regular screening to identify desired genotypes would make it possible to adjust the change in certain alleles and genotypes frequency of occurrence in a particular group, flock, populations, by targeted selection and picking pairs for selection.

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