Genetic structure of the herd by genes GDF9, GH, CAST in merino sheep of the North Caucasus region of Russia

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Abstract. One of the main tasks in sheep breeding is to increase productivity. The use of DNA markers for the selection of animals containing the desired alleles of genes of economically valuable traits is one of the new scientific approaches to solving this problem. The aim of the work was to study the genetic structure of sheep of various breeds by genes GDF9, GH, CAST. The studies were carried out on the basis of the laboratory of molecular diagnostics and biotechnology of farm animals of the Don State Agrarian University and the Center for the collective use of scientific equipment "Bioresources and bioengineering of farm animals" of the All-Russian Scientific Research Institute of Animal Husbandry named after academician L.K. Ernst. To carry out molecular genetic studies, samples of ear pits (tissue from the auricle) with an area of 1 cm² were taken from sheep. The evaluation was carried out by PCR-RFLP method. As a result of DNA genotyping for genes GDF9, GH, CAST, the genetic structure of sheep of such breeds as: Salskaya, Stavropol, Volgograd and Soviet merino was revealed. A and B allelic variants of the GDF9 gene, alleles A and B of the GH gene, alleles A and B of the CAST gene were identified in all studied groups of sheep. Analysis of the data revealed the breed aspect in the distribution of the allele and genotype frequencies of the GDF9, GH, CAST genes in the populations under consideration.

1. Introduction
Sheep breeding is one of the oldest branches of animal husbandry, which has brought great benefits to mankind at the stages of its formation and development. Economic and social conditions shaped the demands of social formations for the dynamics of the number and types of productivity of sheep. After a significant reduction in the number of sheep in many regions of the Russian Federation, their number is stabilizing at a qualitatively new level. In order for the industry to benefit in the development of society, a fundamentally new approach to the development of animal husbandry, based on modern technologies, is required. For example, in sheep breeding, the traditional assessment of the breeding value of animals and a number of other parameters of the breeding process should be supplemented by a genetic monitoring system [1-4].
According to leading experts, the intensification of breeding in sheep breeding is impossible without scientifically based approaches to breeding [5-9]. Obtaining reliable information about the productivity potential of animals at an early age, as well as the possibility of maximizing the full use of their genetic potential, are a necessary condition for increasing the efficiency of selection [10, 11].

The identification and use of marker genes in practical breeding together with classical methods seems to be a significant factor in increasing the efficiency of breeding work. These breeding technologies, which have significant, but not yet fully disclosed potential, are based on research in the field of molecular genetics [12-16].

The generally accepted breeding methods used in sheep breeding often do not allow full use of the genetic potential of existing breeds [17]. For this reason, in recent years, the activities of Russian and foreign specialists in geneticists and breeders have been focused on the search and implementation of innovative methods that have significant potential in symbiosis with traditional breeding. These methods, according to scientists, can be technologies based on research in the field of molecular genetics. The introduction of these technologies into selection and breeding work requires deep scientific research. As a result of the development of a model of the selection process based on knowledge about the molecular genetic characteristics of the population, these technologies can be effectively used in sheep breeding [18, 19].

In sheep breeding, a number of marker genes are known associated with economically important traits of animals. A number of studies have already been carried out to assess the polymorphism of some genes, presumably associated with meat productivity - the growth hormone gene (GH), the calpastatin gene (CAST), and reproductive qualities - the differential growth factor gene (GDF9). However, these studies are in the exploratory phase and need to expand and refine the preliminary findings.

The aim of our work was to study the genetic structure of herds of merino sheep by genes GDF9, GH, CAST.

2. Materials and methods
The research was carried out in sheep herds of the leading breeding farms of the North Caucasus region, on the basis of the laboratory of molecular diagnostics and biotechnology of farm animals of the Don State Agrarian University and the Center for Shared Use of Scientific Equipment "Bioresources and Bioengineering of Farm Animals" of the All-Russian Scientific Research Institute of Animal Husbandry named after Academician L.K. Ernst. To carry out molecular genetic studies, biosamples of skin and cartilaginous tissue were taken from the auricles by pits with an area of 1 cm². The evaluation was carried out by PCR-RFLP method. The sequence of amplification primers is shown in table 1.

| Gene | Sequence of primers | Fragment size |
|------|---------------------|--------------|
| GDF9 | GDF9-F: 5'-GAAGACTGGTATGGGGAAATG-3'; GDF9-R: 5'-CCAATCTGCTCCTACACACCT-3'. | 462 bp |
| GH | GH-F: 5'- GGAGGCAGGAAGGGATGAA-3'; GH-R: 5'- CCAAGGGAGGGAGAGACAGA -3'. | 973 bp |
| CAST | CAST-F: 5'-TGGGGGCCCAATGACGACATCGATG-3'; CAST -R: 5'-GGTGGGACAGCATTTCTGTACACC-3'. | 622 bp |

For restriction of the amplified regions of the genes GDF9, GH, CAST, endonucleases BstHH HaeIII, MspI were used in accordance with the recommendations of the manufacturer SibEnzyme LLC (table 2).
Table 2. Used restriction endonucleases and restriction conditions.

| Gene | Method    | Restrictase | References                                      |
|------|-----------|-------------|------------------------------------------------|
| GDF9 | PCR-RFLP  | BstHH       | Palmer et al., 1998; Gorlov et al., 2016       |
| GH   |           | HaeIII      | Amie Marini et al., 2012; Gorlov et al., 2017 |
| CAST |           | MspI        | Hanrahan et al., 2004; Getmantseva et al., 2019|

According to the results of a molecular genetic study, the presence and frequency of alleles and genotypes for the genes GDF9, GH, CAST were determined.

3. Results and discussion.

As a result of DNA genotyping for genes GDF9, GH, CAST, the genetic structure of sheep populations of such breeds as: Salskaya, Stavropol, Volgograd and Soviet merino was studied. The analysis results are shown in Table 3.

Table 3. Frequency of alleles and genotypes of genes GDF9, GH, CAST.

| Gene    | n   | Allele frequency | Genotype frequency,% |
|---------|-----|------------------|-----------------------|
|         |     | A    | B     | AA  | AB  | BB  |
|         | Salskaya breed |     |       |     |     |     |
| GDF9    | 108 | 0.05 | 0.95  | -   | 10.00 | 90.00 |
| GH      | 108 | 0.73 | 0.27  | 55.56 | 34.26 | 10.19 |
| CAST    | 108 | 0.89 | 0.11  | 78.30 | 21.70 |  -   |
|         | Soviet merino |     |       |     |     |     |
| GDF9    | 72  | 0.03 | 0.97  | -   | 6.94  | 93.06 |
| GH      | 72  | 0.74 | 0.26  | 56.94 | 33.33 |  9.72 |
| CAST    | 72  | 0.88 | 0.12  | 81.90 | 12.00 |  6.10 |
|         | Stavropol breed |     |       |     |     |     |
| GDF9    | 50  | 0.05 | 0.95  | -   | 10.00 | 90.00 |
| GH      | 50  | 0.70 | 0.30  | 50.00 | 40.00 | 10.00 |
| CAST    | 50  | 0.91 | 0.09  | 82.30 | 17.70 |  -   |
|         | Volgograd breed |     |       |     |     |     |
| GDF9    | 110 | 0.08 | 0.92  | -   | 16.36 | 83.60 |
| GH      | 110 | 0.71 | 0.29  | 55.45 | 30.91 | 13.64 |
| CAST    | 110 | 0.85 | 0.15  | 70.30 | 29.70 |  -   |

The results of the analysis of the Salsk sheep population for the GDF9 gene revealed the presence of two alleles A and B. In the studied population, the B allele (95%) and the BB genotype (90%) had a high frequency, and the frequency of the heterozygous AB genotype was 10%. The genetic structure of the Salsk sheep population by the GH gene is characterized by the presence of three genotypes: AA, AB, and BB. In this population, the homozygous BB genotype (10.19%) had a low frequency of occurrence. Analysis of allele frequencies and genotypes of Salsk sheep by the CAST gene revealed the presence of two genotypes AA (78.30%) and AB (21.70%).

The study of the state of the population of sheep of the Soviet Merino breed according to the GDF9 gene showed the presence of two genotypes AB and BB. Most of the animals of the Soviet Merino breed were carriers of the BB genotype (93.06%). The frequency of occurrence of GH gene genotypes in the population of Soviet Merino sheep was characterized by the presence of all possible genotypic combinations of the growth hormone gene: AA, AB, and BB. At the same time, the proportion of homozygotes for allele A was the largest (74%). The detected alleles A and B in the Soviet merino sheep provided the presence of three genotypes of the CAST gene. Most of the animals were carriers of the AA genotype (81.90%).
As a result of DNA genotyping in sheep of the Stavropol breed for the GDF9 gene, the presence of two genotypes AB (10%) and BB (90%) was established. According to the GH gene, the homozygous AA genotype (50.00%) prevailed in the population of the Stavropol breed. The frequency of occurrence of CAST gene genotypes in the population of sheep of the Stavropol breed was characterized by the presence of two genotypes AB (82.30%) and BB (17.70%).

An analysis of the allele and genotype frequencies of Volgograd sheep for the GDF9 gene revealed the presence of two genotypes AA (16.36%) and AB (83.60%). The genetic structure of sheep of the Volgograd breed according to the GH gene was determined by the presence of three genotypes AA, AB and BB. In this population, the homozygous BB genotype had the lowest frequency (13.64%), the AB genotype frequency had an intermediate value (30.91%), and the AA genotype had the highest frequency (55.45%). The genetic structure of sheep of the Volgograd breed according to the CAST gene is determined by the presence of two genotypes AA, AB. The heterozygous AB genotype (29.70%) had the lowest frequency. The allele A and the homozygous genotype AA (70.30%) had the highest frequency.

Malewa A.D. et al. data were presented on the distribution of the genotypes of the growth hormone gene in sheep: AA - 35%, AB - 35%, BB - 28%.

Geoergieva S. et al. presented data on the presence of different genotypes of the CAST gene in dairy-producing sheep bred in Bulgaria. At the same time, genotypes MM, MN, NN were found in the studied sheep population with a frequency of occurrence of 84%, 15% and 1%, respectively.

A and B allelic variants of the GDF9 gene, alleles A and B of the GH gene, and alleles A and B of the CAST gene were identified in all studied groups of sheep. In sheep of the Salsky, Stavropol, Volgograd breeds and the Soviet Merino breed, the BB genotype of the GDF9 gene prevailed. The frequency of occurrence of the AB genotype is within the range of 6.94 to 16.36%. The frequency of occurrence of the AA genotype of the GH gene is the highest (from 50.00% to 56.94%). Genetic analysis of the livestock for the CAST gene showed that the AA genotype prevailed in all studied groups, with a frequency of 70.30% and higher. Homozygotes for the B allele of the CAST gene were not detected in Salsk and Volgograd sheep.

4. Conclusion
The results of studying the genetic structure of the populations of Salskoy, Stavropol, Soviet Merino and Volgograd breeds showed that the distribution of allele frequencies and genotypes of the genes of growth hormone (GH), calpastatin (CAST) and differential growth factor (GDF9) have some peculiarities associated with the breed tested sheep. The established biological characteristics of genotypes must be extrapolated to the economically useful qualities of specific animals and taken into account when assessing the influence of genotypes on productivity indicators and choosing the tactics of breeding programs, as well as the desired genotype of sheep.

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