Meat productivity of sheep of the Altai Mountain breed of different genotypes according to the CAST and GDF9 genes

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Abstract. The successful development of sheep farming in the meat sector is largely determined by the use of molecular genetic methods and modern genotypes. The Altai Mountain breed of sheep optimally combines high wool and meat with unsurpassed adaptability to grazing in the highlands of the Altai Republic throughout the year. The genes of calpastatin (CAST) and differential growth factor (GDF9) are considered as promising markers of quantitative and qualitative signs of sheep meat productivity. It was established that in the Altai Mountain breed the polymorphism of the CAST and GDF9 genes is represented by three genotypes MM, MN, and NN; AA, AG and GG with a frequency of occurrence of 0.23; 0.72 and 0.05; 0.10; 0.35 and 0.55, respectively. The level of homozygosity (Ca) in the CAST gene was 73.1%, GDF9 – 60.6%. The level of effective alleles (Na) was higher in the GDF9 gene (1.65) compared with CAST (1.44). The level of factual (Ho) and theoretical (He) expected heterozygosity for the GDF9 gene was 0.538 and 0.651, respectively, for the CAST gene – 0.290 and 0.368, which indicates a certain lack of heterozygotes in both genes. The analysis of slaughter qualities established the superiority of carriers of the NN genotype over the MM genotype in terms of pre-slaughter and carcass weight, meat ratio by 4.96 and 2.83 kg, respectively (P<0.05), 0.26 units (P<0.05). In the GDF9 gene, the AA-type sheep exceeded the peers of AG and GG-genotypes in the pre-slaughter weight, carcass weight and its output, meat ratio by 1.62 and 7.01 kg, respectively; 1.34 and 3.98 kg (P<0.05); 1.21 and 1.86 abs. percent; 0.16 and 0.39 (P<0.05) units.

1. Introduction
Sheep breeding is a unique industry of livestock farming, characterized by a variety of products: wool, meat, milk, sheepskin [1, 2]. Currently, the successful development of the industry in the meat sector is largely determined by the use of modern approaches to improve the existing sheep breeds and to create new genotypes that allow producing the highest quality products that meet the market demands to the minimum possible cost. In recent years, genomic assessment has been recognized as one of the most effective methods for increasing the genetic potential of sheep. It gains even greater significance while maintaining domestic tribal resources and biodiversity in sheep breeding [3]. Therefore, the problem of...
accumulation and expansion of the information base of genetic data on the Russian breeds is very relevant, because such a database is an informational basis for studying the population structure of breeds, identifying unique genome regions susceptible to positive breeding, identifying genetic variants responsible for adaptation, productive and other economically valuable characteristics.

Genes of calpastatin (CAST) and differential growth factor (GDF9) are considered as promising markers of quantitative and qualitative signs of sheep meat productivity [4, 5, 6].

The sheep CAST gene, with a size of about 100 thousand base pairs (hereinafter – bp), includes four exons and is located on the fifth chromosome. It is part of the calpain-calpastatin enzyme system and acts as a specific inhibitor of calcium-dependent proteases. There is evidence that the pronounced structure and tenderness of meat after the animal slaughter is controlled by the active function of the enzyme calpastatin [7, 8, 9].

The sheep GDF9 gene is localized on the fifth chromosome and has a length of about 2.5 thousand bp, includes 2 exons separated by one intron (1126 bp). It controls the synthesis of a peptide consisting of 453 amino acids. The role of the gene in the formation of signs of reproduction and the growth energy of sheep has been proved [10, 11].

The Altai Mountain breed, formed in the 1930–1940s through complex reproductive crosses, is characterized by high wool and meat productivity and is perfectly adapted to year-round keeping in cold climates and high mountain pastures of the Altai Republic [12].

Despite the wide spreading and relevance of the breed, studies of genes associated with meat productivity have not been conducted in this breed. In this regard, the purpose of this research was to study the polymorphism, the genetic structure of the Altai Mountain breed according to the CAST and GDF9 genes and their relationship with the signs of meat productivity.

2. Material and methods
The research was carried out in the accredited laboratory of immunogenetics and DNA technology of VNIIOK – a branch of the Federal State Budget Scientific Institution “North Caucasian FNATs” (accreditation number ROSS RL/001/21PD29). DNA was isolated from blood samples of sheep of the Altai Mountain breed (n = 40, farm “Usoltseva N.A.” of the Ust-Kansky district of the Republic of Gorny Altai) using the “DIAtom DNAPrep” kit (“Isogen” Ltd, Moscow). To perform PCR gel electrophoresis, “GenePakPCRCore” kits (“Izogen” Ltd, Moscow) were used and DNA samples with a concentration of 3–5 μg/100 μl (OD 260/280 from 1.6 to 2.0) were taken.

PCR was performed on a “Tertsik” programmable thermal cycler (“DNA Technology” Ltd, Russia) using primers for the CAST gene of the following nucleotide sequence: F:5’-tgt-ggc-cca-atg-acg-cca-ttg-atg-3’ and R:5’-ggt-gaa-gca-ctg-atg-acc-3’; for GDF9 – F:5’-gaa-gac-tgg-tat-ggg-gaa-atg-3’ and R:5’-cca-acc-tgc-tcc-tac-aca-ctt-3’.

The position in the genome of the studied single nucleotide polymorphisms in the CAST, GDF9 genes is shown in table 1.

Table 1. Identification of the studied single nucleotide polymorphisms in the genes CAST, GH, GDF9 in NCBI.

| Gene | Position in assembly Oar_rambouillet_v1.0 | Position in assembly 4.0 | Sequence | Identifier NCBI |
|------|-----------------------------------------|-------------------------|----------|----------------|
| CAST | 102036502                               | 93378602                | intron   | rs422618244    |
| GDF9 | 46547268                                | 41770341                | exon     | rs410123449    |

The output of amplicons, their quality was controlled by electrophoretic separation in 2–4% agarose gel. When the amplification of the CAST gene was processed with the restriction enzyme MspI at 37 °C and subsequent electrophoresis, the following genotypes were identified by the presence of bands of a certain molecular weight: CAST<sup>MM</sup>, CAST<sup>MN</sup>, CAST<sup>NN</sup> (figure 1).
The amplification of the amplified region of the GDF9 gene was carried out by BstHH1 endonuclease. Genotypes were identified by the presence of bands of a certain length: GDF9^{AA}, GDF9^{AG}, GDF9^{GG} (figure 2).

The level of homozygosity (Ca), the number of effective alleles (Na), the degree of genetic variation (V), the heterozygosity test (TH) – the level of factual (Ho) and theoretical (He) expected heterozygosity were determined using GenAlEx 6.5 software [13].

To study meat productivity at 7 months of age, according to the GNU “SNIIZHK” (State Scientific Institution “Stavropol Research Institute of Livestock and Feed Production”) technique, in each genotype a control slaughter of three rams corresponding to the average live weight in the corresponding genotype was performed.

Biometric processing of the obtained materials was carried out using the BIOSTAT and MS Office software.

3. Results and discussion

As a result of PCR-RFLP analysis, it was found that in sheep of the Altai Mountain breed, the CAST polymorphism is represented by two alleles and three genotypes, respectively, M and N; MM, NN and MN, GDF9 – by A and G; AA, GG, AG, with different frequency of occurrence.

In the CAST gene, the M allele was detected with a significantly higher frequency; the N allele frequency was lower, which caused highly significant differences in the frequency of homozygous MM and NN genotypes from 0.05 to 0.72. In the GDF9 gene, the G allele and, accordingly, the GG genotype were much more widespread, the frequency of occurrence of which was 0.73 and 0.55, while the A allele was detected with a frequency of 0.27, and the AA genotype was detected with a frequency of 0.10 (table 2).

A greater prevalence of the M allele than the N allele in the CAST gene has been reported in a number of studies. Thus, in sheep of the Dorset Horn breed, the M allele was detected with a frequency of 0.77, while N was 0.23 [5]. In sheep populations raised in Iran and Pakistan two alleles and three genotypes – MM, MN, NN were detected in the Dalagh breed with a frequency of 0.082; 0.891; 0.027, respectively; Lori – 0.320; 0.630; 0.050, Zel – 0.620; 0.260; 0.120;洛希 – 0.77; 0.20; 0.03, Kaji – 0.68; 0.26; 0.06; Thalli – 0.80 (MM) and 0.20 (MN) [14, 15, 16, 17].


Table 2. The frequency of alleles and genotypes of the CAST and GDF9 gene in sheep of the Altai Mountain breed.

| Gene  | Genotype | Frequency of occurrence |
|-------|----------|-------------------------|
|       |          | genotype               | allele  |
|       |          | 0.72±0.07               | M 0.84 |
| CAST  | MM       | 0.23±0.06               | N 0.16 |
|       | MN       | 0.05±0.01               |
|       | NN       | 0.10±0.05               |
| GDF9  | AG       | 0.35±0.07               | A 0.27 |
|       | GG       | 0.55±0.08               | G 0.73 |

A low frequency of occurrence and a complete absence of the AA genotype in the GDF9 gene were noted in other sheep breeds. Thus, when genotyping sheep of five breeds (Shal, Ghezel, Afshari, Lori, Bakhtyari) AA carriers were detected only in three – Shal, Ghezel and Afshari with a frequency of 0.061; 0.017 and 0.036, while this genotype was determined only in ewes, it was absent in rams [18]. Homozygous AA genotypes were not detected among sheep of the Volgograd and Salsk breeds [19].

Comparison of the population-genetic parameters of the studied sheep population revealed a high variability of values, which depended on the gene. The level of homozygosity (Ca) in the CAST and GDF9 genes was 73.1 and 60.6%, respectively, which indicated, to a certain extent, the consolidation of the population, and to a greater extent on the CAST gene. The level of effective alleles (Na) was higher in the GDF9 gene – 1.65, whereas in the CAST gene – 1.44. The actual (Ho) and theoretically (He) expected heterozygosity was also different in the genes studied. Large values were traced in the GDF9 gene, which amounted to 0.538 and 0.651, respectively, for the CAST gene they were lower and equal to 0.290 and 0.368, respectively (table 3).

Table 3. Indicators of genetic variation in the CAST and GDF9 genes in sheep of the Altai Mountain breed.

| Gene  | Ca, %   | Na     | Ho    | He    | V, %  | TH<sup>a</sup> |
|-------|---------|--------|-------|-------|-------|---------------|
| CAST  | 73.1    | 1.37   | 0.290 | 0.368 | 24.4  | −0.08         |
| GDF9  | 60.6    | 1.65   | 0.538 | 0.651 | 36.9  | −0.11         |

<sup>a</sup>F<T – deviation of the factual frequency of occurrence of heterozygotes (F) from theoretically expected (T).

It is interesting to note that a higher degree of genetic variation (V) was characteristic of the GDF9 gene than of the CAST gene. The variability of the heterozygosity test (TH), which, according to Hardy-Weinberg’s law, reflects deviations of the observed frequencies of heterozygous genotypes from theoretically expected ones in the studied genes, was insignificant: from −0.08 for the CAST gene to −0.11 for the GDF9 gene, which indicates some lack of heterozygotes.

A comparative analysis of the slaughter qualities of young sheep of the Altai Mountain breed of different genotypes by the CAST gene established the superiority of carriers of the NN genotype over other genotypes. The greater difference was over the rams of the MM genotype and amounted for slaughter and carcass weight, meat ratio to 4.96 and 2.83 kg (P<0.05), 0.26 units (P<0.05), respectively.

A similar comparison of meat productivity indicators of young animals of different genotypes by the GDF9 gene revealed that the AA genotype rams exceeded their peers of AG and GG genotypes in pre-slaughter weight, carcass weight and its output, meat ratio by 1.62 and 7.01 kg, respectively; 1.34 and 3.98 kg (P<0.05); 1.21 and 1.86 abs. percent; 0.16 and 0.39 (P<0.05) units (table 4).
Table 4. Slaughter qualities of young animals of Altai Mountain breed of different genotypes by CAST and GDF9 genes.

| Gene, genotype | Meat productivity indicators |
|----------------|-----------------------------|
|                | animal weight, kg | carcass mass, kg | output, % | internal fat, g | slaughter mass, kg | meat ratio | slaughter output, % |
| MM             | 40.62 ±2.26        | 18.68 ±1.12     | 45.98     | 674.28         | 19.32 ±1.74       | 3.86 ±0.01 | 47.56 ±0.31       |
| CAST MN        | 44.58 ±2.42        | 20.61 ±0.96     | 46.22     | 685.36         | 21.29 ±1.48       | 4.01 ±0.02 | 47.76 ±0.19       |
| NN             | 45.24 ±2.57        | 21.51 ±0.82     | 47.54     | 739.54         | 22.24 ±1.98       | 4.12 ±0.02 | 49.16 ±0.24       |
| AA             | 46.33 ±2.18        | 22.17 ±0.94     | 47.86     | 744.0          | 22.91 ±2.76       | 4.10 ±0.02 | 49.44 ±0.28       |
| GDF9 AG        | 44.71 ±2.99        | 20.83 ±1.37     | 46.65     | 619.2          | 21.45 ±3.02       | 3.94 ±0.02 | 48.04 ±0.24       |
| GG             | 39.32 ±2.78        | 18.19 ±0.84     | 46.00     | 709.3          | 18.89 ±1.26       | 3.71 ±0.02 | 47.97 ±0.22       |

*P<0.05 CASTER NN-MM.
*P<0.05 GDF9 AA-GG.
*P<0.05 GDF9 AG-GG.

The results obtained in this study indicate that homozygous NN and AA genotypes in the CASTER and GDF9 genes were superior to other genotypes in terms of meat productivity.

The data obtained in this study are consistent with the results of other authors, which indicate a relationship between the quantitative and qualitative characteristics of the meat productivity of sheep with different genotypes in the CASTER and GDF9 genes [20, 21, 22, 23].

Thus, the development of breeding programs with the inclusion of young animals genotyping by the CASTER and GDF9 genes, selection of animal carriers of the desired alleles and their replication through targeted selection of parental pairs will increase the meat productivity of the Altai Mountain breed.

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