Association of Growth Hormone Gene Polymorphisms and Calpastatin Gene with Quality of Sheep Meat

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ABSTRACT

In connection with the increasing interest in the production of young mutton and lamb, priority is given to the study of genes that control meat production. Alleles of genes calpastatin (CAST) and somatotropin (GH) may act as potential markers of sheep meat productivity. However, until now there is no information on the influence of these genes on the indicators of meat productivity of sheep of Russian breeds. Based on this, the purpose of this research was to study the polymorphism of the CAST and GH genes in meat and wool sheep of the ½ Poll Dorset x ½ North Caucasian meat-wool genotype bred in the Stavropol Territory (Russia) and their influence on the traits of meat productivity. Genotyping of sheep for somatotropin and calpastatin genes was carried out by polymerase chain reaction (PCR) with further study of restriction fragment length polymorphism (RFLP). Three genotypes were identified for the GH gene (AA, AB, and BB) and two for CAST (MM and MN). The highest frequency of occurrence for the GH gene was characterized by the heterozygous AB genotype (42.8%), for the CAST gene - the homozygous MM genotype (87.9%). These genotypes were correlated with quantitative and qualitative parameters of meat productivity. The best indicators of meat productivity were in the bright AB, BB, and MN genotypes of the GH and CAST genes, respectively. According to the point assessment of the “marbling” of meat, animals with the indicated genotypes outnumbered their peers by 1.8; 2.1 and 3.7 points.

INTRODUCTION

Sheep breeding is an important branch of world productive animal husbandry, which still plays a significant role in the history of mankind. For many centuries, people have been breeding and raising sheep to satisfy their needs for meat and milk, as well as to obtain wool and leather raw materials (Frantz et al., 2020).

In recent years, the demand for natural wool products has decreased due to the production of chemical textile materials, which are very similar in quality to wool, but cheaper to manufacture, so producers are forced to improve the efficiency of sheep breeding by introducing new technologies based on the maximum use of the meat production potential (Allafi et al., 2020).

The peculiarities of the meat production, including lamb, are of great economic importance. A significant improvement in lamb production and an increase in its quality can be achieved by the use of industrial crossing in commercial herds, which makes it possible to use the heterosis effect indicated in first generation hybrids. The use of “blood infusion” allows to quickly improve the meat qualities of sheep, but does not provide an opportunity to stabilize the inheritance of the desired traits (Pascal et al., 2018; Petrovic et al., 2019). In this regard, for the successful development of meat sheep breeding, new, more modern approaches to the improvement of existing and creation of new breeds, which are distinguished by high productivity and meet market requirements (Selionova et al., 2020).
Recently, the trend towards improving these approaches has gradually changed from traditional methods of phenotypic selection to genotypic methods using molecular markers (Platten et al., 2019). Sheep breeding strategies using DNA markers are positioned to significantly accelerate the rate of genetic gain of the desired production traits, especially those that are difficult to measure during the life of the animal or appear at a later age (Tellam et al., 2012). A genetic marker is a polymorphic sequence that usually has no biological effect, but is easily genotyped and therefore widely used in genetic research. In recent years, using genetic markers, a number of quantitative trait loci have been mapped, which lead to marker-assisted selection (MAS) in animal breeding programs (Boichard et al., 2016). In many countries with developed animal husbandry, marker selection has become an obligatory part of state breeding programs. The greatest success in the practical application of marker selection was achieved in dairy cattle breeding (Trukhachev et al., 2018).

In sheep breeding, information on the main genes or loci, affect the growth characteristics and productive qualities of sheep is relatively limited, and only a few genes carry useful information for targeted marker selection for meat productivity (Zhang et al., 2013). As a result, the accumulation and expansion of knowledge about the genetic structure of Russian breeds sheep is very informative for the further identification of unique genome regions responsible for economically useful traits (Selionova and Podkorytov, 2021). Scientists have already conducted studies to assess the polymorphism of the growth hormone (GH) and calpastatin (CAST) genes, which presumably can be considered markers of quantitative and qualitative signs of high meat productivity in sheep (Armstrong et al., 2018; Pogodaev et al., 2020; Shirokova et al., 2021).

The protein encoded by the GH gene, discovered in the 1920s, is a member of the prolactin/ somatotropin hormones that play an essential role in physiological processes. The GH gene is located on chromosome 11 and includes 5 exons and 4 introns. Influences the proliferation and growth of cells directly or indirectly through the stimulation of insulin-like growth factor (IGF) (Saleh et al., 2020). In most mammals, GH is the product of a single gene and is usually secreted by the pituitary gland. Growth hormone affects such biological functions of a sheep as growth, lactation period, reproductive parameters, and metabolic characteristics (Dettori et al., 2015; Akhatayeva et al., 2020). Insertions and deletions or mutations in the GH gene lead to differences in growth rates (Ünal et al., 2020).

The CAST gene is an inhibitor of calpain, which is responsible for the formation of skeletal muscles and tenderness of meat after slaughter. The calpastatin gene is localized on chromosome 5 in sheep and includes 29 exons and 28 introns (Gregula-Kania et al., 2019). Calpastatin can influence the transformation of muscle protein during animal growth (Montes et al., 2019). Genetic polymorphism of the CAST gene and its relationship with meat quality have been observed in various farm animals, including cattle, goats, and sheep (KÖK and Atalay, 2018; Saccà et al., 2019; Afanasyeva et al., 2019).

Thus, polymorphisms of the GH and CAST genes play a decisive role in the regulation of sheep development and growth, have a direct impact on the carcass quality, and can be used in marker selection (MAS).

Therefore, the purpose of this study was to identify associations of single nucleotide polymorphisms of the GH and CAST genes with the characteristics of carcasses, yield and quality of meat in sheep of meat and wool breeds, which can be used to predict meat productivity and improve the genetic potential of sheep in Russia.

### MATERIALS AND METHODS

#### Ethical statement

The slaughter of animals for histological studies was carried out in accordance with the directive 2010/63/EU of the European parliament and the council of the European Union on the protection of animals used for scientific purposes.

#### The place of the experiment and the object of study

The studies were carried out on the basis of an experimental station located on the territory of the Stavropol Territory (Russia), IV agroclimatic zone, characterized by a moderately humid climate; in the laboratory of immunogenetics and DNA technologies of the All-Russian Research Institute of Sheep and Goat Breeding a branch of the Federal State Budgetary Scientific Institution North Caucasian Federal Scientific Agrarian Center.

The object of the study was meat - wool sheep (ewes, n = 91) of genotype ½ half Dorset x ½ North Caucasian meat - wool. All animals were kept in the same conditions of feeding and housing with the same daily routine, served by the same staff, and were clinically healthy.

#### Genotyping

Genomic DNA was isolated from whole blood samples taken under aseptic conditions from the jugular vein using the “DIAtom tmDNAPrep” reagent kit (IsoGeneLab, Moscow) in accordance with the manufacturer’s protocol.

Determination of polymorphic variants of the somatotropin and calpastatin gene was carried out by
As a result of molecular genetic studies, the presence of polymorphism in the loci of the growth hormone (GH) and calpastatin (CAST) genes in meat and wool sheep was revealed. It was found that the polymorphism of the GH and CAST genes is represented by two alleles A and B; M and N. The difference in the frequency of the A and B alleles of the growth hormone gene was not significant, while the M and N alleles of the calpastatin gene were characterized by a significant difference in the frequency of occurrence. Based on the results of the distribution of allele frequencies in animals, genotypes were determined: three genotypes AA, AB, and BB for the GH gene, two genotypes MM and MN for CAST. In the studied group of sheep, the heterozygous AB genotype (42.8%) had the highest frequency of occurrence for the GH gene (42.8%), homozygous individuals of AA and the desired BB
genotype were found in almost the same proportions (29.7 and 27.5%). For the CAST gene, a slightly different picture of the distribution of genotype frequencies was observed, where the homozygous MM genotype was predominant, the frequency of which was 87.9%, 12.1% were ewes with the heterozygous MN variant, and no individuals with the NN genotype were identified (Table I).

**Table I. Frequency of alleles occurrence and genotypes of somatotropin and calpastatin in meat-wool sheep.**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Number of sheep</th>
<th>Frequency of occurrence</th>
<th>Genotype, %</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH</td>
<td>91</td>
<td>AA AB BB A B</td>
<td>29.7 42.8 27.5 0.51 0.49</td>
<td></td>
</tr>
<tr>
<td>CAST</td>
<td>91</td>
<td>MM MN NN M N</td>
<td>87.9 12.1 - 0.94 0.06</td>
<td></td>
</tr>
</tbody>
</table>

To assess genetic diversity, indicators such as the homozygosity degree (Ca) and the polymorphism level of loci (Na) are also used. The homozygosity degree of a population indicates the number of effective alleles, the polymorphism level is the reciprocal of the homozygosity degree. In our experiment, at the GH locus, the homozygosity degree was characterized by an average value, and by the CAST locus, by a high value. The Na value for the GH locus was 1.99, and for the CAST locus, 1.13. The obtained data indicate a practical absence of the polymorphism level of the CAST locus and a low number of effective alleles and genotypes, and thus a decrease in the genetic diversity of the studied sheep population for this gene.

Proceeding from this, these parameters and other related quantities were calculated during the study.

The value of the observed heterozygosity (Ho) for the GH and CAST gene loci was 0.43 and 0.12, respectively. The value of expected heterozygosity (He), which is less sensitive to the sample size, for the studied loci was 0.50 and 0.88, which indicates the predominance of the random crossing system over inbreeding in this population (Chesnokov and Artemyeva, 2015).

**Table II. Indicators of the genetic structure of the studied animals.**

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Growth hormone (GH)</th>
<th>Calpastatin (CAST)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of homozygotes (n)</td>
<td>52</td>
<td>80</td>
</tr>
<tr>
<td>Number of heterozygotes (n)</td>
<td>39</td>
<td>11</td>
</tr>
<tr>
<td>Observed heterozygosity (Ho)</td>
<td>0.43</td>
<td>0.12</td>
</tr>
<tr>
<td>Expected heterozygosity (He)</td>
<td>0.50</td>
<td>0.88</td>
</tr>
<tr>
<td>Measure of informational polymorphism (PIC)</td>
<td>0.49</td>
<td>0.11</td>
</tr>
<tr>
<td>The degree of homozygosity (Ca), %</td>
<td>50.02</td>
<td>88.72</td>
</tr>
<tr>
<td>The level of polymorphism (Na)</td>
<td>1.99</td>
<td>1.13</td>
</tr>
<tr>
<td>The degree of genetic variation (V), %</td>
<td>55.50</td>
<td>12.53</td>
</tr>
<tr>
<td>Heterozygosity test (TT)</td>
<td>-0.07</td>
<td>-0.76</td>
</tr>
<tr>
<td>Fixation index (Fis)</td>
<td>+0.14</td>
<td>+0.86</td>
</tr>
</tbody>
</table>

The value PIC content is usually used as a measure of polymorphism for a marker locus and depends on the frequency and number of alleles. The GH locus had an average polymorphism, its value was 0.49; for the CAST locus, the PIC value was very low and amounted to 0.11, which indicates a low frequency of rare alleles.

Literature sources contain a sufficient amount of information on studies for the presence of polymorphism in the GH and CAST genes. So, Shirokova et al. (2021) when studying the genetic structure of the population of Salsk sheep, Soviet merino, Statropol and Volgograd breeds for the indicated genes, the following results were obtained. For the GH gene, three genotypes were identified in all populations of the studied animals: AA, AB, and BB with different frequencies of occurrence. According to the CAST gene of sheep of the studied breeds, in addition to the Soviet merino, the presence of two genotypes was common: MM, MN. Three genotypes were identified in sheep of the Soviet Merino breed; MM, MN and NN.

In Colombian Creole sheep, crossed with domestic Mexican sheep, the MM genotype was the most frequent at the CAST locus, followed by two other genotypes, and the frequency of the M allele exceeded that of the N allele (9%). The frequency of the A allele was higher than that of the G for the GH locus, and only genotypes AA and AG were found, the first was the most frequent (64%) (Lenis-Valencia et al., 2021).

Iovenko et al. (2020) determined the level of polymorphism GH and CAST of genes of the Askan sheep and one of its hybrids. Sheep of all studied breeds and the mentioned hybrid were characterized by polymorphism of GH and CAST loci. GH was represented by two genotypes (AA, AB), and CAST - by three genotypes (AA, AB, BB).

For a more objective assessment, we carried out a genetic-statistical analysis of the obtained results, the numerical values of which are shown in Table II.

Heterozygosity (H) and the measure of informational polymorphism (PIC) are the main parameters used in assessing the informativeness of genetic markers.
degree and the number of animals examined. In our case, for the GH gene locus, this indicator was 55.50%, for the CAST gene - 12.53%.

Heterozygosity test (TГ) indicates a lack or abundance of relative heterozygosity, obtained as the difference between actual and theoretical data. A positive TГ value indicates the prevalence of actual heterozygosity over theoretical. Animals of the studied population can also be seen from the data on the coefficient of kurtosis (Fis). There was a deviation of the observed heterozygosity from the expected one with right-sided excess (+0.14; +0.86). The relative deficit of heterozygotes for the studied genes in the studied population can also be seen from the data on the coefficient of kurtosis (Fis). There was a deviation of the observed heterozygosity from the expected one with right-sided excess (+0.14; +0.86).

Considering the meat productivity of ewes, taking into account the combinations of genotypes for the GH and CAST genes, it can be noted that the group of individuals with genotypes AB, BB of the growth hormone gene and MN of the calpastatin gene was distinguished by the best indicators of meat productivity in comparison with animals of other genotypes of both genes.

Analysis of the meat productivity of the studied livestock, depending on the genotypes of the GH gene, showed the superiority of animal carriers of the AB and BB genotypes in live weight before slaughter, slaughter weight and slaughter yield by 2.9, 6.5, 1.4 and 3.5; 7.3; 3.5% over the ewes carriers of the AA genotype. A lower coefficient of meat content, depending on the polymorphism of the GH gene, was characterized ewes carriers of the AA genotype (Table III).

Microstructural analysis of muscle tissue showed that lamb obtained from animals of the AB and BB genotypes of the GH gene was characterized by a large number of muscle fibers by 5.7 and 6.4%, but the fiber diameter was smaller by 7.6 and 9.2% compared to animals of the AA genotype. The muscle fiber of the AB and BB genotypes had a greater amount of fatty interfiber and interfundus inclusions, which led to a higher marbling score by 1.8 and 2.1 points in comparison with animals of the AA genotype, respectively. In addition, the longissimus dorsi muscle obtained from animals of the AB and BB genotypes contained a lower amount of connective tissue by 0.6 and 0.81%, in contrast to animals of the AA genotype, respectively.

Our research on the effect of variation in the growth hormone gene on sheep meat productivity is consistent with the results of other scientists studying the polymorphism of these genes. Iovenko et al. (2020) found that the live weight at ewes of Askan sheep carrying the A4 genotype of the GH gene was 4.5 kg, and of lambs with the homozygous AB genotype 4.9 kg. That is, according to their studies, the heterozygous AB genotype determined an increased level of meat productivity in sheep.

The presence of the heterozygous AB genotype of the growth hormone gene in the Salsk sheep had a positive effect on growth indicators. Animals with the AB genotype of the GH gene significantly outperformed sheep with the AA genotype and showed the best meat production. Pre-slaughter live weight, carcass weight, slaughter weight and slaughter yield of sheep with the AB genotype were 4.97 kg, 4.3 kg, 1.81 kg and 2.17 kg, respectively (Gorlov et al., 2017).

Akhatayeva et al. (2020) in their work identified genetic variations in the GH gene of Chinese black-headed sheep Luxi Blackhead (LXBH) and tested for associations with morphometric parameters of the carcass. It turned out that the tested sheep with genotype DD had better meat productivity compared to genotypes II and ID, which allowed them to make an assumption about the positive effect of the D allele on the growth and development of animals.

### Table III. Relationship of allelic variants of the GH gene and CAST gene with indicators of meat productivity in meat-wool sheep.

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Genotype of GH gene</th>
<th>Genotype of CAST gene</th>
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<tbody>
<tr>
<td></td>
<td>AA</td>
<td>AB</td>
</tr>
<tr>
<td>Live weight before slaughter (kg)</td>
<td>34.0±0.71</td>
<td>35.0±0.25</td>
</tr>
<tr>
<td>Fresh carcass weight (kg)</td>
<td>14.37±0.36</td>
<td>15.30±0.27</td>
</tr>
<tr>
<td>Slaughter weight (kg)</td>
<td>14.65±0.37</td>
<td>15.58±0.27</td>
</tr>
<tr>
<td>Slaughter yield (%)</td>
<td>43.1±0.20</td>
<td>44.5±0.56</td>
</tr>
<tr>
<td>Fleshing index</td>
<td>2.80±0.34</td>
<td>3.23±0.20</td>
</tr>
<tr>
<td>Number of muscle fibers (pcs)</td>
<td>340.74±3.27</td>
<td>360.30±9.72</td>
</tr>
<tr>
<td>Muscle fiber diameter (μm)</td>
<td>30.61±0.96</td>
<td>28.27±0.79</td>
</tr>
<tr>
<td>General assessment of &quot;marbling&quot; (point)</td>
<td>29.72±1.38</td>
<td>31.54±0.95</td>
</tr>
<tr>
<td>Connective tissue content (%)</td>
<td>8.67±0.13</td>
<td>8.07±0.18</td>
</tr>
</tbody>
</table>
Certain differences were also established in terms of the level of meat productivity of animals depending on the genotypes of the CAST gene. Thus, carriers of the MN genotype favorably differed from their peers of the MM genotype of the CAST gene in terms of live weight before slaughter, slaughter weight and slaughter yield by 2.6; 5.2 and 2.5%, respectively (Table III).

The results of histological studies showed that the number of muscle fibers of the longissimus dorsi muscle in animals with the MN genotype was 2.9% higher, the “marbling” score was higher by 1.9 points. However, in terms of the content of connective tissue and the diameter of muscle fibers, they were inferior to animals of the homozygous MM genotype by 0.34 and 3.7%, respectively.

In this study, for the first time, genetic variants in the region of the CAST gene and their relationship with important traits of meat productivity in meat-wool sheep are described. Nevertheless, the results of similar studies by other scientists also indicate a positive effect of calpastatin gene polymorphism on the qualitative and quantitative characteristics of sheep meat.

Greguła-Kania et al. (2019) observed a significant relationship in the percentage of muscle and adipose tissue of the thig of carcasses of lambs with genotypes AA and AE of the CAST gene. Lambs with genotype AA had significantly higher muscle mass and lower percentage of body fat compared to other genotypes.

Jawasreh et al. (2017) found that Awassi sheep carrying the MM genotype of the CAST gene had a higher total bone weight than carriers of the MN genotype, while lambs with the MN genotype had a higher meat-to-bone per carcass ratio, according to compared to the MM genotype.

According to Yilmaz et al. (2014) revealed significant differences in skin thickness and subcutaneous fat between calpastatin genotypes in Kivirchik sheep. In addition, Yilmaz et al. (2014) also found that lambs with genotypes MN and MM had less carcass fat than their peers with genotypes NN.

CONCLUSION

The results of this study allow us to conclude that it is advisable to conduct DNA testing for the studied genes GH and CAST, the polymorphism of which showed that the best meat productivity in the studied population was possessed by individuals carrying alleles B and N in their genome, which can be used for further selection in the formation of highly productive animals. Identification of animals with desirable alleles for breeding, marking high meat productivity, will lead to the creation of new, more useful populations, herds, etc.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES


Gorlov, I.F., Kolosov, Y.A., Shirokova, N.V., Getmantseva, L.V., Slozhkenina, M.I., Mosolova,
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