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# Calpastatin and myostatin genes polymorphism and their association with slaughter traits in the Gashun Soviet Merino breed grown in southern Russia

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**Abstract**

The study aimed to investigate the genetic variability of the Gashun Soviet Merino sheep breed, determining myostatin (*MSTN*) and calpastatin (*CAST*) gene polymorphism by the PCR-RFLP method. Earmark samples were collected from 200 animals, and DNA was isolated using a commercial kit. The *MSTN* gene had *A* and *G* allele frequencies of 0.33 and 0.67, and the *CAST* gene had *M* and *N* allele frequencies of 0.83 and 0.17, respectively. The frequencies of the *AG* and *GG* genotypes for *MSTN* and *MM* and *MN* for *CAST* were 0.66 and 0.34, respectively, in both. Genetic equilibrium was maintained for the *CAST* gene ($\chi^2$=4.1951). For the *MSTN* gene, the Hardy-Weinberg equilibrium was violated ($\chi^2$=24.2). The pre-slaughter body weight, hot carcass weight and slaughter weight were higher in *CAST_MN* genotype than *CAST _MM* by 4.8%, 6.5%, and 6.4%. The hot carcass and slaughter weights of lambs with the *MSTN_AG* genotype were higher than *MSTN _GG* by 4.7% in both. However, the pre-slaughter weight in the animals with *the MSTN _GG* genotype was higher by 3.4%. Data on the *CAST* and *MSTN* gene polymorphism and the genetic structure of the studied sheep population were obtained for the first time, their association with slaughter traits was revealed, and desirable genotypes *CAST_MN* and *MSTN_AG* were determined. The Na, Ne, Ho, He, FIS and $\chi$2 values obtained and the absence of individuals with *CAST_NN* and *MSTN_AA* genotypes substantiated the prospect of further use of these genes in programs of genetic livestock improvement.

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## Introduction

Modern breeding involves introducing advanced genetic methods into livestock farms' breeding services to improve farm animals' productivity (1). There is a gradual shift from traditional animal evaluation methods to the integration of molecular genetic data that improves the accuracy of productivity prediction and opens new opportunities for early breeding (2). Traditional methods based on pedigree and phenotypic data analysis have certain limitations. For example, phenotypic parameters that reflect productivity may only become apparent with age, making early assessment of an animal's genetic potential difficult. In addition, environmental and random factors can distort the true picture, making the estimation less accurate (3). The application of molecular genetic data in animal breeding is a revolutionary step to dramatically improve the productivity and economic profitability of sheep breeding (4). Marker-assisted and genomic breeding programs are intensively implemented in the largest mutton-producer countries,

especially Australia and New Zealand (5-7). According to the state program of agricultural development and regulation of markets for agricultural products, raw materials and food (Resolution of the Government of the Russian Federation No. 2309 dated December 16, 2021), genetics and breeding of farm animals and plants are the key reference point for the development of the agro-industrial complex of the Russian Federation. Increasing the volume of livestock production is one of the main goals of one more federal scientific and technical program for agricultural development (Resolution of the Government of the Russian Federation dated August 25, 2017, No. 996). The livestock breeders of the Russian Federation, especially sheep breeders, face the following challenges: increasing the productivity of local breeds of farm animals. The Russian Federation has considerable potential for increasing the production of all industrial products and enhancing the number of sheep. The territory has natural pasture massifs, which can be used to meet the need for fodder without considerable economic costs. In the relatively recent past, the economy of sheep breeding in Russia was based mainly on the production of wool, the share of which in the total cost of production of this industry was 60-80%, and the selling price of 1 kg of wool was equivalent to 20 kg of body weight mutton. The development of sheep breeding is conditioned by the changing needs of the food market; there is an increasing demand for high-quality mutton and stringent requirements for its quality (8,9). For decades, scientists have tried to get a muscular carcass with marbling traits under a fine-haired Merino caftan through various breeding and selection techniques. Mass breeding of sheep has not been as successful as it should have been. There are many reasons for this condition, but the most important one should be considered: the fear of breeders losing the achieved high indices of wool productivity and the incentive methods used by regional and federal authorities to maintain wool production and others. The low level of Merino's meat productivity is currently one of the main reasons for low profitability and often even the unprofitability of sheep breeding. The marker-associated selection (MAS) methods can help increase meat productivity without compromising the wool quality (10). Current DNA technologies allow us to study genetic biodiversity in populations and identify genes directly or indirectly related to animal productivity (10,11). The discovery of the animal genome has opened new opportunities to increase the productive potential of animals (12). Under the influence of heredity in animals, a certain character of metabolism is formed, which creates the final effect of productivity. However, despite metabolism being the sum of many genetically regulated processes, it is still influenced by the external environment (13). Some of the candidate genes (DNA markers of quantitative trait loci) associated with economic performance allow the estimation of the genetic potential of animal productivity (14). Evaluation of animal genotypes facilitates identifying and accumulating preferred alleles in populations (15). The calpastatin gene (*CAST*) is a specific calpain inhibitor (a family of calcium-dependent calpain proteases) that plays an important role in post-slaughter meat softening (16). Genetic polymorphism of the calpastatin gene (*CAST*) and its association with meat quality have been observed in livestock species, including cattle, goats and sheep (17). In sheep, the *CAST* gene is located at locus 5q15 of the fifth chromosome and contains 28 introns and 29 exons, a length of 89553 bp. The gene polymorphism is located in the first intron between exons 1C and 1D. It was detected in the amplified fragment of 622 bp by PCR-RFLP method using the restriction endonuclease *MspI* that cleaves the nucleotide sequence at the specific site of 5'...C↓CGG...3' (18). Thus, two polymorphic variants were identified due to the presence or absence of a point mutation (SNP) and the substitution of *CCGG* by *CCAG*. If a restriction site is present, two fragments of 336 and 286 bp are formed, corresponding to the allelic *M* variant; if a restriction site is absent, the fragment length remains unchanged (622 bp). Myostatin is one of the most promising genes affecting meat productivity indicators. The protein encoded by this gene is synthesized as a precursor protein and inhibits muscle tissue development. The myostatin gene contains 375 amino acids, is located on chromosome 2 of the sheep genome, locus 2q32.2, and consists of three exons and two introns in all species studied (12,19). Blocking the action of myostatin (*MSTN*) increases the muscularity and strength properties of skeletal muscles (20). The *MSTN* gene was detected in an amplified fragment of 399 bp length by PCR-RFLP using restriction endonuclease *Acl I*, which cleaves the nucleotide sequence at the following specific site: 5'... AA↑CGTT...3'. Two fragments of length 74 and 325 bp corresponded to the *AA* genotype; 74, 325 and 399 bp to the *AG* genotype; and 399 bp to the *GG* genotype. Genetic polymorphism in indigenous breeds can be useful for conserving genetic resources; therefore, it is important to genetically characterize the indigenous breeds (21). The range of DNA markers associated with reproductive, meat and fattening traits constantly expands. At the same time, there is very little scientific knowledge about the polymorphism of myostatin and calpastatin genes in sheep of different breeds bred in the arid regions of southern Russia. Selection of animals by genotype, along with traditional methods of selection, can considerably increase the efficiency of improvement of both the stock of an individual farm and the breed as a whole (17).

Despite the Soviet Merino breed's high importance and prevalence in the arid conditions of southern Russia, no studies have been conducted on the identification of *CAST* and *MSTN* gene polymorphisms, the genetic structure of the Gashun population, or the relationship between different allelic variants of these genes and sheep slaughter traits. Therefore, the aim of this study was to determine the genetic structure of Gashun Soviet Merino sheep by the *CAST* and

*MSTN* genes and to identify the relationship between their polymorphisms and slaughter traits. Results obtained allowed the identification of desirable genotypes and provided prospects for improving breeding work.

## Materials and methods

### Ethical approve

This research was conducted from 05/9/2022 to 30/6/2024 in the collective breeding farm named after Skiba, Rostov region (46.758475; 42.802302) and with the ethical approval of the Don State Agrarian University Institutional Animal Care and Use Committee EA DSAU # 1-2022-09-01.

### Animals and housing

The study subjects were sheep of the Gashun Soviet Merino breed (Figure 1). All animals were clinically healthy and kept in optimal conditions to meet zootechnical norms and zoo-hygienic requirements. The breed selected for the research is the most widespread among fine fleece breeds in Russia due to its main advantages, such as survivability in the conditions of arid zones of southern regions in Russia, resistance to extreme climatic factors and high wool quality. The Gashun type was bred in 1993 in the homonymous Gashunsky breeding farm (Rostov region, 47.128525; 42.965764). Mazayev, Novocaucasian, Wallachian sheep, and American Rambouillet lambs (later lambs of Australian selection) were used as basic breeds. Breeding of Gashun-type resulted in improved productivity indicators of the Soviet Merino sheep. The farm has alkali soil; the water is bitter-salty. According to the Köppen climate classification, the climate there is dry continental with hot summers. The average annual temperature is 10.8 °C, and the rainfall is 380 mm. The traditions of animal husbandry were preserved and developed on this territory during the Soviet period. However, animal husbandry is stagnating because large daily and annual fluctuations of air and soil temperatures characterize the zone of a strongly continental climate. Such soil and climatic conditions are suitable for beef cattle and sheep breeding. This research work analyzed the data from primary zootechnical breeding records and the results of our study, which evaluated parents' productive qualities, controlled slaughter, selection, and laboratory studies of biomaterials. The group of experimental animals included equal amounts of male offspring, received from 3-year-old five ram-producers unrelated to each other and 190 ewe lambs at the age of 2 years. All parental pairs belonged to the Gashun Soviet Merino breed. The organization of reproduction observed conditions of panmixia, which excluded the influence of directional selection of parental pairs. The difference in age of young animals of the experimental group did not exceed 4 days. Full siblings were not included in the experimental group.


Figure 1: Soviet Merino of the Gashun type, where A is the Gashun-type breeding lambs of the Soviet Merino breed, B is the fleece of the main Soviet Merino lambs.

### Sampling

Ear tissue samples (about 1 $cm^2$) were isolated from the experimental animals (n=200) for molecular genetic studies. The biological material was collected during livestock judgement when sheep were earmarked to assign an individual animal number. The samples were stored at -18°C until DNA extraction.

### DNA extraction

DNA extraction from the tissue samples was performed according to the standard protocols using the DNA-Extran-2 kit (Syntol LLC, Russia), the Cleanup Mini kit for DNA extraction and purification from reaction mixtures (Evrogen CJSC, Russia), and the QuantiFluor(R) dsDNA System E2670 kit for quantitative determination of double-stranded DNA in QuantiFluor(R) dsDNA solution (Biom Group, Russia).

### PCR Amplification

The prepared PCR mix included 1x Taq buffer (1 mM $Mg^{2+}$ concentration), dNTP (0.2 mM), forward and reverse primers 10 pmol each, HS Taq DNA polymerase (1-unit activity) and DNA matrix (50-500 ng). The primer sequences involved for *CAST* site [available at], which F: 5'-TGGGGCCCAATGACGCCATCGATG-3' and R: 5'-

GGTGGAGCAGCACTTCTGATCACC-3'; and for the *MSTN* site [available at], which F: 5`-TTATGGGTTCGTGATGGCTGT-3` and R: 5`-TGGAAGCCAGAAATCTAGAGTTAATCA-3`.
Amplification was performed on an ANK-32 thermocycler (Syntol LLC, Russia) using the following program: initial denaturation at 95°C for 5 min followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 62°C (*CAST*) and 58°C (*MSTN*) for 45 s, extension at 72°C for 60 s and final extension at 72°C for 10 min. PCR products (*CAST* of 622 bp and *MSTN* of 399 bp) were identified using a 10-line (100-1000 bp) ladder "Step100" (Biolabmix LLC, Russia) by electrophoresis in 2% agarose gel with dye (EtBr) at 85 V for 60 min using the appropriate kit (DNA-Technology LLC, Russia). The results were visualized using a GenoSens 2150 gel documentation system (Clinx Science Instruments Co., Ltd., China).

**RFLP analysis**

For the restriction analysis, 0.5 µl *MspI* (*CAST*, C↑CGG / GGC↓C) and 0.5 µl *Acl I* (*MSTN*, AA↑CGTT / TTGC↓AA) (SibEnzyme Ltd., Russia) were used. For this purpose, a reaction mixture was prepared. The mixtures were incubated at 37°C for 15 h. The restriction fragments were identified using the 10-line (100-1000 bp) ladder "Step100" (Biolabmix LLC, Russia) by electrophoresis in 2% agarose gel with dye (EtBr) at 85 V for 60 min using the appropriate kit (DNA-Technology LLC, Russia). The results were visualized using a GenoSens 2150 gel documentation system (Clinx Science Instruments Co., Ltd., China).

**Slaughter traits**

A control slaughter of males at 6 months of age (n = 40) was performed, and the results were used to estimate the carcass weight, slaughter weight and slaughter yield in accordance with the current GOST 31777-2012 "Sheep and goats for slaughtering. Mutton, lambs and goats in carcasses. Specifications." The control slaughter was carried out at the Gashunsky breeding farm (Zimovnikovsky district, Rostov region, Russia; 47.128525, 42.965764). Before the controlled slaughter, the animals were fattened for 30 days. Twenty animals from each experimental group were taken for slaughter. They had body weight values close to the average obtained by weighing young animals of the general population.

**Statistical analysis**

Genetic indices (22) were calculated in PopGen 1.32 and Arlequin 3.5.2.2 programs, and statistical processing (23) was performed in the Statistica 10.0 program (Statsoft Inc., USA). The frequency of genotypes was calculated using the formula P=(n/N) (1), where *n* is the number of animals with a given genotype, and *N* is the total number of animals studied in a given population. Allele frequencies were calculated using the formula $p= (2N_{ii}+n_{iy})/2N$ (2), where *p* is the allele frequency, $N_{ii}$ is the number of homozygous animals for a given allele, $N_{iy}$ is the number of heterozygous animals, and N is the total number of animals in the sample. The number of effective alleles (polymorphism level) was calculated using the formula $N_a=(1/C_a)$ (3), where $C_a$ is the coefficient of homozygosity. The number of informative alleles was calculated as those with a frequency greater than 5%. The observed degree of heterozygosity ($H_o$) was calculated for each locus as the ratio of the number of heterozygotes to the total number of animals examined $H_o=(1/n\Sigma h_i)$ (5), where *n* is the number of animals; $h_i$ is the number of heterozygotes. The expected degree of heterozygosity ($H_e$) for each locus was calculated using the formula $He= (1-\Sigma p_i^2)$ (6), where $p_i$ is the frequency of the *i*-th allele occurrence. The $F_{is}$ fixation index is the inbreeding coefficient in individuals in relation to the group. It was determined by the formula $F_{is}=(H_e-H_o)/H_e$ (7), where $H_e$ is the expected degree of heterozygosity; $H_o$ is the observed degree of heterozygosity. The genetic equilibrium in the studied populations was analyzed according to the Hardy-Weinberg distribution using the $\chi^2$ method. The Kolmogorov-Smirnov and Lilliefors tests (the Shapiro-Wilk W test additionally) were used to test the normality of the distribution of the analyzed parameters. One-way parametric ANOVA, where the dependent variables were the corresponding measurement results and the independent variable (factor) was the genotype, was applied to the data conforming to the Gaussian distribution law, with the requirement of Levene and Brown-Forsythe tests for homogeneity of variances for the compared samples being taken into account. Tukey's test was used to compare indicators between groups (Post-hoc analysis) and to determine the significance between groups in the analysis of variance. The significance compared to mean values at $P<0.05$: *** = $P<0.001$; ** = $P<0.01$.

**Results**

The M and N allelic variants of the *CAST* gene and genotypes in the sheep breed studied were determined by the restriction analysis using the *MspI* restriction endonuclease. Representative bands for the *MM* and *MN* genotypes for the GSM sheep breed are shown in figure 2: 622 bp, 336 bp, and 286 bp for the MN genotype and 336 bp and 286 bp for the MM genotype.

The digestion using restriction endonuclease *Acl I* allowed us to identify the *A* and *G* allelic variants of the *MSTN* gene and determine two genotypes presented in the studied sheep population (Figure 3): 74, 325, and 399 bp for the AG genotype and 399 bp for the GG genotype.

As can be seen in table 1, the DNA genotyping of the experimental sheep population with respect to the *CAST* gene found the presence of two genotypes: *CAST_MM* (66%) and *CAST_MN* (34%), with the *M* allele having the highest frequency (83%). The studies of the sheep population

in terms of the *MSTN* gene showed the presence of two genotypes: *MSTN_AG* (66%) and *MSTN_GG* (34%). The majority of animals were carriers of the *MSTN_AG* genotype. The highest frequency was found for the *G* allele (67%).

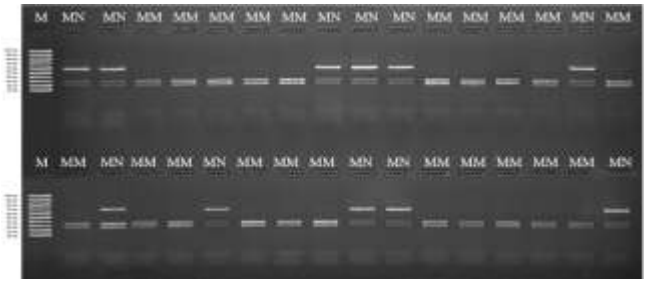

Figure 2: Electrophoregram of PCR-RFLP results (*CAST* gene).

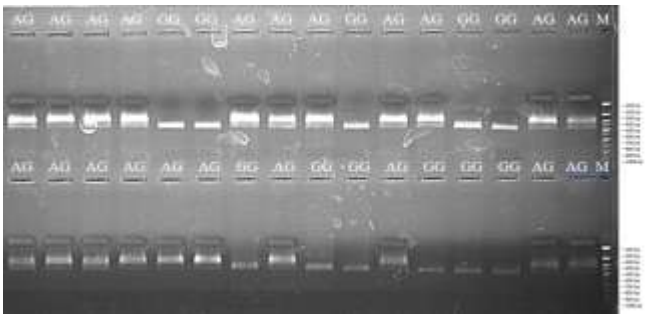

Figure 3: Electrophoregram of PCR-RFLP results (*MSTN* gene).

Table 1: Allele and genotype frequencies in the population under study

| Gene | Allele frequency | | Genotype frequency | | | | | |
|---|---|---|---|---|---|---|---|---|
| *CAST* | *M* | *N* | MM | | MN | | NN | |
| | | | n | % | n | % | n | % |
| | 0.83 | 0.17 | 132 | 66 | 68 | 34 | 0 | 0 |
| *MSTN* | *A* | *G* | AA | | AG | | GG | |
| | | | n | % | n | % | n | % |
| | 0.33 | 0.67 | 0 | 0 | 68 | 66 | 132 | 34 |

As shown in table 2, the individuals of the analyzed sample had an average observed ($H_o$) heterozygosity of 0.340 and expected ($H_e$) heterozygosity of 0.312 for the *CAST* gene and 0.660 ($H_o$) and 0.552 ($H_e$) for the *MSTN* gene. It was necessary to check whether the actual genotype frequencies corresponded to the theoretically expected ones to assess the significance of the selective difference between genotypes. We used the $\chi^2$ criterion for this purpose. The analysis of the obtained data allowed us to conclude that the genetic equilibrium was maintained in the studied sheep population for the *CAST* gene ($\chi^2$ = 4.1951). At the same time, the Hardy-Weinberg equilibrium was violated in the studied population for the *MSTN* gene ($\chi^2$ = 24.2).

Table 2: Heterozygosity level of sheep in the population under study (n = 200)

| Gene | Na | Ne | Ho | He | $F_{IS}$ | $\chi^2$ |
|---|---|---|---|---|---|---|
| *CAST* | 1 | 1.45 | 0.340 | 0.312 | 0.088 | 4.1951 |
| *MSTN* | 2 | 2.23 | 0.660 | 0.552 | -0.200 | 24.2 |

Further studies on the association between allelic variants of the studied genes and the meat productivity of sheep showed the advantage of lambs with heterozygous *CAST_MN* genotype over lambs with *CAST _MM* genotype in terms of the pre-slaughter weight (by 4.8%, P<0.001), carcass weight (by 6.5%, P<0.001) and slaughter weight (by 6.4%, P<0.001). The superiority of the *MSTN_AG* genotype individuals over the homozygous *MSTN_GG* counterparts was revealed in the *MSTN* gene lambs in terms of the carcass weight (by 4.7%, P<0.01) and slaughter weight (by 4.7%, P<0.01). However, the homozygous *MSTN _GG* genotype was significantly superior to the heterozygous *MSTN _AG* genotype (by 3.4%, P<0.01) concerning the pre-slaughter weight, which may be due to better development of internal organs. There was no significant difference in internal fat weight between genotypes for both *CAST* and *MSTN* genes. In Figures 4 and 5, the differences in slaughter traits of lambs with different genotypes are shown ("2D Box-Whiskers Plots"): the point corresponding to the Mean is surrounded by a vertically arranged rectangle ("Box"), the height of which corresponds to Mean ± s.e.m., and whiskers – Mean ± SD. The F-ratio with the degrees of freedom and P-value are also indicated under each diagram. "Boxes" with different letters – the differences are significant (P<0.05: **P<0.01, *** P<0.001); and with the same letters – are not significantly different (Figures 4 and 5).

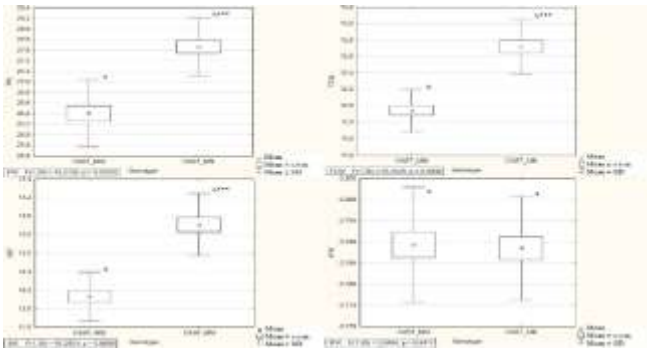

Figure 4: Diagrams of differences in slaughter traits of experimental *CAST* gene lambs of different genotypes (kg), where PW is the pre-slaughter weight, FCW is the fresh (hot) carcass weight, SW is the slaughter weight, IFW is the internal fat weight.

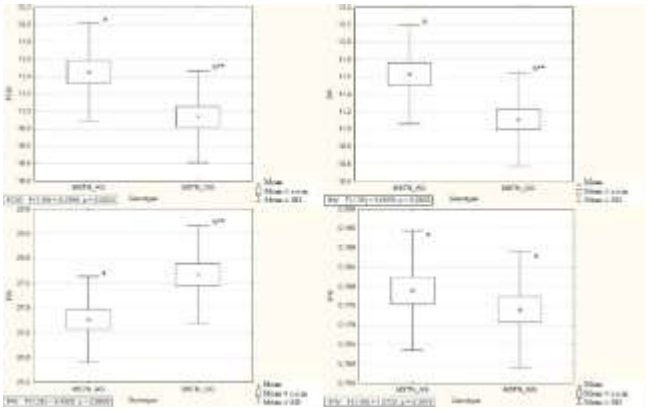

Figure 5: Diagrams of differences in slaughter indices of experimental *MSTN* gene lambs of different genotypes (kg), where PW is the pre-slaughter weight, FCW is the fresh (hot) carcass weight, SW is the slaughter weight, IFW is the internal fat weight.

**Discussion**

Our obtained *CAST* genotypic and allelic frequencies for the studied sheep population were in agreement with Suleman *et al.* (18), who observed only two genotypes, i.e. *MM* (80%) and *MN* (20%), in the Thalli sheep breed raised in the Thall area of Punjab (Pakistan). The allelic frequency for M was 90%. In our study, also only two genotypes, *CAST_MM* (66%) and *CAST_MN* (34%), were identified, and the *M* allele had the highest frequency (83%). It is important to note that our previous studies on the Soviet Merino sheep population bred in LLC Belozernoye (Salsk district, Rostov region, Russia; 46.838770, 41. 337891; 188 km from the Skiba farm, Zimovnikovski district, Rostov region, Russia; 46.758475, 42.802302) showed the presence of all three genotypes *MM*, *MN* and *NN* with frequencies of 0.82, 0.12 and 0.06, respectively; the *M* allele also had the highest frequency (88%) (24). In the populations of Lohi (central Punjab, Pakistan) and Kajli (Sargodha and Gujrat districts of Punjab, Pakistan) sheep breeds (18), the genotypic and allelic frequencies were 0.77, 0.20, 0.03 and 0.68, 0.26, 0.06 for *MM*, *MN*, *NN* genotypes; 87 and 81% for the *M* allele, respectively. Similar results were obtained in Akkaraman lambs reared in Turkey (25), i.e. 0.81, 0.18 and 0.01 for *MM*, *MN* and *NN* genotypes; 90% for *M* allele. However, no significant association was found between *CAST-MspI* genotypes and early body weight traits in Akkaraman lambs (at birth and on the 30th, 60th and 90th days). In Ibrahim *et al.* (26) on Barki sheep, no relationship was found between calpastatin polymorphism and the growth and carcass traits. However, we found a significant relationship (24) between calpastatin polymorphism and growth traits in our previous work and between calpastatin polymorphism and slaughter traits (pre-slaughter weight, fresh carcass weight and slaughter weight) in the current investigation, which does not contradict but complements the available scientific data (27).

Studies by Bayraktar & Shoshin (28) on the Awassi sheep breed population (Kirkuk, Iraq) also identified three genotypes in the *CAST* gene (*MM*: 0.70; *MN*: 0.16; and *NN*: 0.14) and two genotypes in the *MSTN* gene (*AA*: 0.10; *GG*: 0.90), with no association being found between different genotypes and the body weight, body length, chest depth, heart girth and wither height for either *CAST* or *MSTN*. However, in our work, we found a significant association between the studied SNPs and slaughter indices (pre-slaughter weight, hot carcass weight and slaughter weight), which does not contradict but complements the available scientific data.

Yilmaz *et al.* (29), in their study on sheep populations of different breeds reared in West Anatolia (Turkey), found that the frequency of the *CAST_NN* genotype was significantly lower in Kıvırcık (0.04) and Karacabey Merino (0.07) than in Sakız (0.40) and was absent in the Gökçeada population. In this paper, the research team also assumed that the low frequency or absence of *the NN* genotype, which negatively affects body weight, exists in the population due to the selection implemented many years ago as part of the National Merino Program.

Jawasreh *et al.* (30) found that Awassi sheep (Jordan) carrying the *MM* genotype had a higher total bone weight than *MN* genotype lambs. In contrast, lambs carrying the *MN* genotype had a higher meat-to-bone ratio than *MM* genotype lambs. Jawasreh *et al.* (31) reported that since calpastatin contributes to improved final body weight, this gene of the *MM* genotype is best used in marker-assisted molecular selection programs aimed at improving final body weight and longissimus muscle width in Awassi sheep.

Studies on the Nilagiri sheep, an endangered breed of South India, aimed at detecting polymorphisms in exon 3 of the *MSTN* gene showed that the genotypes were distributed as follows: the wild-type DNA molecule *MM* (0.689) and *Mm* (0.311) at a total absence of fragments with mutation *mm* (32). No relationship was found between wild homozygous and heterozygous types and growth performance up to one year of age. However, Madras Red sheep (Tamil Nadu, India) with homozygous (*MM*) genotypes were significantly ($P<0.05$) heavier than heterozygous (*Mm*) genotypes at nine and 12 months of age, respectively (33). The authors attributed the lack of relationship between genotype and growth performance to the founder effect, inadequate pasture availability, genetic proximity, evolutionary factors and living in similar agroclimatic conditions.

A number of studies suggest that *MSTN* in exon 3 is monomorphic for various reasons (sample size, environmental effects, geographical location and mating strategies), such as the studies by Georgieva *et al.* (16) on animals from the Synthetic Population of Bulgarian Milk breed, Bozhilova-Sakova *et al.* (20) on animals of an Indigenous Bulgarian sheep breed (Karakachan),

Khederzadeh *et al.* (21) on Zandi sheep from three populations (the Zandi sheep breeding station located in Khojir National Park and Saveh and Damavand cities of Iran), or Dimitrova *et al.* (8) on animals of the Northeast Bulgarian Merino sheep breed, which reduces the possibility of studying the relationship between *MSTN* and productivity indicators.

However, Saygili *et al.* (34) reported the presence of three *MSTN* genotypes (with allele frequency $m = 56\%$) in a population of the Morkaraman sheep breed (Turkey) and a significant effect of this polymorphism on weaning age and average daily gain.

Alnajm *et al.* (11) also found three *MSTN* genotypes in two native Iraqi sheep breeds (*MM*, *Mm*, and *mm* were 0.70, 0.19, and 0.11 in the Awassi breed, but they were 0.67, 0.13, and 0.20 in the Naimi breed, respectively). The frequency of the *M* allele (0.81 and 0.76) was higher than that of the *m* allele (0.19 and 0.24).

According to studies by Iroanya *et al.* (35) in four breeds of indigenous Nigerian sheep, the myostatin gene has three allelic variants (*AA*, *AG* and *GG*) controlled by two alleles (*A* and *G*), with the *G* allele and *GG* genotype having the highest frequencies (83% and 73%, respectively). In contrast, in our study, only 2 allelic variants, *MSTN_AG* and *MSTN_GG*, were detected in the samples studied, and the frequency of the heterozygous form is higher than that of the homozygous form (66% and 34%, respectively). The *G* allele had the highest frequency (67%), which agrees with the results of Iroanya *et al.* (35).

Our results compared with other scientists' studies (11;16;18;21;25;28;29) support the conclusion that the *CAST_MM* genotype is the dominant genotype and the *M* allele is the dominant allele in small ruminant breeds belonging to different geographical areas. However, the distribution of genotypes differs, which may be due to, among other things, different degrees of inbreeding in farms.

Studying genetic variability in populations is essential for developing a rational breeding strategy. The distribution of alleles to the higher side of one of them, with the Hardy-Weinberg equilibrium being violated, indicates the effectiveness of an intensive breeding program on the population (29). Given the low value of the *N* allele (*CAST*) frequency revealed in our investigation, further studies are advisable to identify the causes of the observed drift and possibly the need for artificial selection aimed at maintaining and increasing the *N* allele frequency in the population to avoid its elimination (35). The imbalance in the equilibrium position for *MSTN* may be due to some confounding factors such as selection, migration and sample size. The $H_o$ is greater than the $H_e$ in both genes, indicating that the herd studied is in a state of slow improvement and can be used in animal breeding programs and genetic variation (11).

The high population variability and the diversity of SNP mutations may contribute to the discrepancy between the obtained results regarding the contribution of *CAST* and *MSTN* genes in productivity traits (31).

## Conclusion

Data on the *CAST* and *MSTN* gene polymorphism and the genetic structure of the population of Soviet Merino sheep of Gashun type bred in the South of Russia have been obtained for the first time. The Na, Ne, $H_o$, $H_e$, FIS and $\chi^2$ values obtained and the absence of individuals with *CAST_NN* and *MSTN_AA* genotypes prove that earlier selection work was conducted without methods of genomic or marker-associated selection being applied or without the genetic structure being taken into account and is probably connected with insufficient quantity of stud lambs in the herd, which can cause genetic drift later.

The association of polymorphic variants of genes with meat productivity indicators was revealed, and desirable genotypes *CAST_MN* and *MSTN_AG* were determined. The conducted research substantiated the prospect of their further use in programs of genetic livestock improvement, which allows the evaluation and prediction of breeding traits of sheep immediately after their birth and significantly increases the efficiency of breeding farms.

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## Conflict of interest

There is no conflict of interest.

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# تعدد أشكال جينات الكالبستاتين والميوستاتين وارتباطهما بخصائص الذبح في سلالة ميرينو غاشون السوفيتية المربات في جنوب روسيا

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**الخلاصة**

هدفت الدراسة إلى التحقيق في التباين الجيني لسلالة أغنام ميرينو غاشون السوفياتية، وتحديد تعدد أشكال جينات الميوستاتين (*MSTN*) والكالباستاتين (*CAST*) بطريقة *PCR-RFLP*. تم جمع عينات مخصصة من ٢٠٠ حيوان، وتم عزل الحمض النووي باستخدام العدة التجارية. كان لجين *MSTN* ترددات أليل *A* و *G* تبلغ ٠,٣٣ و ٠,٦٧، وكان لجين *CAST* ترددات أليل *M* و *N* تبلغ ٠,٨٣ و ٠,١٧، على التوالي. كانت ترددات الأنماط الجينية *AG* و *GG* لـ *MSTN* و *MM* و *MN* لـ *CAST* *0.66* و ٠,٣٤ على التوالي في كليهما. تم الحفاظ على التوازن الجيني لجين *CAST* *($\chi^2$ = 4.1951)*. بالنسبة لجين *MSTN*، تم انتهاك توازن هاردي-واينبرغ (*$\chi^2$ = 24.2*). كان وزن الجسم قبل الذبح ووزن الذبيحة الساخنة ووزن الذبح أعلى في النمط الوراثي *CAST_MN* منه *MM* _ *CAST* بنسبة ٤,٨٪ و ٦,٥٪ و ٦,٤٪. كانت أوزان الذبيحة الساخنة والذبح للحملان ذات النمط الجيني *MSTN_AG* أعلى من *MSTN* _*GG* بنسبة ٤,٧٪ في كليهما. ومع ذلك، كان وزن ما قبل الذبح في ذات النمط الجيني *MSTN* _*GG* أعلى بنسبة ٣,٤٪. تم الحصول لأول مرة على بيانات حول تعدد أشكال الجينات *CAST* و *MSTN* والتركيب الجيني لمجموعات الأغنام المدروسة، وتم الكشف عن ارتباطها بصفات الذبح، وتم تحديد الأنماط الجينية المرغوبة *CAST_MN* و *MSTN_AG*. تم الحصول على قيم *Na* و *Ne* و *Ho* و *He* و *FIS* و *$\chi$2* وغياب الأفراد الذين لديهم أنماط وراثية *CAST_NN* و *MSTN_AA* أثبتت احتمال زيادة استخدام هذه الجينات في برامج تحسين الثروة الحيوانية الوراثية.