

# Phenotypic effects of polymorphism of the calpastatin gene (CAST), associated with growth and development indicators, in West Siberian mutton breed

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**Abstract**— One of the modern ways to improve the breeding qualities is selection using the genetic markers or the marker-assisted selection. At the present stage of animal breeding development, and sheep breeding in particular, marker-assisted selection methods have high practical application, which is based on identifying the interrelation of genes with productivity indicators. Genetic markers associated with growth and meat productivity indicators have been identified, one of which is the CAST gene (calpastatin). The purpose of our research was to study the phenotypic effect of the polymorphism of the CAST gene, associated with growth and development indicators, in sheep of the West Siberian mutton breed. The frequency of the occurrence of alleles and genotypes of the CAST gene was detected by PCR analysis in stud rams ( $n = 30$ ) and ewes ( $n = 70$ ) of the West Siberian mutton breed. It has been established that the most frequent genotype in the population of sheep of West Siberian mutton breed is the MM genotype, the frequency of which reaches 0.710 or 72%, the alternative CAST NN genotype is found in singular animals – 5%. Sheep with the CAST MN genotype were distinguished by the best average daily weight gain from birth to weaning by 6.9% and live weight by 20% ( $P < 0.01$ ), in relation to the sheep with genotype NN. There were no significant differences in growth and development indicators between the MM and MN genotypes.

**Keywords**— *West Siberian mutton breed, sheep, genes, calpastatin, live weight.*

## I. INTRODUCTION

Genomic selection is one of the modern ways to improve the breeding qualities of animals. It allows you to identify and distinguish heterozygous genotype from homozygous; has a high coefficient of heritability and does not depend on environmental factors, as well as on gender and age [1]. One of the promising areas of genomic selection is marker-assisted selection.

The genes that are considered as markers, has allelic variants that are associated with the phenotypic manifestation of qualitative and quantitative indicators of productivity [2, 3]. Genes-markers are most important for assessing traits, formation and manifestation of which is limited by sex,

occurs relatively late or whose development depends on environmental factors. That could be, for example, the fertility resistance, stress-resistance, qualitative and quantitative indicators of wool, meat, milk production, etc. [4].

At the present stage of animal breeding development, and sheep breeding in particular, marker-assisted selection methods have high practical application, which is based on identifying the interrelation of genes with productivity indicators [3], one of which is the CAST gene (calpastatin) [4].

In the conditions of the Altai Territory (Western Siberia), the West Siberian mutton breed of sheep (patent No. 5728, 11.01.2011) was established as a result of a long selection and breeding work in 2011. These sheep are well adapted to breeding in harsh climatic conditions with a long winter, low temperatures and harsh continental climate. The sheep are characterized by endurance, precocity, high reproductive qualities and fertility up to 160%. They are distinguished by a long, thick, white-colored grease coat and noble crimp with wool fineness of 21–24 microns. The output of scoured wool is 57–58%, with a hair length of 9 cm in ewes and up to 11.5 cm in rams; the slaughter yield reaches more than 50%. The breed is in demand in many regions of the Russian Federation.

Further improvement is possible only by conducting targeted breeding work, based both on traditional methods and the use of genetic markers, in particular the CAST gene.

In this regard, the purpose of our research was to study the phenotypic effects of the polymorphism of the calpastatin gene (CAST), associated with growth and development indicators, in West Siberian mutton breed of sheep.

## II. METHODS

Our studies were carried out on the basis of the “Mayak” farm of the Rodinsky district, Altai region, in accordance with the thematic plan-task for the implementation of research works (No. AAAA-A18-1180903003003-7; from

03.09.2018) commissioned by the Ministry of Agriculture of Russia. The studied material was tissue samples from rams (n = 30) and ewes (n = 70) of West Siberian mutton breed. The indicators of growth and development presented in the article were obtained in the initial period of postnatal development; therefore, animals are designated as ram-lambs and ewe-lambs.

The DNA was isolated from the blood of canned “EDTA K3” tube using an extraction kit “Ampli Prime DNA-sorb-B”, according to the instructions of the manufacturer – “NextBio”. Sheep DNA typing for the CAST gene was performed according to the recommendations of N.A. Shirokov et al. [5]. Amplification was performed in the C1000 “BioRad” amplifier. The resulting gene amplification products were treated with restriction endonucleases RsaI, MspI, SacII (SibENZIM, Novosibirsk) according to the manufacturer's instructions. Visualization and identification of genotypes was determined by electrophoresis in 2% agarose gel in UV light.

In accordance with the guidelines of L.A. Zhivotovsky [6], the frequency of occurrence of genotypes, alleles and their error was calculated. Growth rates of young stock were studied by individual weighing at birth, and then in accordance with the stages of the study. Based on the weighing results, the live weight was determined. Absolute, daily average and relative weight gains were calculated using generally accepted formulas.

Early maturity was evaluated in accordance with the “Procedure and conditions for the appraisal of fine-fleeced, semi-fine-fleeced and meat productivity breeds of sheep” (October 5th, 2010, №335).

Statistical processing of digital data was carried out using the method of variation statistics using the program “StatSoft STATISTICA 10.0.1011.0 Russian Portable”.

### III. RESEARCH RESULTS

At the physiological level, the calpastatin gene is an endogenous inhibitor of calpains. It has been established that the CAST gene influences the growth and development of the heart muscle, determines the weight of the animal and the quality of the meat. There are reports on the interrelations of CAST genotypes with the growth rate of young sheep, primarily due to a greater increase in muscle mass. It should be noted that the polymorphism of the calpastatin gene has significant species and breed differences [7, 8]. In this connection, we studied the polymorphism of the CAST gene in sheep of the West Siberian mutton breed. The results are presented in Figure 1.

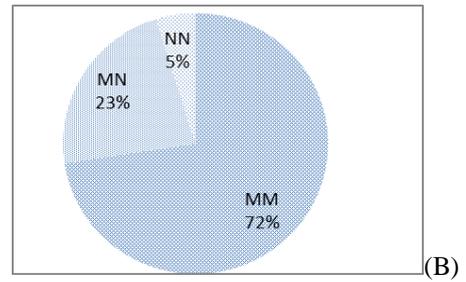
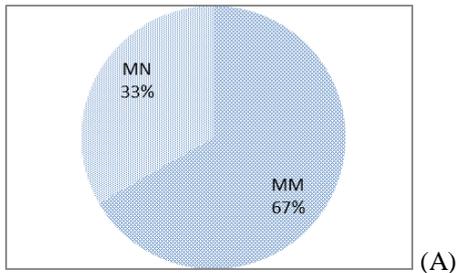


Fig. 1. Polymorphism of the CAST gene in stud rams (a) and in ewes (b) of West Siberian mutton breed

Research has established that the prevailing genotype in sheep of West Siberian mutton breed is the CAST MM genotype, the frequency of which reaches 0.710 or 72%, while the alternative CAST NN genotype is found in singular animals – 5% (Fig. 1). The share of allele M accounts for 0.837, and N – 0.163. A distinctive feature of the stud rams, in comparison to the ewes, is the absence of animals with the CAST NN genotype [9], which suggests that this genotype is most likely excluded from the population due to selection.

The results of our research on the CAST gene polymorphism are consistent with the studies on other sheep breeds. So, in sheep of Dalagh, Zel, Karakul, Soviet Merino, Salsk, Tuva and Prikatun type of Gornoaltai breeds, the frequency of allelic M and N options varies from 80% to 89% and 1.1% to 20%, respectively. According to various data, the MM genotype is found in 65.5-88.0% of the animals, the MN genotype in 11.0-29.0%, and NN – from 1% to 6% [10, 11, 12, 13, 14].

In animal husbandry practice, the most valuable is the information that characterizes the interrelations of various options of gene polymorphism and level of productivity. Therefore, we have analyzed the growth and development of young West Siberian mutton breed, with different options of the calpastatin gene polymorphism (Fig. 2).

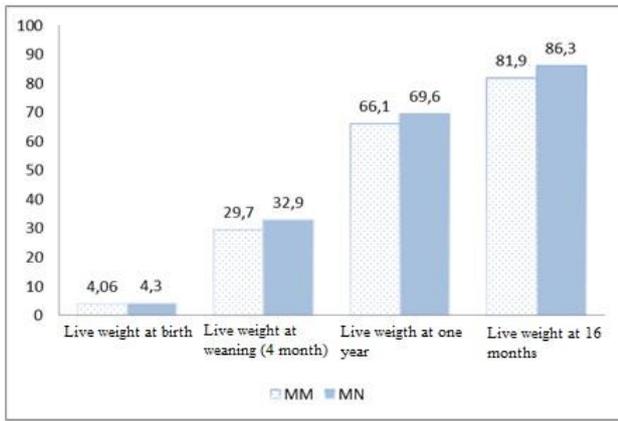
The calpain system is necessary for the fetus growth and the prenatal development of skeletal muscle [15], which is reflected in the body weight of lambs at birth.

Our studies showed that live weight at birth of ewe-lambs, with the CASTMM and CASTNN genotypes, did not differ and averaged  $4.26 \pm 0.12$  kg, while animals with the CASTMN genotype were 1% higher; and the ram-lambs were higher by 7%.

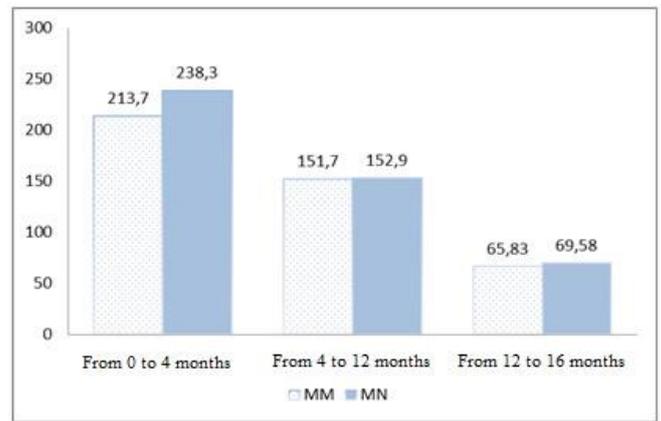
At the age of 4 months, the live weight of the ewe-lambs, with the CASTMN and CASTMM genotypes, was higher by an average of 6% ( $P < 0.05$ ), compared to animals with the CASTNN genotype, the difference between the MM and MN alleles was insignificant.

The ram-lambs, with the heterozygous genotype CASTMN, were characterized by a higher body weight – 11%, compared to the lambs with the genotype CASTMM.

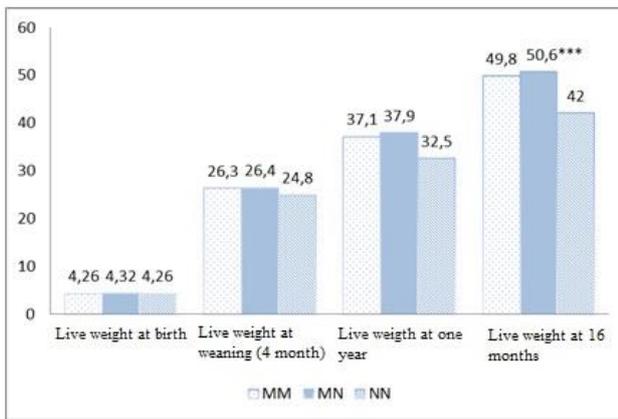
The established pattern was preserved in subsequent periods. Ram-lambs with the CAST MN genotype reached a live weight of  $69.6 \pm 4.62$  and  $86.3 \pm 5.54$  kg, and the ewe-lambs –  $37.9 \pm 1.51$  and  $50.6 \pm 1.68$  kg, at 12 and 16 months, respectively. In a comparative aspect, the live weight of rams with the CASTMN genotype is on average 5%, and the ewes is 2 and 20% ( $P < 0.01$ ) higher, compared to the homozygous animals with the CASTMM and CASTNN genotypes.



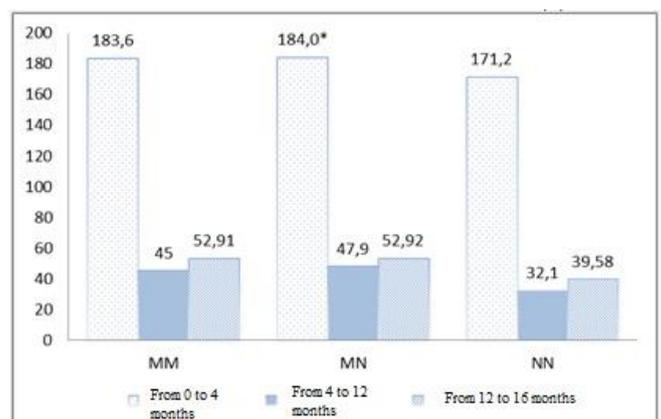
(A)



(A)



(B)



(B)

Fig. 2. Live weight dynamics of ram-lambs (A) and ewe-lambs (B) of West Siberian mutton breed with different options of CAST gene polymorphism, in kg

Fig. 3. Average daily gain in live weight of ram-lambs (A) and ewe-lambs (B) of West Siberian mutton breed with different variants of CAST gene polymorphism, in g

Along with the live weight, the early maturity of young animals is of great economic importance in the meat and wool sheep breeding, which is determined by the increase in the live weight from birth to slaughter, compared to the minimum requirements of class 1 (for the ewe-lambs of West Siberian mutton breed is 24 kg and for the ram-lambs – 28 kg).

So, the ewes with the MM and MN genotypes exceeded the requirements of class 1 on average by 10% and corresponded to the animals of the elite class and were assessed at 5 points. Ewes with the NN genotype exceeded only by 3.5%, and had a score of 4.5.

The precocity of the rams with the MM and MN genotypes was estimated at 5 points, which is higher by 5.9% and 17.5%, in comparison with class 1 animals.

Higher live weight in ewe-lambs with CAST MN and CAST MM genotypes, in comparison to homozygous animals that have the CAST NN genotype, is most likely associated with changes in the regulation of the rate or efficiency of proteolysis, since the activity of the calpain complex causes cell protein metabolism, digestion and homeostasis [15].

The dynamics of average daily gains in live weight correspond to the change in live weight of sheep in the age aspect (Fig. 3).

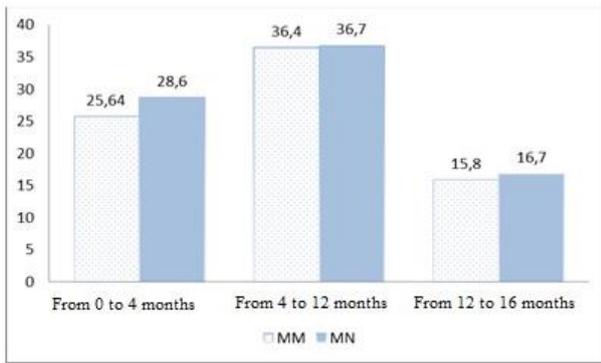
The maximum gains in live weight were recorded in the age range from birth to 4 months for ewes and rams with the CAST MN genotype —  $184.0 \pm 2.26$  ( $P < 0.05$ ) and  $238.3 \pm 2.91$  g, respectively; the minimum ones for the ewes with the genotype CAST NN –  $171.2 \pm 2.34$  g. It should be noted that the average daily increment between animals with the genotypes MN and MM was insignificant.

With hetero and homozygous genotypes MN and MM in the ewe-lambs, the increase in live weight was higher than in animals with the CASTNN genotype by 49% and 40%, respectively, from the age of 4 month (the age of weaning) and up to 16 months; at 16 months – an average of 34%.

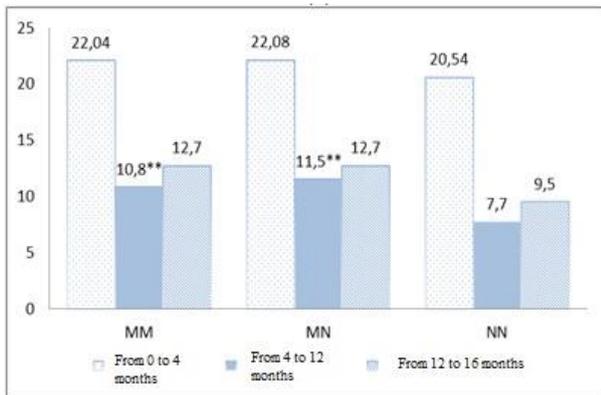
The patterns of growth in the live weight of animals are most significantly revealed when comparing the relative and absolute growth over a certain period. In this regard, we calculated the absolute and relative gains in live weight of lambs (Fig. 4, 5).

The highest values of absolute gains in live weight were observed in the period from the birth to 4 months in the youngsters of all groups, regardless of genotype. Significant differences in the absolute increase between animals were noted in the age period from the waning to 12 months: in ewes with CAST MM genotypes by 40.3% ( $p < 0.01$ ), CASTMN by 49.4% ( $p < 0.01$ ), compared to animals with genotype CASTNN.

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$  - the difference is statistically significant in comparison between groups



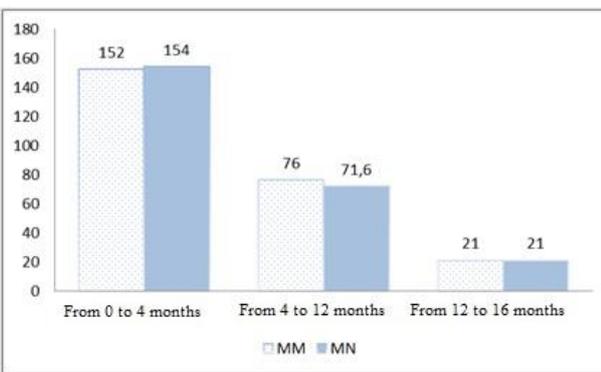
(A)



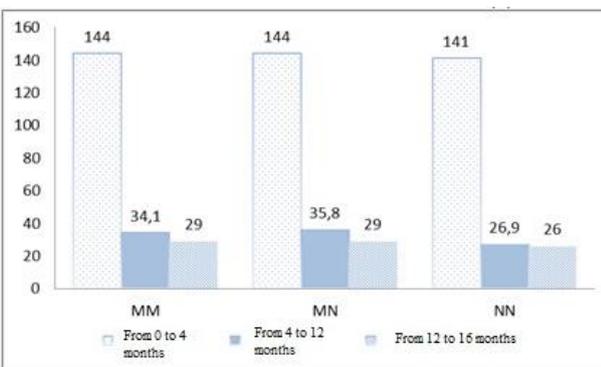
(B)

Fig. 4. Absolute increase in live weight of ram-lambs (A) and ewe-lambs (B) of West Siberian mutton breed with different variants of CAST gene polymorphism, in kg

The results of the relative growth rate of the experimental animals are presented in Figure 5.



(A)



(B)

\*P <0.05; \*\*P <0.01; \*\*\*P <0.001 - the difference is statistically significant in comparison between groups

Fig. 5. Relative increase in live weight of ram-lambs (A) and ewe-lambs (B) of West Siberian mutton breed with different variants of CAST gene polymorphism, in kg

The results of studies of the relative growth rate also indicate better growth of ewe-lambs genotypes CAST MM and MN, than with the genotype NN. The higher magnitude of the relative gains in live weight has the genotype MN ewe-lambs. The relative growth rate was on average higher by 2.1%; 24.8% and 10.3%, in comparison to genotype NN. Significant differences in the magnitude of the relative gains in animals with genotypes CAST MM and MN were not found.

The higher body weight dynamics and growth rate of animals with the MN and MM genotypes established in our studies, compared to the NN genotype, correspond to data obtained by E. Dehnavi et al. [16] in sheep of the Zel and O.Yilmaz breed [17], as well as to the research of N. F. Bakoeva, N. V. Shirokov [10] that was conducted on Soviet merino breed in the Rostov region.

#### IV. CONCLUSION

The results of studies of the relative growth rate also indicate better growth of ewe-lambs genotypes CAST MM and MN, than with the genotype NN. The higher magnitude of the relative gains in live weight has the genotype MN ewe-lambs. The relative growth rate was on average higher by 2.1%; 24.8% and 10.3%, in comparison to genotype NN. Significant differences in the magnitude of the relative gains in animals with genotypes CAST MM and MN were not found.

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