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

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SLN presented the concept and designed the study. FIO and KDV analysed and interpreted the results. SAV and KNI wrote the manuscript. DII and VCP reviewed the manuscript.

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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 growth hormon and calpastatin genes. The slaughter weight of individuals of the *AB, BB* genotype of the *GH* gene and the *MN* genotype of the *CAST* gene is higher by 6.3, 7.3, and 5.2%, 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).

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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 (Greguła-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

PCR-RFLP. The amplification reaction was performed using the "GenPakPCRCore" kit (IsoGeneLab, Moscow) on a programmable four-channel thermal cycler "Tertsik" (Moscow).

The following nucleotide sequences were used as primers:

For the *GH* gene: F: 5'-GAAACCTCCTTCCTCG-CCC-3', R: 5'-CCAGGGTCTAGGAAGCCACA-3' (amplification fragment - 934 bp). The amplification conditions were as follows: denaturation - 95 ° C (5 min.), then 33 annealing cycles - 95 °C, 60 °C, and 72 ° C (45 sec each), elongation - 72 °C (10 min.);

For the *CAST* gene: F: 5'-TGGGGCCCAATGAC-GCCATCGATG-3', R: 5'-GGTGGAGCAGCACTTCT-GATCACC-3' (amplification fragment - 622 bp). The amplification conditions were as follows: denaturation - 95 ° C (4 min.), then 35 annealing cycles - 95 °C, 62 °C and 72 °C (45 s each), elongation at 72 °C (7 min).

The polymorphism of the *GH* and *CAST* genes was determined using the HaeIII and MspI restriction endonuclease reagent kits (manufactured by "SibEnzyme" LLC, Moscow) in accordance with the instructions. The size of the obtained restriction fragments was analyzed by electrophoresis in 4% agarose gel using staining with ethidium bromide.

Data analysis

The main characteristics of the genetic structure of the studied populations were calculated using the formulas:

Observed heterozygosity (Ho)= N/n, where Ho is the observed heterozygosity, N is the number of heterozygotes, n is the sample size.

Expected Heterozygosity (He)= 1-(p2+q2), where He is the expected heterozygosity, p is the frequency of the A allele, q is the frequency of the B allele.

Measure of informational polymorphism (PIC) = $1 - \Sigma(Pi)^2$, where PIC is a measure of informational polymorphism; Pi is the frequency of occurrence of the i-th allele.

Homozygosity degree (Ca), % =$\sum p^2$*100, where Ca is the coefficient of homozygosity (%), p is the frequency of an allele.

Polymorphism level (Na) = 1/Ca, where Na is the locus polymorphism level; Ca is the homozygosity degree at the locus.

The degree of genetic variation (V), % =1 – Ca/1 – N/1x100, where V is the degree of genetic variation, N is the number of examined animals, Ca is the homozygosity coefficient.

Heterozygosity test (TΓ)= Ho - He, where TΓ is a heterozygosity test; Ho - observed heterozygosity; He - expected heterozygosity.

Fixation index (Fis)= 1 – (Ho/He), where Fis is the fixation index, Ho is the observed heterozygosity; He is the expected heterozygosity.

Indicators of meat productivity were studied after the control slaughter of the studied animals at the age of 8 months. In the course of the study, the following signs of meat productivity were determined: live weight before slaughter, weight of fresh carcasses, weight of internal fat, slaughter weight, slaughter yield, varietal and morphological composition of carcasses. For histological studies, biological material (muscle tissue) was selected from the carcasses of the studied animals. After the cessation of fibrillation of muscle fibers, that is, 45-60 min after the slaughter of the animal, a sample of 2 cm3 was cut out at the level of the neck of the last right rib in the middle part of the longissimus dorsi muscle in the transverse and longitudinal direction. The muscle tissue samples were fixed with 10% neutral formalin for at least 24 h. Sections in the transverse and longitudinal directions, 12 μm thick, were prepared on a sledge microtome. For an overview, histosections were stained with hematoxylin-eosin according to the Ehrlich method, connective tissue according to the Van Gieson method, and adipose tissue with Sudan black. Stained histosections were embedded in Canadian balsam under a cover glass and subjected to microscopic examination using a Biomed S-1 biological microscope with an eyepiece magnification of × 10 and an objective of × 4, × 10, and × 40. Microphotography of histological preparations was performed using a Canon Power Shot A 460 IS camera. Photographing of the slides was carried out using a digital camera (video eyepiece) Scopetek DCM510 for a microscope. The obtained images were processed using the supplied Scope Photo program.

RESULTS AND DISCUSSION

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.

Gene	Number of sheep	Frequency of occurrence				
		Genotype, %			Alleles	
GH	91	AA	AB	BB	A	B
		29.7	42.8	27.5	0.51	0.49
CAST	91	MM	MN	NN	M	N
		87.9	12.1	-	0.94	0.06

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, Stavropol 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. 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.

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

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.

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.

The degree of genetic variability is expressed through the coefficient V, which depends on the homozygosity

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 (ТГ) indicates a lack or abundance of relative heterozygosity, obtained as the difference between actual and theoretical data. A positive ТГ value indicates the prevalence of actual heterozygosity over theoretical. Animals of the studied population are characterized by a lack of heterozygotes for both the *GH* gene and the *CAST* gene, as evidenced by the ТГ index (-0.07 and -0.76). 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 *AA* 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 higher in comparison with the *AA* genotype; 1.83; 4.83 kg and 2.04%, 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.

Indicator	Genotype of *GH* gene			Genotype of *CAST* gene	
	AA	AB	BB	MM	MN
Live weight before slaughter (kg)	34.0±0.71	35.0±0.25	35.2±0.36	34.73±0.29	35.63±0.47
Fresh carcass weight (kg)	14.37±0.36	15.30±0.27	15.43±0.35	14.92±0.42	15.71±0.54
Slaughter weight (kg)	14.65±0.37	15.58±0.27	15.72±0.36	15.21±0.43	16.0±0.53
Slaughter yield (%)	43.1±0.20	44.5±0.56	44.6±0.61	43.8±0.89	44.9±0.87
Fleshing index	2.80± 0.34	3.23± 0.20	3.32± 0.15	3.27± 0.23	3.35± 0.34
Number of muscle fibers (pcs)	340.74±3.27	360.30±9.72	362.67±8.45	358.82±8.64	369.26±14.33
Muscle fiber diameter (μm)	30.61±0.96	28.27±0.79	27.78±1.38	29.07±1.59	27.99±1.08
General assessment of "marbling" (point)	29.72±1.38	31.54±0.95	31.76±1.23	31.34±1.03	33.21±0.57
Connective tissue content (%)	8.67±0.13	8.07±0.18	7.87±0.48	8.0±0.23	7.66±0.46

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 thigh 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

Afanasyeva, A., Sarychev, V. and Goncharenko, G., 2019. *Phenotypic effects of polymorphism of the Calpastatin gene (CAST), associated with growth and development indicators, in West Siberian mutton breed*. International scientific and practical conference "Digital agriculture-development strategy" (ISPC 2019). Atlantis Press. pp. 116-120. https://doi.org/10.2991/ispc-19.2019.26

Akhatayeva, Z., Li, H., Mao, C., Cheng, H., Zhang, G., Jiang, F., Meng X., Yao, Y., Lan, X., Song E. and Zhang, D., 2020. Detecting novel Indel variants within the GHR gene and their associations with growth traits in Luxi Blackhead sheep. *Anim. Biotechnol*., pp. 1-9. https://doi.org/10.1080/10495398.2020.1784184

Allafi, F., Hossain, M.S., Lalung, J., Shaah, M., Salehabadi, A., Ahmad, M.I. and Shadi, A., 2020. Advancements in applications of natural wool fiber. A review. *J. Natl. Fibers*, **2021**: 1-16. https://doi.org/10.1080/15440478.2021.1964131

Armstrong, E., Ciappesoni, G., Iriarte, W., Da Silva, C., Macedo, F., Navajas, E.A., Brito, G., San, Julián R., Gimeno, D. and Postiglioni, A., 2018. Novel genetic polymorphisms associated with carcass traits in grazing Texel sheep. *Meat Sci*., **145**: 202-208. https://doi.org/10.1016/j.meatsci.2018.06.014

Boichard, D., Ducrocq, V., Croiseau, P. and Fritz, S., 2016. Genomic selection in domestic animals: Principles, applications and perspectives. *C. R. Biol*., **339**: 274-277. https://doi.org/10.1016/j.crvi.2016.04.007

Chesnokov, Y.V. and Artemyeva, A.M., 2015. Evaluation of the measure of polymorphism information of genetic diversity. *Agric. Biol*., **50**: 571-578 (eng). https://doi.org/10.15389/agrobiology.2015.5.571eng

Dettori, M.L., Pazzola, M., Pira, E., Paschino, P. and Vacca, G.M., 2015. The sheep growth hormone gene polymorphism and its effects on milk traits. *J. Dairy Res*., **82**: 169. https://doi.org/10.1017/S0022029915000047

Frantz, L.A., Bradley, D.G., Larson, G. and Orlando, L., 2020. Animal domestication in the era of ancient genomics. *Nat. Rev. Genet*., **21**: 449-460. https://doi.org/10.1038/s41576-020-0225-0

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

N.I., Bakoev, N.F., Leonova, M.A., Kolosov, A.Y. and Zlobina, E.Y., 2017. Association of the growth hormone gene polymorphism with growth traits in Salsk sheep breed. *Small Rumin. Res*., **150**: 11-14. https://doi.org/10.1016/j.smallrumres.2017.02.019

Greguła-Kania, M., Gruszecki, T.M., Junkuszew, A., Juszczuk-Kubiak, E. and Florek, M., 2019. Association of CAST gene polymorphism with carcass value and meat quality in two synthetic lines of sheep. Meat Sci., **154**: 69-74. https://doi.org/10.1016/j.meatsci.2019.04.007

Iovenko, V., Vdovychenko, Y., Pysarenko, N., Skrepets, K. and Hladii, I., 2020. Genetic diversity and population structure of breeds of Askanian sheep by analysing polymorphisms in qualitative trait loci. *Agric. Sci. Pract*., **7**: 3-13. https://doi.org/10.15407/agrisp7.01.003

Jawasreh, K.I., Jadallah, R., Al-Amareen, A., Abdullah, A.Y., Al-Qaisi, A., Alrawashdeh, I.M., Al-Zghoul, M., Ahamed, M. and Obeidat, B., 2017. Association between MspI calpastatin gene polymorphisms, growth performance, and meat characteristics of Awassi sheep. *Indian J. Anim. Sci*., **87**: 635–639.

KÖK, S. and Atalay, S., 2018. The use of various *SNPs* in *CAST* and *CAPN1* genes to determine the meat tende rness in Turkish grey cattle. *Kafkas Üniv. Vet. Fakül. Derg.*, **24**: 1-8.

Lenis-Valencia, C., Hernández-Herrera, D. and Álvarez-Franco, L., 2021. Polymorphisms of the *CAPN*, *CAST, LEP, GH, GHR, IGF-1* and *MSTN* loci of Colombian Creole hair x Pelibuey sheep sheep crossbreeds. *Vet. Stanica*, **52**: 2021. https://doi.org/10.46419/vs.52.1.6

Montes, V., Donicer, L.V.C. and Hernández, H.D., 2019. Polymorphisms of the calpain and calpastatin genes in two populations of Colombian creole sheep. *Rev. MVZ Córdoba*, **24**: 7113-7118.

Pascal, C.T., Nechifor, I., Cristian, C., Florea, M. and Valusescu, D., 2018. Estimation of the heterosis effect for the production of meat in goats. *Lucrări Ştiinţ.-Univ.Ştiinţe Agric. Med. Vet. Ser. Zooteh.*, **70**: 72-78.

Petrovic, M.P., Caro-Petrovic, V., Ruzic-Muslic, D., Maksimovic, N., Pavlovic, I., Cekic, B. and Cosic, I., 2019. The phenomenon of heterosis and experience in crossing different breeds of sheep in Serbia. *Biotechnol. Anim. Husb*., **35**: 311-321. https://doi.org/10.2298/BAH1904311P

Platten, J.D., Cobb, J.N. and Zantua, R.E., 2019. Criteria for evaluating molecular markers: Comprehensive quality metrics to improve marker-assisted selection. *PLoS One*, **14**: e0210529. https://doi.org/10.1371/journal.pone.0210529

Pogodaev, V., Aduchiev, B., Kononova, L., Aslanukova, M. and Kardanova, I., 2020. Features of polymorphism of calpastatin and somatotropin genes in young and sheep, obtained from crossing ewes of Kalmyk fat-rumped sheeps and dorper rams. *E3S Web Conf. EDP Sci*., **175**: 03020. https://doi.org/10.1051/e3sconf/202017503020

Saccà, E., Corazzin, M., Bovolenta, S. and Piasentier, E., 2019. Meat quality traits and the expression of tenderness-related genes in the loins of young goats at different ages. *Animal*, **13**: 2419-2428. https://doi.org/10.1017/S1751731119000405

Saleh, A., Hammoud, M., Dabour, N., Hafez, E. and Sharaby, M., 2020. *Genetic variability in IGFBP-3 and GH genes and their association with body weight and growth performance at birth, weaning and six-month in sheep PREPRINT (Version 1) available at Research Square*. https://doi.org/10.21203/rs.3.rs-86803/v1

Selionova, M.I. and Podkorytov, N.A., 2021. Polymorphism of the gene GDF9 in sheep of Prikatun type of Altai Mountains breed and its correlation with indices of meat rate productivity. *Theor. Pract. Meat Process*., **6**: 4-9. https://doi.org/10.21323/2414-438X-2021-6-1-4-9

Selionova, M.I., Chizhova, L.N., Surzhikova, E.S., Podkorytov, N.A., Podkorytov, A.T. and Voblikova, T.V., 2020. Meat productivity of sheep of the Altai Mountain breed of different genotypes according to the *CAST* and *GDF9* genes. *IOP Conf. Ser. Earth Environ. Sci. IOP Publ.,* **613**: 012130. https://doi.org/10.1088/1755-1315/613/1/012130

Shirokova, N.V., Kolosov, A.Y., Kolosov, Y.A., Getmantseva, L.V., Bakoev, N.F., Vorontsova, E.S. and Kolosova, N.N., 2021. Genetic structure of the herd by genes *GDF9, GH, CAST* in merino sheep of the North Caucasus region of Russia. *IOP Conf. Ser. Earth Environ. Sci. IOP Publ.*, **677**: 052113. https://doi.org/10.1088/1755-1315/677/5/052113

Tellam, R.L., Cockett, N.E., Vuocolo, T. and Bidwell, C.A., 2012. Genes contributing to genetic variation of muscling in sheep. *Front. Genet.*, **3**: 164. https://sci-hub.do/10.3389/fgene.2012.00164, https://doi.org/10.3389/fgene.2012.00164

Trukhachev, V.I., Selionova, M.I., Krivoruchko, A.Y. and Aibasov, A.M.M., 2018. Genetic markers of meat productivity of sheep (*Ovis aries* L.). I. Myostatin, calpain, calpastatin. *Agric. Biol*., **53**: 1107-1119. https://doi.org/10.15389/agrobiology.2018.6.1107eng

Ünal, E.Ö., Işık, R. and Soysal, M.İ., 2020. Identification

of novel single nucleotide polymorphisms in the growth hormone (*GH*) gene in Anatolian water buffalo (*Bubalus bubalis*) populations in Turkey. *Rev. Bras. Zoot.*, pp. 49. https://doi.org/10.37496/rbz4920190260

Yilmaz, O., Cemal, I., Karaca, O. and Ata, N., 2014. Association of *Calpastatin* (*CAST*) gene polymorphism with weaning weight and ultrasonic measurements of loin eye muscle in Kıvırcık lambs. *J. Fac. Vet. Med. Kafkas Univ.*, **20**: 675-680. https://doi.org/10.9775/kvfd.2014.10816

Zhang, L., Jiasen, L., Fuping, Z., Hangxing, R., Lingyang, X., Jian, L., Shifang, Z., Xiaoning, Z., Caihong, W., Guobin, L., Youmin, Z. and Lixin, D., 2013. Genome-wide studies of associations for growth and meat production in sheep. *PLoS One*, **8**: e66569. https://doi.org/10.1371/journal.pone.0066569