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RESEARCH ARTICLE

Use of Progressive Biotechnological and Molecular-Genetic Methods in the Development of New Approaches to the Intensification of Animal Husbandry Production

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ABSTRACT

Ensuring food security, increasing the productivity of farm animals and guaranteeing product quality are the strategic priorities of the agro industrial complex. The use of advanced biotechnological and molecular genetic methods makes it possible to control the manifestation of the most valuable economically useful traits in the offspring. In domestic and global zootechnical science and practice, the most important requirements of breeding programs are: increased production and product quality; increase productivity and economic efficiency, and preserve genetic diversity. The aim of the study was to conduct a molecular genetic analysis of the GH gene polymorphism in Soviet Merino sheep using a set of biotechnological methods and bioinformatic approaches. To identify allelic variants and genotypes significantly associated with meat productivity traits and, on this basis, to develop and test new approaches to livestock production intensification, including the implementation of marker-assisted selection.

INTRODUCTION

Contemporary breeding programs involve the active implementation of advanced genetic approaches within livestock breeding services to enhance the productivity of farm animals [19, p. 4]. At present, a progressive shift is observed from conventional animal evaluation methods toward the integration of molecular genetic data, which not only increases the accuracy of productivity prediction but also opens new opportunities for early selection [20, p. 614]. Classical approaches based on pedigree analysis and phenotypic data have inherent limitations. For example, phenotypic traits reflecting productivity often manifest only with age, complicating early assessment of an animal's genetic potential. In addition, environmental influences and stochastic factors may distort the true picture, reducing the accuracy of evaluation [17, p. 6].

The application of molecular genetic data in animal breeding represents a significant advancement, enabling substantial improvements in productivity and economic efficiency in sheep farming [13, p. 291]. Marker-assisted and genomic selection programs are being intensively implemented in leading lamb-producing countries, particularly in Australia and New Zealand [21, p. 354; 18, p. 1820].

Ensuring food security, increasing livestock productivity, and guaranteeing product quality are strategic priorities of the agro-industrial complex. Current trends in agricultural science and production are characterized by the active adoption of molecular genetic methods that enable the development of effective breeding strategies in animal husbandry. These strategies encompass two main directions: improving animal productivity and the quality of raw materials obtained, as well as producing foods with targeted functional properties. The key challenge lies in implementing these approaches in the shortest possible time and with minimal costs [16, p. 2; 12, p. 1107; 8, p. 12].

Therefore, further research is critically needed to accumulate data on gene function and to assess the applicability of genetic polymorphism for monitoring population genetic diversity and predicting productivity [14, p. 1; 15, p. 701]. In this context, the use of molecular genetic methods for predicting productive traits will accelerate the accumulation of genes associated with desirable characteristics, while systematic selection of animals carrying relevant genetic markers will increase the frequency of high-performing individuals in future generations [1, p. 35; 10, p. 40; 11, p. 72].

Molecular markers are expected to improve the efficiency of breeding programs aimed at developing highly productive animals, enabling livestock producers to adopt more advanced approaches to the early selection of individuals with genetically determined desirable traits. These markers will serve as a primary tool for geneticists and breeders in selecting animals for the production of foods with targeted functional properties [2, p. 44; 3, p. 11; 4, p. 291].

Thus, both currently and in the long term, addressing the challenges of genetic prediction of livestock productivity, rational processing of animal-derived raw materials, and the production of high-quality, competitive food products remains highly relevant. The development and implementation of scientifically grounded methods to enhance livestock productivity under the conditions of southern Russia are of substantial economic importance [6, p. 80; 7, p. 84].

The Growth Hormone Gene (GH) encodes an anabolic hormone that regulates metabolic processes in the organism. It is located on chromosome 11 and consists of five exons and four introns. Growth hormone has a molecular mass of approximately 22 kDa and comprises a polypeptide chain of 191 amino acid residues. Animal growth is regulated by a complex system in which the somatotropic axis plays a key role [10, p. 39].

MATERIALS AND METHODS

The study was conducted on Soviet Merino sheep (*Ovis aries*). The genetic structure of the Soviet Merino population was analyzed in a flock at the Skiba collective farm located in Mokryi Gashun settlement, Zimovnikovsky District, Rostov Region (Fig. 1; 46.880924, 42.754562).

Figure 1. Location of the Skiba collective farm

The Skiba collective farm is situated in the southeastern part of Zimovnikovsky District within the Ergeni Upland. The climate is sharply continental and characterized by frequent strong winds. The farm specializes in sheep breeding and has the status of a pedigree breeding enterprise. All parental animals belonged to the Soviet Merino breed. Random mating conditions (panmixia) were maintained during reproduction, excluding the effect of assortative mating. The age difference among lambs in the experimental group did not exceed four days.

During the study, live weight dynamics were assessed through serial weighing of ram lambs at birth and subsequently at 4 and 6 months of age. To evaluate meat productivity traits, a control slaughter of representative ram lambs of the studied genotypes was performed at 6 months of age in accordance with the methodological guidelines of the North Caucasus Research Institute of Animal Husbandry (2009).

The primer sequences used for amplification of the GH gene were as follows:

GH-F: 5′-GGAGGCAGGAAGGGATGAA-3′;

GH-R: 5′-CCAAGGGAGGGAGAGACAGA-3′

The PCR reaction mixture for amplification of the GH gene was prepared in a total volume of 20 μL using the commercial kit GenPak PCR Core (Izogen, Russia), designed for DNA amplification. The mastermix contained all necessary components for a single reaction, including hot-start Taq DNA polymerase, a mixture of highly purified 2′-deoxynucleoside-5′-triphosphates (dATP, dTTP, dGTP, dCTP), and a loading dye for electrophoresis.

PCR conditions were as follows: initial denaturation ("hot start") at 95°C for 5 min (1 cycle), followed by 33 cycles of denaturation at 95°C for 45 s, annealing at 62°C for 45 s, and extension at 72°C for 45 s, with a final extension at 72°C for 10 min. The amplified fragment length was 277 bp.

Restriction fragments of the GH gene were separated by electrophoresis in a 3.8% agarose gel at 130 V in 1×TBE buffer containing 0.5 μg/mL ethidium bromide for 50–60 min and visualized under UV light using a gel documentation system. Following digestion of PCR products with the restriction endonuclease AluI (SibEnzyme, Russia) at 37°C for 3–5 h, the following genotypes were identified: AA—277, 202, 110, 100, 94, 68, 49, 22, 8, and 4 bp; AB—277, 256, 202, 110, 100, 94, 68, 49, 22, 8, and 4 bp; BB—256, 202, 110, 100, 94, 68, 49, 22, 8, and 4 bp.

The results were visualized using a GenoSens 2150 gel documentation system.

Genotype frequencies were calculated using the following formula:

$$P = \frac{n}{N} \quad (1),$$

where *n* is the number of animals with a given genotype and *N* is the total number of animals studied in the population.

Allele frequencies were calculated using the following formula:

$$p = \frac{(2 \cdot N_{ii} + N_{iy})}{2 \cdot N} \quad (2),$$

where *p* is the allele frequency, N_{ii} is the number of homozygous individuals for a given allele, N_{ij} is the number of heterozygous individuals, and *N* is the total number of animals in the sample.

The number of informative alleles was determined as the number of alleles with a frequency exceeding 5%. The number of effective alleles was calculated using the following formula:

$$N_e = \frac{1}{1 - H_e} \quad (3),$$

where H_e is the expected homozygosity.

RESULTS AND DISCUSSION

Re i striction analysis identified allelic variants A and B of the *GH* gene and determined the genotypes present n the studied sheep population (Fig. 2).

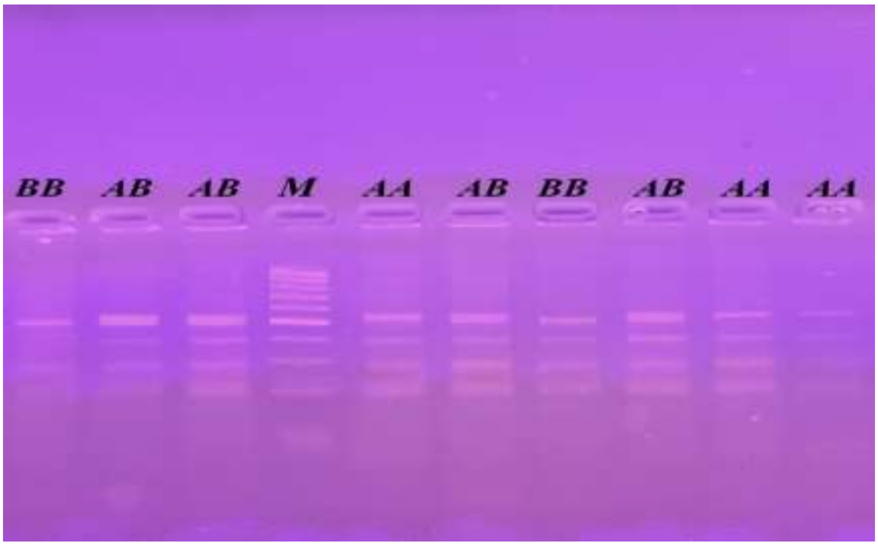

Figure 2. PCR–RFLP analysis of the *GH* gene in a 3.8% agarose gel

Growth hormone acts in concert with other candidate genes that influence bone formation, birth weight, muscle development, and morphological traits. Data on genotype and allele frequencies of the GH gene are presented in Figures 3 and 4.

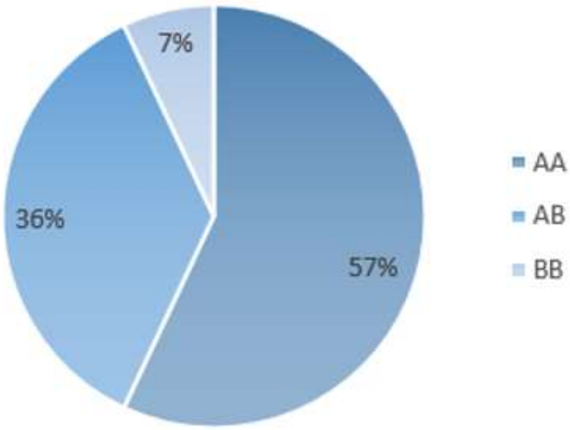

Figure 3. Genotype frequencies of the GH gene

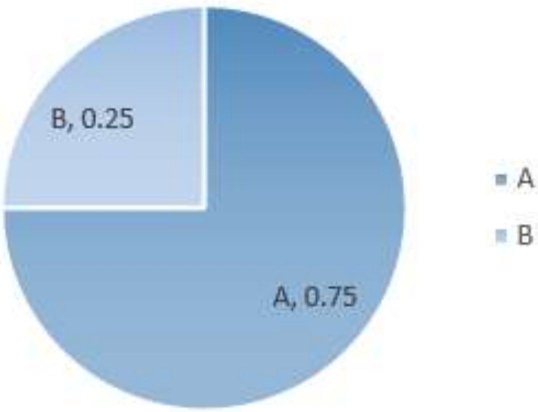

Figure 4. Allele frequencies of the GH gene

Analysis of genotyping results showed that polymorphism of the *GH* gene is represented by two alleles, GH^A and GH^B, and three genotypes, GH^{AA}, GH^{AB}, and GH^{BB}, with different frequencies. In the studied breed, the somatotropin gene is characterized by a high frequency of the GH^A allele (0.75) and a low frequency of the GH^B allele (0.25). Based on the PCR–RFLP results, experimental groups of ram lambs were formed according to *GH* gene genotypes. The influence of polymorphic variants of this gene on growth dynamics was investigated (Table 1).

Table 1. Live weight of ram lambs with different *GH* genotypes

Age, months	Genotypes		
	GH^{AA}	GH^{AB}	GH^{BB}
Live weight at birth, kg	3,64±0,21	4,18±0,17	3,72±0,09
Live weight at weaning, kg	25,73±0,76	29,63±0,87	26,56±0,29
Live weight at 6 months, kg	31,84±0,89	38,28±0,31	32,31±0,37

According to the data presented in Table 1, at birth the highest live weight was observed in heterozygous individuals with the GH^{AB} genotype compared to homozygous counterparts GH^{AA} and GH^{BB}. By four months of age, animals with the GH^{AB} genotype maintained this advantage, exceeding the live weight of GH^{AA} homozygotes by 12% and GH^{BB} homozygotes by 11.6% ($P > 0.999$).

By six months of age, heterozygous animals (GH^{AB}) showed a statistically significant superiority in live weight: 20.2% higher than GH^{AA} homozygotes ($P > 0.99$) and 18.4% higher than GH^{BB} homozygotes ($P > 0.99$). At the same time, live weight differences between animals with homozygous genotypes GH^{AA} and GH^{BB} were negligible.

During ontogenesis, animals with the GH^{AB} genotype demonstrated higher growth and development rates compared to individuals with GH^{AA} and GH^{BB} genotypes. These findings suggest that the observed intergroup differences in live weight are associated with the effect of the heterozygous GH^{AB} genotype of the growth hormone gene.

In improving productive traits in fine-wool sheep, increasing meat productivity is of primary importance, as under current socio-economic conditions the profitability of sheep farming largely depends on the efficiency of mutton production.

To substantiate the feasibility of DNA-based diagnostics, slaughter traits of Soviet Merino ram lambs with different genotypes of the growth hormone gene were evaluated. It was established that individuals with the GH^{AB} genotype exhibit significantly higher meat productivity and outperform animals with GH^{AA} and GH^{BB} genotypes across most analyzed parameters.

In terms of pre-slaughter weight, ram lambs with the GH^{AB} genotype exceeded their homozygous counterparts by 19.6% and 17.9% ($P > 0.95$).

Table 2. Results of control slaughter of ram lambs with different GH genotypes

Indicators	Genotypes		
	GH^{AA}	GH^{AB}	GH^{BB}
Pre-slaughter live weight, kg	30,89±1,39*	36,94±1,47	31,33±1,48*
Weight, kg: hot carcass chilled carcass internal fat	 12,26±0,47*** 12,02±0,46*** 0,130±0,006***	 17,13±0,65 16,61±0,63 0,186±0,007	 12,78±0,44*** 12,52±0,43*** 0,141±0,007**
Slaughter weight, kg	12,15±0,46***	16,79±0,64	12,66±0,43***
Slaughter yield, %	39,3±0,6***	45,5±0,6	40,5±0,8**

In terms of slaughter weight, ram lambs with the GH^{AB} genotype exceeded GH^{AA} and GH^{BB} counterparts by 38.2% and 32.6%, respectively ($P > 0.95$).

A similar trend was observed for slaughter yield, with the GH^{AB} genotype exceeding GH^{AA} and GH^{BB} by 6.2% and 5.0%, respectively ($P > 0.95$). Figure 5 presents diagrams illustrating differences in slaughter traits among ram lambs of different *GH* genotypes. Post hoc analysis was performed using Tukey's HSD test.

Based on the obtained results, the GH^{AB} genotype can be recommended as a preferable genotype in breeding programs for Soviet Merino sheep aimed at improving meat productivity.

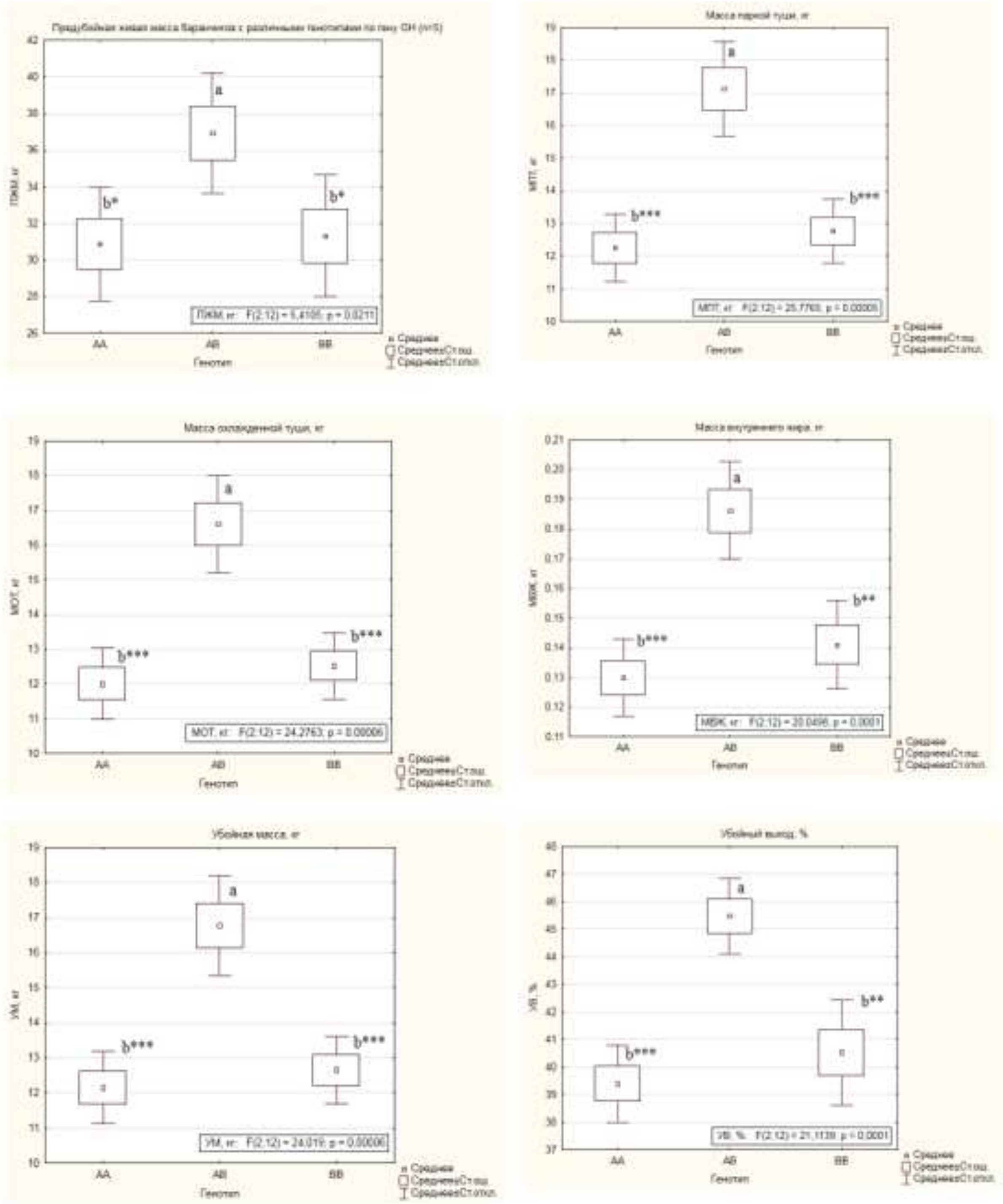

Figure 5. Diagrams of differences in slaughter traits among experimental ram lambs with different *GH* genotypes

CONCLUSION

1. The polymorphism of the *GH* gene is represented by two alleles, GH^{A} and GH^{B}, and three genotypes, GH^{AA}, GH^{AB}, and GH^{BB}, with different frequencies. This gene is characterized by a high frequency of the GH^{A} allele and a low frequency (0.25) of the GH^{B} allele.
2. In terms of pre-slaughter weight, ram lambs with the GH^{AB} genotype exceeded homozygous counterparts by 19.6% and 17.9% ($P > 0.95$). In terms of slaughter weight, GH^{AB} individuals surpassed GH^{AA} and GH^{BB} counterparts by 38.2% and 32.6%, respectively ($P > 0.95$). A similar trend was observed for slaughter yield, with the GH^{AB} genotype exceeding GH^{AA} and GH^{BB} by 6.2% and 5.0%, respectively ($P > 0.95$).

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REFERENCES

B. Aduchiev.K. V. Pogodaev.A. n.P., Sergeeva N.V. Meat productivity of sheep obtained from crossing sheep of the Stavropol breed with sheep with a bloodline of ½ Kalmyk chicken + ½ dorper // Zootechny. 2021. № 1. According to S. N. 35-39. DOI: 10.25708/ZT.2021.74.55.008

Meskhi B.Ch., Rudoy D.V., Olshevskaya A.V., Vershinina A.V., Odabashyan M.Yu., Prutskova A.S. Bioinformatics: A Key Section of 21st Century Genetics // Collection of Scientific Papers of the III International Forum "Youth in the AIC". 2022. https://doi.org/10.23947/young.2022.9-11

M. Aybazov, M. N. Analysis of the experience of European countries in the breeding of small cattle, the production of mutton and milk, and the development of a legislative framework for animals //Agricultural Journal. 2024. No. 2 (17). According to S. N. 44-61. DOI: 10.48612/FARC/2687-1254/005.2.17.2024

Genetic markers of meat productivity of the Romanov sheep breed: IGFBP-3, gho and CAST / M. N. Kostylev [et al.] / / Agrarian Science. 2020. № 11-12. According to S. N. 36-40. DOI 10.32634/0869-8155-2020-343-11-36-40

T. Deniskova. E. S. Petrov. N. A. Sermyagin. A. N. A. Dotsev. V. N. M. Fornara. According to senior scientists, Reyer H. K., Wimmers. V. Bagirov. A. N., Brem G., Zinovieva N. A. N. Search for genomic variants associated with live weight in sheep based on the analysis of high-density snp genotypes. Agricultural Biology 2021;56(2):279-291. DOI: 10.15389/agrobiology.2021.2.279rus

Identification of candidate genes associated with the growth and development of sheep from a crossbred population using a genome-wide association search / T. N. E. Deniskova, O. N. A. N. Koshkina, S. N. N. Petrov [et al.] / / Agrarian Science of the Euro-North-East. – 2024. - T. N. 25, No. 2. -pp. 236-250. – DOI 10.30766/2072-9081.2024.25.2.236-250. – EDN QCGYYF.

Koshkina, O. N. A. N. Development and approbation of a test system for determining the polymorphism of dgkh and ppp1r1c genes associated with live weight of sheep / O. A. N. Koshkina, T. N. E. Deniskova, N. A. N. Zinoviev / / Agrarian Science. – 2023. – No. 12. -pp. 80-84. – DOI 10.32634/0869-8155-2023-377-12-80-84. – EDN AADFGF.

K. Kulikova, A. N. Polymorphism of the calpastatin gene (cast) in mountain and steppe sheep of the interbreed types of the Tuvan short-tailed breed // Bulletin of the Bashkir State Agrarian University. 2018. № 1(45). According to S. N. 84-89.

Features of polymorphism of the CAST, gh and gdf9 genes in Kalmyk sheepskin and crossbreeds of the ½ Kalmyk sheepskin + ½ dorper / V. A. N. Pogodaev, A. N. N. Arilov, N. V. N. Sergeeva, E. N. According to S. N. Surzhikov / / Sheep, goats, wool business. – 2023. – No. 3. -pp. 12-15. – DOI 10.26897/2074-0840-2023-3-12-15. – EDN MZJVFY.

Olshevskaya A.V., Sarkisyan D.S., Saakyan S.R., Kutyga M.A. Clonal Micropropagation of Rare and Valuable Plant Species // Materials of the All-Russian Scientific and Practical Conference "Agrarian Science at the Present Stage: State, Problems, and Prospects". 2022

Polymorphism of CAST, gh, gdf9 genes in Gorno-Altaisk sheep. Selionova [et al.] / / Siberian Bulletin of Agricultural Science. 2020. Vol. 50. No. 1. According to S. N. 92-100.

Polymorphism of CAST, gh, gdf9 genes in Dagestan mountain sheep / A. N. A. N. Ozdemirov [et al.] / / The South of Russia: ecology, development. 2,021. Vol. 16. No. 2(59). According to S. N. 39-44.

Distribution of the frequency of occurrence of calpastatin gene alleles in sheep of different breeds (review) / Z. K. Gadzhiev, E. N. According to S. N. Surzhikov, T. N. N. Mikhailenko [et al.] / / Agrarian Scientific Journal. – 2023. – No. 5. -S. 72-78. – DOI 10.28983/asj.y2023i5pp72-78. – EDN YQMPXH.

Trukhachev, V. N. Prize winner. Genetic markers of sheep meat productivity (ovisariesl.). Message I. Myostatin, calpain, calpastatin / V. N.Winner of the award named after Trukhachev, M. N.

Prize winner. Selionova, A. N. Y. N. Krivoruchko, A. N.M. N.M.N. Aybazov / / Agricultural Biology. – 2018. - T. N. 53. – No. 6. -pp. 1107-1119.

Association of a single nucleotide polymorphism in the calpastatin gene with carcass and meat quality traits of beef cattle / F. Schenkel, S. Miller, Z. Jiang et al. // Journal Animal Sciences. 2006. Vol. 84. P. 291–299.

Bellige, R. Myostatin and its implications on animal breeding / Bellige, R., D. Liberles, S. Iaschi, P. O'Brien and G. Tay // A Review. Anim. Genet. – 2005. – №36. –p.1–6.

Hu, Z.L. Building a livestock genetic and genomic information knowledgebase through integrative developments of Animal QTLdb and CorrDB / Z.L.Hu, C.A.Park, J.M.Reecy // Nucl. Ac. Res. – 2019. – V. 47. – № D1. – P. D701– 710. https://doi.org/10.1093/nar/gky1084

Investigation of the Genetic Diversity of Dagestan Mountain Cattle Using STR-Markers / V. V. Volkova, A. S. Abdelmanova, T. E. Deniskova [et al.] // Diversity. – 2022. – Vol. 14, No. 7. – P. 569. – DOI 10.3390/d14070569. – EDN BSWQBQ.

Meuwissen, T. Genomic selection: A paradigm shift in animal breeding / T. Meuwissen, B. Hayes, M. Goddard // Animal Frontiers. – 2016. – Vol. 6. – № 1. – P. 6.

Sahu, A. R. et al. Novel report on mutation in exon 3 of myostatin (MSTN) gene in Nilagiri sheep: an endangered breed of South India //Tropical animal health and production. – 2019. – T. 51. – №. 7. – C. 1817-1822.

Wakchaure, R. Marker Assisted Selection (MAS) in Animal Breeding: A Review / R. Wakchaure, S. Ganguly, P.K. Praveen, A. Kumar, S. Sharma, T. Mahajan // Journal of Drug Metabolism & Toxicology. – OMICS International, 2015. – Vol. 06. – № 05.

Yadav, A.K. Importance of Molecular Markers in Livestock Improvement : A Review / A. K. Yadav, S. S. Tomar, A. K. Jha, J. Singh. – 2017. – Vol. 5. – № 4. – P. 614–621.

Yilmaz, O., Sezenler, T., Ata, N., Yaman, Y., Cemal, I., Karaca, O. 2014. Polymorphism of the ovine calpastatin gene in some Turkish sheep breeds. Turkish Journal of Veterinary & Animal Sciences, 38(4), Article 2. https://doi.org/10.3906/vet-1401-13.