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GENETIC MARKERS OF MEAT PRODUCTIVITY OF SHEEP (*Ovis aries* L.). I. MYOSTATIN, CALPAIN, CALPASTATIN

(review)

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Abstract

The study of genetic and biochemical bases of phenotypic polymorphism that determine meat productivity of agricultural animals is relevant for animal breeding. Breeders of USA, Europe and Australia use genes associated with quantitative and qualitative traits of meat cattle, such as *CAPN* and *CAST* (calpactin and calpain cascade), *MSTN* (myostatin), *GDF5* (growth differentiating factor), *TG5* (thyreoglobulin), *LEP* (leptin), *FABP4* (protein binding fatty acids) in selection programs (A.V. Eenennaam, 2006; Y.F. Liu et al., 2010; U. Singha et al., 2014; A.Ciepłoch et al., 2017). The main trend in the development of sheep breeding in recent decades throughout the world is a steady growth in mutton production, which determines an increase in the proportion of specialized meat breeds and increasingly growing requirements to parameters of meat productivity of meat sheep and meat wool sheep (A.M. Holmanov et al., 2015; M.I. Selionova, 2015). In this regard, search for candidate genes associated with these parameters is given more atttantion (D.W. Pethick et al., 2014). The presented review summarizes data on several factors which affect meat productivity in shhep. First, myostatin biological activity, gene structure and effect on the indices of sheep meat productivity are under consideration. Myostatin gene located on chromosome 2 and includes three exons and two introns is highly polymorphic (J.G. Hickford et al., 2010; M.R. Ansary, 2011; H. Han et al., 2013). Its mutations g+6723G>A and g+2449G>C have positive effects on the development of muscles and lead to a significant increase in meat with a decrease in fat content in the carcass (A. Clop et al., 2006; P.L. Johnson et al., 2009; I.A. Boman et al., 2010; A.Y. Masri et al., 2011; M. Hope et al., 2013; J. Wang et al., 2016). Another factor determining meat productivity in sheep is a proteolytic calpain-calpastatin system (CCS) (D.E. Goll et al., 2003; H.Y Chung, 2003). Calpastatin gene is located on chromosome 5 and includes 4 exons and 3 introns (B.R. Palmer, 1998). Calpain and calpastatin genes are presented by a variety of alleles, which differ in the frequency in different breeds (F.E. Shahroudi et al., 2006; S.O. Byun et al., 2009; M.A. Azari et al., 2012; G. Shahabodin et al., 2012; R.R. Arora et al., 2014; N. Shahram et al., 2014; N.S. Kumar et al., 2015). There is a relationship between point mutations in *CAPN* gene and fatty hips, kidneys, heart and a significant association of these mutations with lower fat deposition in the carcass. Intensity of growth rate in sheep young is primarily due to a greater increase in muscular weight which also correlates with *CAST* gene (M.R. Nassiry et al., 2006; A. Mahdavi Mamaghani et al., 2008; M. Tahmoorespur et al., 2012; Q. Fang et al., 2013). These results testify to expedience for myostatin, calpain and calpastatin genes typing in breeding genotypes with higher meat productivity.

Keywords: *Ovis aries* L., sheep, meat productivity, myostatin, *MSTN*, calpain, *CAPN*, calpastatin, *CAST*, genetic polymorphism, SNP, genome editing

Studies of the genetic and biochemical basis of phenotypic polymorphism characteristics of meat productivity have already been conducted for many decades. It is known that most productivity indicators are under the joint control of a significant number of genes. Polymorphism of candidate genes involved in the

formation of certain indicators of productivity and desirable genotypes is detected using the standard methods of molecular genetic analysis (AFLP — amplified fragment length polymorphism, ISSR — inter-simple sequence repeats) [1]. The greatest success has been achieved in dairy cattle breeding. The main genes determining the quantitative and qualitative indicators of milk productivity are identified. Marker-assisted selection (MAS) in many countries with developed dairy farming has become an integral part of national breeding programs [2].

The identification of genes associated with the meat productivity of animals was somewhat slower, however, also successful. For example, in the countries of America, Europe and Australia, the test for genes of calpactin and calpain cascade (*CAPN1*, *CAST*), myostatin (*MSTN*), growth differentiation factor (*GDF5*), thyroglobulin (*TG5*), leptin (*LEP*), a protein binding fatty acids (*FABP4*) is applied [3-6].

A constant increase in the production of mutton, which determines the increase in the share of specialized meat breeds and the increasing requirements for meat productivity for sheep of meat-wool and wool breeds, has become the main trend in the development of sheep breeding in recent decades worldwide [7, 8]. However, the increase in sheep meat productivity on the basis of MAS should be recognized as the least developed topic so far. By now, the use of molecular genetic tests in breeding programs has not led to a noticeable improvement in the economically significant indicators in the production of lamb. At the same time, the identification of and direct selection for the corresponding genes can be promising, since the indicators of meat productivity are characterized by low heritability. For example, for merino breeds and their hybrids with meat breeds, the coefficient of heritability of carcass mass, its yield, and the content of meat in it is 0.20-0.40, for specialized meat breeds — 0.38-0.54 [9-11].

The work objective of this review was to analyze the current state of research on the identification of genes that control the meat productivity of sheep and consider the main directions of the application of molecular genetic tests in sheep breeding to increase the qualitative meat production.

To understand the mechanism of genetic variability that affects the development of skeletal muscles of sheep, it is important to consider its functioning and features of normal development. It is known that muscle tissue is one of the main components of the body of superior vertebrates (it accounts for up to 40 % of the weight), 25 % of protein metabolism reactions flow in it [12]. The muscle tissue is dominated by the so-called 'slow' muscle fibers (type I). They are characterized by the fact that they slowly get tired, contain a large number of mitochondria and myoglobin, which gives the tissue a reddish color and determines the oxidative type of metabolism. On the contrary, 'fast' fibers (types IIb and IIx) have a glycolytic anaerobic type of metabolism, contain less myoglobin and mitochondria that causes the lighter color of these fibers [13]. The type and number of muscle fibers are laid in the early and middle stages of fetal development. In the late stages of embryogenesis and the first period of postnatal development, hypertrophy of all muscle fibers occurs due to the fusion of multinuclear myofibrils with mononuclear satellite cells [13]. These structural transformations determine the maximum similarity of the skeletal muscles of newborns and adults, including the type of muscle fibers [14]. In this case, mutations that affect the development of muscle tissue, as a rule, change the amount, composition of muscle fibers, as well as the degree of their hypertrophy.

The myostatin gene (*MSTN*) is one of the best-known candidate genes for meat productivity in sheep [15], goats [16], and cattle [17, 18]. Interest in *MSTN* appeared in the study of its mechanism of action in mammals and fish [19] and participation in ensuring the racing efficiency of dogs [20]. Myostatin is also

known as growth and differentiation factor 8 (GDF8) [21, 22]. A.C. McPherron et al. [23], studying the mouse genome and coding proteins belonging to one of the most important families of growth factors (transforming growth factor β-family, *TGF-β*), conducted one of the first detailed studies of the myostatin gene and mechanisms that ensure its biological activity. Later, the structure of the myostatin gene was determined for other species of farm animals [24, 25].

In the study of the *MSTN* gene structure in sheep, Y.F. Gong et al. [26] found that it is located on the 2nd chromosome and consists of three exons and two introns. In exons 1, 2 and 3, 508 nucleotides (including 373 coding), 374 and 1893 nucleotides (381 coding) are located respectively, in introns 1 and 2 — 1833 and 2030 nucleotides, respectively. The coding regions of the gene are highly conserved. In the sheep breeds of New Zealand Romney, Texel, Corriedale, Dorper, Perendale, Suffolk, Poll Dorset, Dorset Down, Merino, 28 nucleotide substitutions (data from NCBI GenBank, access DQ530260), including a well-described mutation c.*1232G<A (MSTN g+6227G<A), are revealed. Among these 28 substitutions, only one is in exon 1 (C.101G<A) and potentially leads to the replacement of glutamic acid (Glu) with glycine (Gly) in the codon 34. Three SNPs are located in the promoter area, three in the 5′-UTR area, 11 in intron 1, five in intron 2, and five in the 3′-UTR [27]. In Soviet Merino sheep, 28 SNPs are also described, two of which were discovered for the first time: c.940G>T in exon 3 and c.*16C>A in the 3-flanking region of the gene. Mutation c.940G>T converts the 314th codon of glutamic acid into a stop codon (GAA>TAA), which leads to the shortening of the protein product by 62 amino acids [28]. It has been reported about additional but not yet described mutations in the promoter region, introns 1 and 2 and the 3′-UTR region [29-31]. The obtained data show that the genetic variability of myostatin may be greater and a more detailed study of the impact of identified SNPs on the development of skeletal muscle of sheep is necessary.

In sheep of different breeds and animals of other species, the myostatin gene is highly homologous. The areas with affinity to the genes of the *MEF2* (myocyte enhancer factor-2, a key promyogenic transcription factor) and *AR* (androgen) were found in the promoter of *MSTN* in sheep, which indicates the possibility of participation of the protein products of these genes in the regulation of myostatin expression [32]. The regulation of transcription of the *MSTN* gene also involves the MyoD, Myf5, Mrf4, and P21, Smad factors [33-35]. Blocking the pathway from the myostatin gene to its product and further to the target muscle cells having an appropriate transmembrane receptor is accompanied by the expressed positive effect on the metabolism of skeletal muscle cells [36-38]. During embryonic myogenesis, myoblast progenitors are activated by the MyoD factors. After the interaction with the myostatin, the factor 21 (p21) starts; it inhibits the activity of proteins of the Cdk2 cyclin and the Rb retinoblastoma, which suppresses the proliferation of myoblasts and satellite cells in the G_1 phase. Reduction of myoblast proliferation in vitro under the influence of different myostatin concentrations was proved. In the absence of myostatin, the Rb protein is retained in the hyperphosphorylated form, which leads to an increase in the degree of myoblast proliferation [39]. Confirmation of the described mechanism was obtained by C. Liu et al. [40]. In their work, a vector with a built-in short RNA region (short-hairpin, shRNA) was used to block the myostatin expression in the cell culture of sheep primary myoblasts. It allowed reducing the activity of endogenous MSTN by 73.3 %, increase the proliferation of primary myoblasts by 28.3 % and significantly reduce the expression of MyoD proteins (by 37.6 %, $p = 0.025$), myogenin (by 33.1 %, $p = 0.049$), p21 (by 49.3 %, $p = 0.046$) and Smad3 (by 50.0 %, $p = 0.007$).

The negative regulatory role of myostatin in the development of skeletal muscles was demonstrated in the experiment to produce sheep with the expressed phenotypic effect of 'double muscles'. The knock-out method (KO) by means of microinjection of the genetic vector shRNA and the somatic cell nuclear transfer technology (SCNT) was used to block the expression of the *MSTN* gene. Five live lambs were received from 429 KO-embryos; three of them have reached the age of productive use. In transgenic animals with the myostatin gene knockout, the diameter of myofibrils (muscle fibers) and live weight were significantly higher at the age of 6 months [41].

The high efficiency of simultaneous use of nuclease acting as a transcription activator (transcription activator-like effector nucleases, TALENs) and single-stranded oligonucleotide DNA sequence (single-stranded DNA oligonucleotides, ssODN) for genome editing in sheep was revealed [42]. Testing on the cell line of primary fibroblasts of sheep NEK 293T showed that the joint transfection of TALENs and ssODN induced precise editing of the myostatin gene. *MSTN*-modified cells were successfully used as donor nuclei for embryo cloning [42]. With the help of TALENs and ssODN, cattle and sheep with KO-myostatin (knock-out MSTN) were also derived in the UK and the USA. The authors used the btGDF83.1L + 83.1 NR [43] construction and the RCIscript-GoldyTALEN transcription vector (Addgene ID 38142, Addgene, USA). Twelve lambs were derived from 26 edited blastocysts, 9 of which proved to be viable and demonstrated significantly higher growth energy due to greater growth of myofibrils [44]. M. Crispo et al. [45] demonstrated the high efficiency of the CRISPR/Cas9 system for editing *MSTN* and producing knockout sheep with increased body weight and expressed skeletal muscle development. A total of 53 MSTN-KO blastocysts were transplanted to 29 recipients, 22 viable lambs were received from them, 10 of which confirmed the genetic mutation of myostatin. In lambs with myostatin expression disabled, the average daily muscle mass growth was significantly higher [45].

Genetic manipulations that inactivate MSTN in transgenic mice and fish cause the same effect: in knockout individuals, body weight and its growth were significantly higher than in the control [23, 46]. Thus, by microinjection of the vector with antisense RNA of the myostatin gene, transgenic fish with the phenotype of 'double musculature' was obtained [47]. In KO-homozygous transgenic individuals, the number of myostatin and protein mRNAs was 33 % and 26 % respectively of their content in non-transgenic individuals, while the number of mRNAs myogenic regulatory factors, the MyoD, myogenin (MyoG), Mrf4, and Myf5, was significantly higher. Blocking the expression of myostatin in knockouts caused an increase in the area of muscle fibers by 2 times and body weight by 45% compared to non-transgenic individuals [47]. In transgenic mice, the *MSTN* knockout resulted in an almost 4-fold increase in skeletal muscle mass, and excessive expression of follistatin activated activin receptors regulating the synthesis of the llb type of fibers and led to myoblast hypertrophy with a significant increase in skeletal muscle plasticity [48-51].

The revealed biological role of myostatin and demonstration of genomic editing prospects for obtaining animals with high meat productivity without embedding recombinant DNA in the genome determined the interest in this protein and its gene as a potential genetic marker. The first studies of the effect of the myostatin gene on meat productivity were performed on sheep of the Texel breed with more developed muscles than in other breeds. The aim of the experiment was to determine a genetic mechanism controlling this distinctive phenotypic feature of the breed [15]. In the 3′-region mRNA of the myostatin gene, SNP.*1232G>A was revealed (previously referred to as g+6723G>A),

which is significant for the formation of muscle fibers. It was found that it or a mutation leading to the replacement of nucleotide G to A (allele *A*) creates the tenor of the recognition for the three miRNAs (miR-1, miR-206, and miR-122), thereby blocking the point of translation initiation of mRNA and the myostatin gene is inhibited. It leads to muscle hypertrophy and an increase in the amount of flesh in the carcasses. Similar results were obtained on the hybrids of Texel with the breeds of Poll Dorset and Welsh Mountain [52-54]. This effect of the mutation is confirmed on other breeds of sheep. Thus, at genotyping of 338 Charolais lambs by two SNPs in *GDF8* (g+2449G>C and g+6723G>a), comparison of SNP-genotypes, productivity indicators, and phenotypic traits in 56,500 lambs revealed a significant relationship of SNPs with muscle depth ($p < 0.001$). The muscle depth in animals with g.+6723AA compared to having the genotypes g+6723GG and g+6723AG was more with high confidence ($p < 0.002$). The additive and dominant effects of the allele g+6723A were 1.20±0.30 mm and –0.73±0.36 mm [55], respectively.

The positive effect of mutation g+6723G> of the A myostatin gene in a homozygous state is reported [56]. The authors revealed a significant advantage of homozygotes *AA* in carcass and flesh yield in the lumbar region and hind limbs. No differences in physical and chemical parameters of muscles musculus longissimus lumborum (LL) and musculus semimembranosus (SM) in sheep of different genotypes were found. At the same time, after cooking, steaks from SM from animals with the *AA* genotype received a much higher tasting score. The connection of mononucleotide substitutions revealed in the amplicon 304 bps from the promoter region of sheep *MSTN* with the signs of meat productivity in New Zealand Romney sheep was found [57]. General linear models of the mixed effect showed that the individuals with the genotype c.-2449GC had higher flesh yield ($p = 0.032$) and the bone-flesh ratio ($p = 0.028$) than those with the genotype c.-2449GG. The genotype c.-2379CC was associated with an increase in birth weight ($p = 0.003$) and weaning weight ($p = 0.028$), whereas the genotype c.-2379TC is not associated with growth rate. The haplotype H3 was associated with a decrease in birth weight ($p = 0.002$) and at weaning ($p = 0.011$), and the haplotype H2 was associated with increased flesh yield in the carcass ($p = 0.012$). The authors highlight the prospects for the selection of carriers of c.-2449GC for increasing the meat productivity of sheep [57].

In studies on Norwegian White Sheep breed, a new mutation (c.960delG) in the coding region of the myostatin gene was described. It was found that it shifts the reading frame at position 320 and leads to the premature stop codon at position 359, which causes greater muscle development and lower fat content. The detected mutation had a greater phenotypic effect than c.*1232G>a (g+6723G>A) [58]. In Makuei sheep, bred in Iran, in intron 1 of the myostatin gene, new mononucleotide polymorphisms are described and registered in the NCBI GenBank under the number KJ526625, which are in the positions 224 bps, 226 bps, and 242 bps and lead, respectively, to replacements of c.224C>T, c.226A>G, and c.242g>T. Genetic and statistical analysis showed that the replacement of c.226A>G is related to the heart size and the girth of the metacarpus, while this and other described mutations are not associated with the height at the withers and sacrum and with the body length [59]. In the Baluchi breed, the mutation in intron 1 of *MSTN* significantly influenced the live mass of animals and the flesh yield in the carcass [60].

Discussing the prospects of breeding on certain alleles and genotypes on *MSTN* to increase the meat productivity of sheep, some negative aspects should be noted. Getting animals with the effect of ‘double muscles’ was accompanied by a decrease in the size of vital organs (heart, lungs, kidneys) and the fragility

of the bones of the hind limbs. The animals were more susceptible to respiratory diseases, urolithiasis, alveolar hypoxia, hypoxemia and dystocia at the same time. In addition, rapid muscle growth had a negative impact on reproduction, increased embryonic mortality, abortion, and stillbirth rates [61]. The authors highlight that strict genetic control in the selection of parent pairs is necessary to avoid undesirable effects [6, 62].

The proteolytic calpain-calpastatin system (CCS) is involved in the realization of signs of meat productivity of sheep. It is represented by a family of Ca^{2+}-dependent neutral proteases, which are present in most animal tissues and involved in the regulation of cellular processes, including signaling and synthesis of cytoskeletal proteins, and muscle tissue homeostasis. The increase in the rate of skeletal muscle growth is regulated by a decrease in the rate of muscle protein degradation by reducing the activity of the calpain locus (calpain, *CAPN*) and simultaneously increasing the activity of calpastatin cascade proteins (calpastatin, *CAST*). In addition, the calpain-calpastatin complex regulates proteolytic and cytolytic reactions after slaughter, determining the rate of destruction of Z-discs of skeletal muscles and weakening of the bonds between muscle fibers, which plays a key role in the decay of tubulin and titin during maturation and the formation of the so-called tenderness of meat [63, 64]. The biological role of the calpain-calpastatin system in protein autolysis makes the calpain and calpastatin genes important candidates in the development of genetic approaches to the production of tender texture meat [65, 66].

The first protein of the calpain family was studied in 1976. It was found that calpain is represented by two heterodimeric types, each of which contains similar and very different subunits, the K30 and K80, respectively. Calpain A, or μ-calpain, is maximally activated in vitro at a concentration of Ca^{2+} 50-100 μm, calpain B, or m-calpain, at a concentration of 1-2 μm. However, at the optimal concentration of Ca^{2+}, the activity of calpain B is higher than that of calpain A [65, 67]. Calpain activity and Ca^{2+} concentration vary depending on the temperature and storage time of the muscles after slaughter. After 120 h at 30 °C, the content of free calcium in the longest back muscle was 40% higher than at 2 °C. The activity of m-calpain decreased more slowly than that of μ-calpain [68]. Calpastatin, the endogenous calpain inhibitor, binds in the presence of Ca^{2+} only with heterodimeric calpain molecules. Single-chain calpains are not inactivated by calpastatin due to the lack of a second binding site. Amino acid sequences of μ-and m-calpain in vertebrates are highly conservative (mammalian calpain homology is more than 90 %), and the set of substrates they split is similar and sometimes identical [68]. Genome-wide sequencing of the calpain gene and the study of regulatory and conservative subunits of this protein allowed determining their high similarity in many animal species. Thus, the identity of the nucleotide sequence in sheep of 192 bps in the calpain gene (exons 5 and 6 with intermediate intron) with a similar sequence in the goat, cattle, bison, and pig was 99, 97, 97, and 89 %, respectively [69]. For amplification of a portion of the cattle calpain 3 gene, primers designed to detect the genome of the sheep were used, and comparable results were obtained [70].

On different breeds of sheep, it was shown that the calpain gene is polymorphic, and the frequency of genotypes varies. In the Iranian breed Bandur, three genotypes — *AA*, *AB*, and *BB* with a frequency of 0.672; 0.295 and 0.033, respectively, were revealed [68]. In the Dalagh breed, the distribution of these genotypes, designated as G1, G2, and G3, was 0.082; 0.891 and 0.027 [71]. Similar results were obtained for fat-tailed and Karakul sheep [72, 73]. In the study of the genetic structure of 11 phenotypically dissimilar breeds of sheep in India with different geographical distribution for a number of candidate genes of

meat productivity (*CAPN4*, *CAST*, *FABP3*, and *DGAT1*), the average heterozygosity for them and their haplotypes was determined, 0.328 and 0.545, respectively [74].

The study of the influence of the allelic state in the calpain gene on the parameters of meat productivity of sheep allowed finding significant differences in the fat content in carcasses obtained from animals of different genotypes. Thus, the association of point mutations in exon 10 *CAPN3* with the fat layer around the thighs, kidneys, and heart in animals with different SNP was found [75]. In experiments carried out on lambs of the Romney breed in New Zealand, a reliable association of one of the genotypes with a lower fat content (in the absence of connection with the parameters of carcasses by weight and measurements) was revealed in exon 10 *CAPN3*, which determined a higher grade and price. The data obtained allowed the authors to conclude on the prospects of using the *CAPN3* gene in the breeding program of this breed to improve the consumer qualities of lamb [76]. At the same time, Y. Muto et al. [77], assessing the biochemical value of polymorphism in the *CAPN3* gene in sheep, did not found a significant effect of mutations on the studied signs. None of the four identified mononucleotide substitutions, including exon 10, did cause changes in the amino acid sequence of the protein *CAPN3*. No significant differences in the degree of mRNA expression and the amount of the *CAPN3* protein in animals of different genotypes were found [77].

As already noted, calpastatin is a specific inhibitor of m- and μ-calpains in mammalian tissues, causing the tenderness of meat when ripe after slaughter. The structure of the sheep calpastatin gene was first studied in 1998 [78]. It is localized on the 5th chromosome, includes 4 exons, and its size is about 100 thousand bps. Sequencing of consensus sequences of the calpastatin gene of sheep showed high similarity with those of goats, cattle, and pigs (94-98, 92-93 and 82-83 %, respectively) [79]. In sheep of the Dorset Horn breed, two alleles (*M* and *N*) of the calpastatin gene, which occur at a frequency of 0.77 and 0.23, respectively, were revealed [78]. In populations of sheep from Iran and Pakistan, two alleles were also detected, the frequency of genotypes *MM*, *MN*, *NN* in the breeds of Dalagh was 0.082, 0.891, 0.027; Lori — 0.320, 0.630, 0.050; Zel — 0.620, 0.260, 0.120; Lohi — 0.77, 0.20, 0.03; Kajli — 0.68, 0.26, 0.06, and Thalli — 0.80 (*MM*) and 0.20 (*MN*) [71, 80-82]. In subsequent studies, it was found that the calpastatin gene in sheep is genetically more diverse and represented by many other alleles. A study of the nucleotide sequence of exon 6 by PCR-ISSR analysis and subsequent DNA sequencing allowed determining five new alleles, one of which was a missense mutation and led to the replacement of glycine by leucine (Gln/Leu), which could potentially affect the function of the protein [83]. In the New Zealand Romney breed, in the study of exon 6 and introns 5 and 12 of the calpastatin gene, in addition to those described earlier, four new alleles in exon 12 were found. Generalization of the results obtained by other researchers and their own data allowed the authors to distinguish nine different haplotypes for the calpastatin gene of sheep and make an assumption about their greater informative value in comparison with single nucleotide substitutions (SNPs) for the analysis of the relationship between genes and such a polygenic factor as meat tenderness [84].

Analysis of the gene sequence of calpastatin in the ancient Iranian breeds, differing in the number and form of fat deposits along the caudal vertebrae (thin-tailed sheep Zel; fat-tailed breeds Lori-Bakhtiari; Chall; hybrids with an average severity of tail Zel-Atabay), revealed four SNP in intron 5 (C24T, G62A, G65T, and T69–) and three in exon 6 (c.197A>T, c.282 G>T, and c.296

C>G) [78]. All three polymorphisms in exon 6 were missense mutations that resulted in the replacement of glycine with leucine (Gln/Leu at position 66), glutamine with asparagine (Glu/Asp at position 94) and proline with arginine (Pro/Arg at position 99). The authors suggested that amino acid substitutions may affect the physical and chemical properties of the CAST protein, including hydrophobicity, amphiphilicity, total charge and activity of Ca^{2+}-channels. In total, eight haplotypes (*CAST-1*, *CAST-2*, *CAST-3*, *CAST-4*, *CAST-6*, *CAST-8*, *CAST-10*, *CAST-11*) were identified, with *CAST-1* and *CAST-2* occurring at a frequency of 0.365 and 0.295, previously not described haplotype *CAST-8* – with a frequency of 0.129. The highest heterozygosity (0.802) in the studied haplotypes was found in the Lori-Bakhtiari breed. Differences in the frequency of *CAST-10* and *CAST-8* between the fat-tailed breed of Lori-Bakhtiari and thin-tailed Zel were highly significant ($p < 0.001$), which indicates the possibility of using these haplotypes as genetic markers in the study of the specificity of breeds [79]. The study of the polymorphism of exon 1 of the calpastatin gene of sheep of the fat-tailed Kurdi breed using PCR-ISSR allows revealing the genotypes *aa*, *ab*, and *ac*, occurring at a frequency of 0.55; 0.32 and 0.13 respectively. The average daily weight gain in lambs with the genotype *ab* was 215.2 g, which was significantly higher than for the genotypes *aa* (204.9 g, $p < 0.05$), and *ac* (172.6 g, $p < 0.01$) [85]. The connection of *CAST* genotypes with the growth rate of young sheep is reported, primarily due to a greater increase in muscle mass [86-88].

Thus, the volume of conducting research indicates an increased interest in the search for candidate genes that mark meat productivity in sheep, and the use of genomic editing methods to obtain animals with the expressed effect of increasing muscle mass. In this regard, the genes of myostatin, calpain, and calpastatin are promising for further study in different breeds, the development of reliable DNA tests and their use for genotyping animals as a mandatory reception in breeding programs.

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