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ASSOCIATION OF SINGLE NUCLEOTIDE POLYMORPHISMS OF GH, LEP, MSTN GENES WITH GROWTH CHARACTERISTICS IN MEAT-WOOL AND WOOL SHEEP

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The main trend in the development of sheep breeding in the last decade all over the world has become a constant increase in the production of mutton. This study was designed to study polymorphisms of genes *GH, LEP, MSTN* in two breeds of North Caucasian Meat-Wool and Soviet Merino sheep in Russia, as well as to identify potential single nucleotide polymorphisms (SNPs) associated with growth traits to improve the genetic potential of sheep. Parts of the *GH, LEP, MSTN* genes were amplified in North Caucasian Meat-Wool and Soviet Merino sheep to identify SNPs by Sanger sequencing and using a polymerase chain reaction protocol. These genotypes were correlated with meat traits such as birth weight, growth rate, weaning weight. Genetic and variance analysis of the data obtained was also carried out. Sheep age and parity had a significant effect ($p < 0.05$) on birth weight, growth rate and weaning weight. Sequencing revealed missense mutations in the somatotropin, leptin and myostatin genes in the structure of the sheep genome of the studied breeds. Missense mutations of the *GH* gene (c.476G> A) and the *LEP* gene (c.541G> T), as well as a synonymous replacement of the *MSTN* gene (c. 212C> A), were revealed. According to the results of studies in the North Caucasian Meat-Wool sheep, three GH^{CC}, GH^{CT}, GH^{TT} genotypes for the *GH* gene and three LEP^{GG}, LEP^{GT}, LEP^{TT} genotypes for *LEP* were identified. The studied region of the *MSTN* gene in sheep of the

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North Caucasian meat and wool breed turned out to be monomorphic. According to the results of studies in Soviet Merino sheep, three genotypes GH^{CC}, GH^{CT}, GH^{TT} for the *GH* gene, three genotypes $MSTN^{CC}$, $MSTN^{CA}$, $MSTN^{AA}$ for the *MSTN* gene, two genotypes LEP^{GG}, LEP^{GT} for the *LEP* gene were established. The analysis of associations showed a significant effect ($p < 0.05$) of the GH^{CT} and LEP^{GT} genotypes on the signs of sheep growth. Interestingly, the presence of the T-allele in sheep of the North Caucasian Meat-Wool breed tended to increase in weight during weaning (+2.2 kg) both for the *GH* gene and for the *LEP* gene (+ 1.6 kg). Interestingly, similar results were observed in sheep of the Soviet Merino breed, where the missense mutation led to an increase in weaning weight (+ 1.2 kg) for the *GH* gene and for the *LEP* gene (+1.3 kg). A synonymous *MSTN* gene substitution does not lead to a substitution of the coding amino acid, but may further lead to gene expression. The *GH*, *LEP*, and *MSTN* genes are proposed markers for improving growth traits in meat-wool and wool sheep, which will increase the ability to understand the genetic architecture of the genes underlying SNPs that influence such traits.

Keywords: growth hormone gene *(GH)*, leptin gene *(LEP)*, myostatin gene *(MSTN)*, sheep

INTRODUCTION

In modern economic conditions, the importance of the meat productivity of sheep is extremely high. Lamb is an essential food with a high nutritional value. In addition, lamb has an excellent taste in compared to other types of meat, since it contains proteins, fats (with a significant amount of stearic complex), amino acids, saturated and unsaturated fatty acids, enzymes, vitamins, macro- and microelements (TSHABALALA *et al*. 2003). In terms of its chemical composition, mutton differs very slightly from beef: its protein is also absorbed by 91%, in terms of the content of vitamins and the amount of lipids in tissues. These products are similar, and in terms of calorie content, mutton even surpasses beef (BELHAJ *et al. 2021*).

Traits of meat productivity are a priority in the breeding of farm animals. Among the complex of measures aimed at improving the efficiency of the livestock industry, an important role is assigned to the methods of molecular genetics. Marker-associated breeding in most countries with developed animal husbandry is an essential part of national breeding programs. A significant number of genes associated with the beef productivity of cattle have been identified. In America, Europe, Australia, testing is used for the genes of the calpastatin-calpain cascade *(CAPN1, CAST)*, myostatin *(MSTN)*, growth differentiating factor *(GDF5)*, leptin *(LEP)* (TRUKHACHEV *et al. 2018*).

In sheep breeding, such studies have been developed recently. Significant successes in this direction have been achieved in sheep breeding in a number of foreign countries. The largest producers of mutton meat, such as Australia and New Zealand, effectively implement marker-oriented and genomic selection programs (MASRI *et al, 2011*). According to the growing interest in the production of young mutton and lamb, priority is given to the study of genes that control meat productivity.

The myostatin gene *(MSTN)* is one of the most studied genes for meat productivity in sheep (CLOP *et al. 2006*), goats (ZHANG *et al, 2012*), and cattle (GROBET *et al, 1997*). The

myostatin gene (MSTN) is one of the most studied genes for meat productivity in sheep (CLOP *et al*. 2006), goats (ZHANG *et al*. 2012), and cattle (GROBET *et al*., 1997). The myostatin gene is located on the second chromosome, consists of three exons, two introns, and is characterized by high genetic polymorphism. The nucleotide sequences of the MSTN gene were revealed in Norwegian sheep bred in England, Australia, New Zealand, and India. The relationship between the polymorphism of the MSTN gene and the indicators of meat productivity in sheep of the Texel, White Suffolk, New Zealand Romney, Chinese Meat Merino, and a number of other breeds has been established (KIJAS *et al*. 2007; GAN *et al*. 2008; BOMAN *et al*. 2009; DONALDSON *et al*. 2014; WANG *et al*., 2015).

Particularly relevant is the qestion of considering the polymorphism of marker genes and candidate genes encoding hormones that control growth and energy metabolism (WAKCHAURE *et al, 2015*). Growth hormone *(GH)* and leptin *(LEP)* are key hormonal regulators of energy metabolism. Polymorphism of genes encoding these proteins is associated with reproductive qualities, parameters of meat, wool, and milk productivity in cattle (HERNANDEZ *et al, 2017*), goats (SILVA *et al. 2012*) and sheep (AQLAN *et al.,* 2009; BARZEHKAR *et al.,* 2009; IBRAHIM *et al.* 2016; GORLOV *et al, 2017*).

The *GH* gene (growth hormone, somatotropic hormone, somatotropin) is one of the main regulators of somatic growth with a wide range of biological effects. In sheep, the growth hormone *(GH)* gene encoding somatotropin is mapped to the eleventh chromosome.

The leptin gene *(LEP)* acts as one of the hormones responsible for the regulation of fat metabolism and is an important component of the physiological signaling system that regulates appetite and fat metabolism. Leptin is a protein hormone primarily produced by adipocytes. LEP consists of 3 exons and 2 introns. The length of the protein is 167 amino acids and is synthesized mainly in white adipose tissue (BOUCHER *et al*. 2006). In sheep, the LEP gene, which encodes the protein leptin, is located on the fourth chromosome. *LEP* gene polymorphism is associated with the intensity of growth and body weight (HAJIHOSSEINLO *et al.* 2012*)*, milk production (MAHMOUD *et al.,* 2014) and reproductive qualities (BAKHTIAR *et al.* 2017).

At the same time, the world development of marker-oriented and genomic selection is conducting a further search for polymorphisms, leading to functional genetic variants and affecting productivity. Determination of the corresponding genes and direct selection for them can be promising, since the traits of meat productivity are characterized by low heritability. For merino breeds, their crosses with meat breeds, the coefficient of heritability of carcass weight, carcass yield, and pulp content in it is 0.20-0.40, for specialized meat breeds - 0.38-0.54 (MORTIMER *et al*, 2014).

Nowadays, very scanrty research have been devoted to the study of polymorphism of the *GH, LEP, MSTN* genes in sheep of Russian breeds. In addition, studies aimed at identifying the associations of these genes with indicators of meat productivity in sheep of the North Caucasian meat-wool and Soviet merino sheep are under study. Therefore, identification of genetic markers in meat-wool and wool sheep bred in Russia may be of particular interest. The aim of this study was to study the polymorphism of the *GH, LEP, MSTN* genes in sheep of the North Caucasian meat-wool and Soviet merino sheep breeds. At this stage of research, we identified single nucleotide polymorphisms *(SNPs)* associated with growth traits, which can later

be used to predict the growth rate in sheep, develop breeding programs to improve the genetic potential and strategies for managing sheep genetic resources in Russia.

MATERIALS AND METHODS

Ethical statement

All procedures were carried out in accordance with Directive 2010/63 / EU of the European Parliament and of the Council of the European Union on the protection of animals used for scientific purposes.

Animal resources and phenotypes

Our study has carried out on the basis of the All-Russian Scientific Research Institute of Sheep and Goat Breeding, a branch of the Federal State Budgetary Scientific Institution "North Caucasus Federal Research Agrarian Center" and in the laboratories of the Federal Government Health Institution, the "Stavropol Scientific Research Anti-Plague Institute".

The object of the study, the sheep of the North Caucasian meat-wool breed and the Soviet Merino breed, were raised in the Stavropol Territory (Russia). The selection of the genetic material was carried out on sheep at the age of four months. As a biomaterial, blood was used for DNA genotyping in sheep. In total, 60 samples have been taken (30 samples of North Caucasian meat- wool sheep, and 30 samples of Soviet merino).

Genetic analysis

Genetic analysis was performed using Sanger capillary sequencing. DNA isolation was carried out by nucleosorption using a certified "DNA sorb-B" kit (InterLabService, Russia).

Reagents produced by InterLabService (Russia) were used for PCR: dNTP mixture, PCR wax 15% (melting temperature 37° C), PCR-mixture-2 red (2.5-fold PCR buffer, cresol red, 5.5 mM MgCl2). Oligonucleotide primers for amplification of the *LEP*, *GH*, and *MSTN* gene regions (Table 1.) were selected based on the results of other studies (MEENA *et al.*, 2017; FARAG *et al. 2016*; AHANI AZARI *et al. 2012*).

Amplification was carried out on a plate-type thermal cycler (Bio-Rad, USA) (Table 2.). The size of the resulting amplicons was detected using the Kit of reagents for electrophoretic detection in agarose gel, as well as a DNA marker of molecular weights 50 p.n.bps (InterLabService, Russia). The PCR products are purified using the Agencourt AMPure XP reagent kit (Beckman Coulter Inc, USA).

Table 1.- Sequences of primers for amplification of gene regions GH, LEP, MSTN

Gene	Primer	Fragment length, bps
GH	F: 5'-GAAACCTCCTTCCTCGCCC-3' R: 5'- CCAGGGTCTAGGAAGCCACA-3'	365 bps
LEP	F: 5'- AGGAAGCACCTCTACGCTC -3', R: 5'- CTTCAAGGCTTCAGCACC -3'	471 bps
MSTN	F: 5'- CCGGAGAGACTTTGGGCTTGA-3' R: 5'- TCATGAGCACCCACAGCGGT-3'	337 bps

Table 2. Conditions for carrying out amplification for genes LEP, GH, and MSTN

Stage	*GH*			*LEP*			*MSTN*		
	t, °C	Time	The number of cycles	t, °C	Time	The number of cycles	t, °C	Time	The number of cycles
Temperature retention	94	5 min	1	94	5 min	1	94	5 min	1
Cycling	95	30 sec.	35	94	30 sec.	35	94	60 sec.	35
	65	30 sec.		59	30 sec.		59	60 sec.	
	72	45 sec.		72	30 sec.		72	2 min	
Final elongation	72	5 min	1	72	5 min	1	72	4 min	1

DNA sequencing

The sequencing reaction has performed using the BigDye ™ Terminator v3.1 Cycle Sequencing Kit according to the manufacturer's instructions. The reaction products are purified by precipitation with 75% isopropyl alcohol. Sequencing was done using an ABI PRISM 3500 Genetic Analyzer.

The genome assembly Oar_v4.0 was used for mapping. Gene annotation is performed using the Ensemble (www.ensembl.org) and NCBI (www.ncbi.nlm.nih.gov) genomic browsers. Genetic and statistical analysis of the research results was carried out using MS Excel software.

Genetic analysis

The main task of population-genetic analysis is to characterize the genetic structure of a population, including an assessment of the degree of genetic diversity.

Genotypic frequency

Genotype frequency is the ratio of the genotypes of the *GH*, *LEP*, and *MSTN* genes to the general population. The mathematical model of the genotype frequency (NEI and KUMAR, 2000) is presented as follows:

$$X_i=G_i/N,$$

where Xi is the frequency of the genotype, Gi is the number of animals of a particular genotype, N is the total number of animals.

Allelic frequency and heterozygosity

Allele frequency is the ratio of an allele to a common allele at a locus in a population. The mathematical model for the allele frequency (NEI and KUMAR, 2000) is presented as follows:

$$Xi= (2n_{ii} + n_{ij})/2N,$$

where Xi is the frequency of the i-th allele, n_{ii} is the number of the sample of the ii genotype, n_{ij} is the number of the sample of the genotype ij, and N is the total number of animals.

Gene diversity or the degree of heterozygosity was calculated using the formula (NEI and KUMAR, 2000) as follows:

$$h = 2n (1 - \Sigma x_i 2) / (2n-1),$$

where h is the degree of heterozygosity, x_i is the i-th allele frequency, n is the total number of individuals.

Analysis of variance was carried out using a spreadsheet processor MS Excel and an integrated mathematical package Matlab.

As a result of the analysis of variance procedure, the following values of interest to us were obtained:

SS_A – factorial (intergroup) variance - the sum of the weighted squares of the central deviations of the partial mean by gradation from the total average over the entire complex;

SS_E – random (intragroup) variance - the sum of the squares of the central deviations of the resulting feature values from its partial mean;

SS_T – total variance - the sum of the squares of the central deviations of the resulting trait values from the total average over the entire complex;

F – test F-statistic with a Fisher distribution;

p – type I error probability (p-value);

F_{st} – Fisher's standard value.

If the condition is met:

$$(1)\begin{cases} F \geq F_{st}, \\ p < \alpha \end{cases}$$

that is, there is every reason to reject the null hypothesis and accept the alternative. If condition (1) is not met, then the null hypothesis is accepted.

Based on the analysis of variance, the strength of the factor influence on the resulting trait was calculated. The indicator of the influence strength was calculated using the expression:

$$(2)\ \eta_x^2 = \frac{SS_A}{SS_T}.$$

The error in the indicator of the influence strength in a one-factor dispersion complex was determined by the expression:

$$(3)\ m_{\eta_x^2} = \left(1-\eta_x^2\right)\frac{r-1}{N-r},$$

where N is the total volume of the dispersion complex; r is the number of the factor gradations.

The final indicator of the analysis of variance was the indicator of the reliability of the influence power, determined by the expression:

$$(4)\ F_\eta = \frac{SS_A}{SS_E}\cdot\frac{N-r}{r-1} \geq F_{st} \quad ,$$

The influence of the factor (belonging to different genotypes) on the value of the resulting trait (body weight, average daily gain in body weight) is considered significant if inequality (4) is satisfied for the F_{st} value corresponding to a given significance level (α).

RESULTS

The study identified two distinctive patterns of conformation (alleles *G* and *T*; *C* and *T*; *C* and *A*) in the *GH, LEP,* and *MSTN* genes in the studied sheep breeds.

According to the results of sequencing DNA patterns, a missense mutation was identified in the coding part of exon 3 of the *LEP* gene at position 92503453 and a missense mutation in the coding part of exon 5 of the *GH* gene at position 47485936, as well as a synonymous substitution in the coding part of the *MSTN* gene at position 118145377.

Further, as a result of studies of the North Caucasian meat-wool breed sheep, were identified three genotypes of the *GH* gene (*CC, CT, TT*) and *LEP* (*GG, GT, TT*) (Table 3). The *MSTN* gene in this breed turned out to be monomorphic since all animals were of the same $MSTN^{CC}$ genotype. In Soviet merino sheep were identified three genotypes of the *GH* gene (*CC, CT, TT*) while only two genotypes (*GG, GT*) were found for the *LEP* gene (Table 3).

Table 3. Genotypes frequency of GH, LEP, and MSTN genes in sheep of North Caucasian meat-wool and Soviet merino breeds

Indicator	Genotype frequency, %								
	GH			*LEP*			*MSTN*		
	CC	*CT*	*TT*	*GG*	*GT*	*TT*	*CC*	*CA*	*AA*
North Caucasian meat - wool breed	53.3	30.0	16.7	60.0	26.7	13.3	100.0	-	-
Soviet merino	53.3	33.3	13.4	73.3	26.7	-	70.0	20.0	10.0

However, there is a similarity in the structure of allele frequencies of both breeds (Table 4.). The degree of heterozygosity is the average percentage of heterozygous individuals in the population and characterizes the level of allele polymorphism. The degree of heterozygosity of *GH* polymorphisms in sheep of the North Caucasian meat-wool and Soviet merino sheep breeds is 0.443 and 0.427, respectively (Table 4). The data on the genetic diversity of sheep for the *LEP* gene have values of 0.400 and 0.244, respectively. The value of the degree of heterozygosity of *MSTN* gene polymorphisms in sheep of the Soviet Merino breed was 0.301. It is known that high heterozygosity indicates the greatest genetic diversity within the population (NEI and KUMAR, 2000).

Table 4. Alleles frequency of GH, LEP, and MSTN genes in sheep of North Caucasian meat-wool and Soviet merino breeds (n = 60)

	GH		Degree of Heterozygosity	*LEP*		Degree of Heterozygosity	*MSTN*		Degree of Heterozygosity
	C	*T*		*G*	*T*		*C*	*A*	
North Caucasian meat - wool breed	0.68	0.32	0.443	0.73	0.27	0.400	1	0	-
Soviet merino	0.7	0.3	0.427	0.86	0.14	0.244	0.82	0.18	0.301

Based on the obtained values of heterozygosity, it was found that the polymorphisms of the *GH*, *LEP*, and *MSTN* genes had a high degree of genetic diversity. In breeding programs, high heterozygosity is desirable since high heterozygosity reflects the genetic variability of genes in the population and provides more opportunities for the selection of genes in the population. The revealed regularity suggests that sheep of the North Caucasian meat-wool breed have more opportunities for genetic improvement than sheep of the Soviet merino breed if the selection program for these markers was implemented.

To determine the strength of the influence of one or another complex of genotypes of the somatotropic axis on the values of the studied growth traits, an analysis of variance with the designation of the null hypothesis was applied. For animals of the North Caucasian meat- wool breed, ANOVA was carried out according to 7 gradations of the factor, for animals of the Soviet Merino breed - according to 11 gradations of the factor, belonging to a particular genotype (Table 5). The gradations are due to the combination of genotypes of the *GH* and *LEP* genes for a sample of animals of the North Caucasian meaty wool breed and a merger of genotypes of genes *GH, LEP,* and *MSTN* for a sample of animals of the Soviet merino breed.

Table 5. Results of variance analysis for a sample of the North Caucasian meat-wool and Soviet merino breeds (α-0.05)

ANOVA parameter		Birth weight	Weaning weight	Growth rate from birth to weaning
North Caucasian meat-wool	SS_A	2.458	14.652	8230.532
	SS_E	5.977	168.214	10405.16
	SS_T	8.435	315.867	18635.74
	F	1.579	3.365	3.032
	p	0.199	0.016	0.025
	F_{st}	2.528	2.528	2.528
	η_x^2	0.291±0.185	0.467±0.139	0.442±0.146
	F_η	1.576	3.365	3.032
Soviet merino	SS_A	4.224	24.864	1327.323
	SS_E	1.757	23.165	1955.037
	SS_T	5.982	48.030	3282.360
	F	4.568	2.039	1.290
	p	0.002	0.087	0.303
	F_{st}	2.378	2.378	2.378
	η_x^2	0.706±0.155	0.518±0.234	0.404±0.313
	F_η	4.568	2.039	1.290

In sheep of the North Caucasian meat-wool breed, the composition of the genotypes of the *GH* and *LEP* genes does not have a noticeable effect on the average value of weight at birth. However, analyzing the weight at weaning at the age of 4 months, the indicator of the reliability of the strength of the influence of the genotype on the average value of the resulting traits

exceeds the standard value of the Fisher criterion. Therefore, it can be argued that for sheep of the North Caucasian breed, the meat and wool composition of the genotype significantly affects the weight of sheep at the age of 4 months, as well as the growth rate from birth to weaning (Table 5).

In Soviet merino sheep, the combination of genotypes of genes *GH*, *LEP*, and *MSTN* has a significant effect on live weight at birth. In this case, the highest indicator of the strength of the influence of the factor is observed with the reliability of this indicator. Regarding the data on weight at weaning at the age of 4 months and the growth rate, the composition of genotypes does not significantly affect the average value (Table 6). More clearly, the average values of growth signs, depending on the gradations of the factor, for animal breeds of the North Caucasian meat-wool and Soviet merino are shown in the diagram (Figure 1, 2).

Table 6. Signs of growth of North Caucasian meat-wool sheep by genotypes of genes GH, LEP ($^{*}p \leq 0,05$)

Gene	Genotype	Signs of growth		
		Birth weight, kg	Growth rate, g	Weaning weight, kg
GH	*CC*	4.6±0.11	198.3±5.5	28.4±0.69
	CT	4.9±0.17	214.1±5.42	30.6±0.69
	TT	4.3±0.321	184.33±6.24	27.6±1.1
LEP	*GG*	4.5±0.13	200.3±5.04	28.5±0.64
	GT	4.8±0.19	210.9±6.19	30.1±0.78
	TT	4.78±0.27	189.4±13.43	28.0±1.7

Figure 1a shows that the gradations of the factor significantly affect the average values of weight at birth in sheep of the Soviet Merino breed. This is especially pronounced in a group of animals with a complex genotype: *CT + GT + CC*.

Figures 2b and 2c show that the gradations of the factor significantly affect the average values of the resulting trait weight at weaning. This is especially noticeable in a group of animals with a heterozygous complex of *CT + GT* genotypes, which is confirmed by the data in Table 5.

The results of variance analysis confirm the assumptions of our studies that the polymorphism of the *GH*, *LEP*, and *MSTN* genes affects the body weight at birth and weaning in both the North Caucasian meat- wool sheep and the Soviet merino sheep (Figure 1, 2).

We found that in sheep of the North Caucasian meat - wool breed, the GH^{CT} genotype is associated with a higher weight at weaning (30.6 kg) than the homozygous GH^{CC} and GH^{TT} genotypes (28.4 and 27.6 kg, respectively) (Table 6). The LEP^{GT} genotype is distinguished by a higher weaning weight compared to the LEP^{GG} and LEP^{TT} genotypes (30.1 kg versus 28.5 and 28.0 kg). It should be noted that at birth, animals with the LEP^{GT} and LEP^{TT} genotypes had practically the same average weight. These indicators confirm the corresponding values of the growth rate (Table 6).

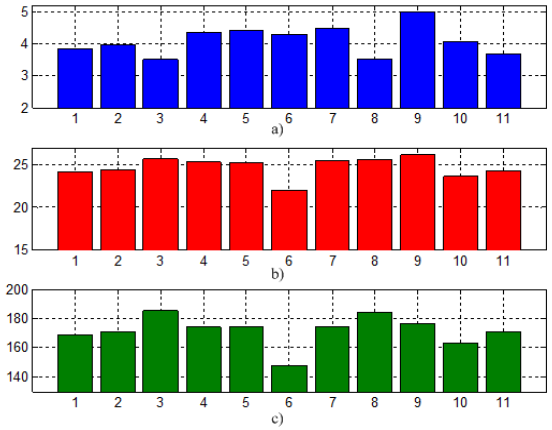

Figure 1. Average values of growth traits for various complexes of genotypes of Soviet Merino breed

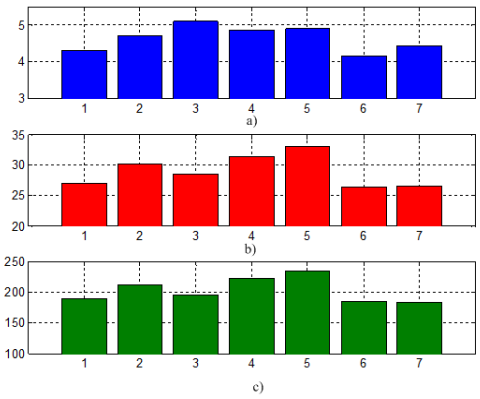

Figure 2 - Average values of growth traits for various complexes of genotypes of North Caucasian meat-wool breed

a) Birth weight; b) Weaning weight; c) Growth rate from birth to weaning

1	*CC+GG+CC*	1	*CC+GG*
2	*CC+GG+CA*	2	*CC+GT*
3	*CC+GG+AA*	3	*CC+TT*
4	*CC+GT+CC*	4	*CT+GG*
5	*CC+GT+CA*	5	*CT+GT*
6	*CC+GT+AA*	6	*TT+GG*
7	*CT+GG+CC*	7	*TT+TT*
8	*CT+GG+CA*		
9	*CT+GT+CC*		
10	*TT+GG+CC*		
11	*TT+GG+AA*		

The revealed relationship is traced between *GH* polymorphism with birth weight and weaning weight in Soviet merino sheep (Table 7). The GH^{CT} genotype showed significantly higher weaning weights than the GH^{CC} and GH^{TT} genotypes (25.6 kg versus 24.4 and 23.8 kg). The $MSTN^{CA}$ genotype has a higher weaning weight when compared with the $MSTN^{CC}$ and $MSTN^{AA}$ genotypes (25.67 kg versus 24.38 and 23.74 kg).The LEP^{GT} genotype was also with a higher weaning weight compared to the LEP^{GG} genotype (25.8 kg versus 24.5 kg). The homozygous LEP^{TT} genotype was not found in animals of this breed (Table 7).

*Table 7. Signs of growth of Soviet Merino sheep by genotypes of genes GH, LEP and MSTN (*p ≤ 0,05)*

Gene	Genotype	Signs of growth		
		Birth weight, kg	Growth rate, g	Weaning weight, kg
GH	*CC*	4.03±0.09	169.6±1.8	24.4±0.25
	CT	4.48±0.15*	175.75±2.04	25.6±0.31*
	TT	3.9±0.13	165.2±2.07	23.8±0.24
LEP	*GG*	4.1±0.11	170.0±3.3	24.5±0.27
	GT	4.51±0.18	177.3±2.01	25.8±0.35*
MSTN	*CC*	4.0±0.08	169.09±1.48	24.38±0.23
	CA	4.49±0.17*	176.81±1.26	25.67±0.33*
	AA	3.82±0.11	167.78±10.93	23.74±0.22

DISCUSSION

At present, reports on the high heritability of meat productivity traits are concentrated in cattle, which suggests selection for these traits based on marker breeding methods. Identifying genetic markers and candidate genes for improving meat production will help to select the best animals at an early age with potentially greater production potential.

On the other hand, there have been many studies of the association of mutations with traits of meat productivity, but they are comparatively limited to information for targeted marker selection. Accordingly, research is needed to identify the gene variation in sheep and assess their impact on productivity. This study describes for the first time the genetic variants of the *GH, LEP*, and *MSTN* genes in North Caucasian Meat-Wool and Soviet Merino sheep and their relationship with the traits of meat productivity. In general, the studied signs of growth in sheep of the North Caucasian Meat-Wool and Soviet Merino sheep breeds coincide with other studies (MEENA *et al.,* 2017; FARAG *et al.,* 2016; JIA *et al.,* 2014).

The creation of amplicons of the North Caucasian Meat-Wool and Soviet Merino breeds was based on the sequencing of the *GH, LEP*, and *MSTN* genes of Egyptian breeds (FARAG *et al.,* 2016), Indian Malpura breed (MEENA *et al.,* 2017), and Iranian Dalag breed (AHANI AZARI *et al.,* 2012). The success of the amplification shows that these breeds were similar to the studied breeds of the North Caucasian Meat-Wool and Soviet Merino.

In the process of molecular genetic analysis of the growth hormone fragment (*GH*) gene in sheep of the North Caucasian Meat - Wool breed and the Soviet Merino breed, a nonsynonymous substitution was revealed (*Oar_v4.0 g.47485936C> T; p.Arg159Gln*), which is located in the coding part of exon *V*. The frequency of occurrence of the reference allele *C* in this position was 2.2 times higher than the frequency of occurrence of the mutant allele *T* in sheep of the North Caucasian Meat - Wool breed and 2.3 times in sheep of the Soviet Merino breed.

In the region of exon III of the leptin gene (*LEP*), a nonsynonymous substitution was revealed (*Oar_v4.0 g.92503453G> T; p.Val181Leu*). According to the results of experimental studies, it was found that the most common is the reference allele *G*, its frequency of occurrence in sheep of the North Caucasian Meat-Wool and Soviet Merino breeds is 2.7-2.8 times higher than the frequency of occurrence of the mutant allele *T*. However, in sheep of the North

Caucasian Meat-Wool breed, the mutant allele *T* occurs 8.1% more often than sheep of the Indian Malpura breed (MEENA *et al.*, 2017).

Studies of the search for mutant alleles in Soviet Merino sheep also confirmed the missense mutation (rs420693815) of the *LEP* gene, which occurs 8.4% more often than in Indian Malpura sheep. The homozygous LEP^{TT} genotype was not found in animals of this breed, which is comparable to other studies in the Edilbaev breed of sheep, where this LEP^{TT} genotype was also absent (SENINA *et al.*, 2020*).* It should be noted that foreign researchers previously reported on the discovery of the substitution *Oar_v4.0 g.92503453G> T* in New Zealand merino sheep, Romney Marsh sheep, Coupworth, Corridale, Poll Dorset, Suffolk. However, data on the frequency of occurrence of alleles for these breeds are not given (ZHOU *et al.*, 2009).

As a result of molecular genetic analysis of a fragment of exon III of the myostatin gene in sheep of the Soviet Merino breed, has found a synonymous substitution (*Oar_v4.0 g. 118145377 C> A*). This substitution does not affect the coding amino acid. However, such a phenomenon as a *shift in codon frequency*, where there is a deviation of synonymous codons used from a uniform one, can further lead to gene expression. Indeed, *MSTN* could be classified as a gene with a high level of expression for more accurate translation or increasing the efficiency of the gene itself (PLOTKIN and KUDLA, *2011*). A similar result of studies of the *MSTN* gene had reflected in the study of polymorphism in Sanjabi sheep (SOUFY *et al.*, 2009).

Studies of a fragment of exon III of the myostatin gene in sheep of the North Caucasian Meat-Wool breed showed that the *MSTN* locus was monomorphic. A similar result of the *MSTN* gene was observed in the Iranian Karakul sheep (EFTEKHARI SHAHROUDI *et al.*, 2006), Dalag sheep (AHANI AZARI *et al.*, 2012), and the Bulgarian dairy sheep breed (GEORGIEVA *et al.*, 2015).

Results of the associative analysis of *GH, MSTN,* and *LEP* genotypes and growth traits confirm our hypothesis about the presence of genetic potential for improving the production of lamb in the North Caucasian Meat-Wool and Soviet Merino sheep breeds. Genetic variations in productivity have been observed clearly, highlighting the importance of finding genetic creators for marker assisted breeding programs in the breed. There are currently no studies investigating the relationship between *GH, LEP,* and *MSTN* variants and growth traits in North Caucasian Meat-Wool and Soviet Merino sheep due to comparisons of current studies.

According to POULSEN *et al*. (2017), it is essential to note that there is an assumption about the possible separation of genetic variants of genes into good and bad genotypes according to growth traits, which will facilitate future breeding in the direction of improving production (POULSEN *et al.*, 2017). This proposal can be used in variants *GH, LEP,* and *MSTN* to achieve optimal progress in lamb production from North Caucasian Meat-Wool and Soviet Merino sheep breeds based on the results of the current study.

CONCLUSION

As a result of this study, single nucleotide polymorphisms in the *GH, LEP*, and *MSTN* genes are proposed as markers for improving growth traits in meat-wool and wool sheep, which will increase the ability to understand the genetic architecture of the genes underlying the SNPs that affect such traits.

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ASOCIJACIJA SNP-ova GH, LEP, MSTN GENA SA KARAKTERISTIKAMA RASTA KOD MESNATIH I MERINO OVACA

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Izvod

Glavni trend u razvoju ovčarstva u poslednjoj deceniji u celom svetu postao je stalni porast proizvodnje ovčetine. Ova studija je osmišljena za proučavanje polimorfizama gena GH, LEP, MSTN kod dve rase severnokavkaske mesne vune i sovjetskih merino ovaca u Rusiji, kao i za identifikaciju potencijalnih polimorfizama pojedinačnih nukleotida (SNP) povezanih sa osobinama rasta radi poboljšanja genetskog potencijal ovaca. Delovi gena GH, LEP, MSTN su amplifikovani u severnokavkaskim ovcama za meso-vunu i sovjetskim merino ovcama da bi se identifikovali SNP pomoću *Sanger* sekvenciranja i korišćenjem PCR protokola. Ovi genotipovi su bili u korelaciji sa osobinama mesa kao što su težina pri rođenju, stopa rasta, težina pri odbiću.Takođe je sprovedena genetička analiza i analiza varijanse dobijenih podataka. Starost i paritet ovaca su imali značajan uticaj ($p < 0,05$) na porođajnu težinu, brzinu rasta i težinu pri odbijanju. Sekvenciranjem su otkrivene mutacije gena somatotropina, leptina i miostatina u strukturi genoma ovaca ispitivanih rasa. Otkrivene su mutacije gena GH (c.476G> A) i gena LEP (c.541G> T), kao i sinonimna zamena MSTN gena (c. 212C> A). Prema rezultatima istraživanja na severnokavkaskim mesno-vunastim ovcama, identifikovana su tri genotipa GHCC, GHCT, GHTT za GH gen i tri LEPGG, LEPGT, LEPT genotipa za LEP. Pokazalo se da je proučavani region MSTN gena kod ovaca severnokavkaske rase meso-vuna monomorfan. Prema rezultatima studija na sovjetskim merino ovcama ustanovljena su tri genotipa GHCC, GHCT, GHTT za GH gen, tri genotipa MSTNSS, MSTNSA, MSTNAA za MSTN gen, dva genotipa LEPGG, LEPGT za LEP gen. Analiza asocijacija je pokazala značajan uticaj ($p < 0,05$) genotipova GHST i LEPGT na znake rasta ovaca. Zanimljivo je da je prisustvo T-alela kod ovaca severnokavkaske mesno-vunene rase imalo tendenciju povećanja težine tokom odbijanja (+2,2 kg) i za GH gen i za LEP gen (+1,6 kg). Zanimljivo je da su slični rezultati primećeni kod ovaca sovjetske merino rase, gde je mutacija dovela do povećanja težine odbića (+1,2 kg) za GH gen i za LEP gen (+1,3 kg). Sinonimna supstitucija MSTN gena ne dovodi do supstitucije kodirajuće amino kiseline, ali može dalje dovesti do ekspresije gena. Geni GH, LEP i MSTN su predloženi markeri za poboljšanje osobina rasta kod mesno-vunenih ovaca, što će povećati sposobnost razumevanja genetske arhitekture gena koji su u osnovi SNP-a koji utiču na takve osobine.

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