Association of polymorphic variants of the mstn gene with live weight in kalmyk curled sheep and their crosses

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Abstract. The article provides a rationale for the feasibility of genetic marking of productivity at an early age. The phenotypic effect of genotypes of the polymorphic myostatin gene on live weight was studied in sheep of the Kalmyk curly breed and their crossbreeds with the Charollais and Dorper breeds. To achieve this, the following tasks were addressed: genotyping and recording of performance indicators of animals of the Kalmyk curly breed (KC), Charollais (C), Dorper-Kalmyk crossbred ewes (½D×½KC), purebred and crossbred young animals; determination of the influence of MSTN gene genotypes on live weight and average daily gain. The research subjects were KC breeding rams (n=6), C breeding rams (n=2), KC ewes (n=40); ½D×½KC crossbred ewes (n=40). The number and gender distribution of the offspring obtained were as follows: KC (n=26); ½C×½KC (n=32), ½C×¼D×¼KC (n=50). DNA extraction was performed from whole blood of the sheep. Animal genotyping was conducted using the PCR-RFLP method. A 337 bp exon 3 fragments of the MSTN gene was amplified using primers, and the results were analyzed using HRM analysis technology. It was found that among KC breeding rams, carriers of the M allele were more frequently identified, while among C breeding rams, carriers of the N allele were predominant, and among purebred and crossbred ewes, the prevalence of the M allele carriers was observed. Among purebred KC young animals and ½C×¼D×¼KC crossbreeds, carriers of the N allele predominated, whereas among ½C×½KC crossbreeds, carriers of the M allele were predominant. Population genetic analysis revealed a significant redistribution in favor of an increase in the frequency of the heterozygous MN genotype among the three-breed ½C×¼D×¼KC young animals, while among the two-breed ½C×½KC, the NN genotype was prevalent.

1 Introduction

The accelerated development of sheep farming should be considered a matter of national importance, the solution of which will allow for a scientifically justified and, in the interests

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of the entire population, the satisfaction of the solvent demand for lamb through domestic production. The main trend in the development of sheep farming in recent decades worldwide is the sustainable growth of lamb production, which determines the increase in the share of specialized meat breeds and increasing requirements for lamb quality [1-2].

The discovery in the field of DNA technologies has provided a powerful impetus for the creation of fundamentally new approaches in animal breeding. Ensuring the population's access to high-quality food products is impossible without optimizing and standardizing the product's safety and quality. Currently, our country is implementing new biotechnological methods for evaluating various productivity traits of farm animals based on genetic information analysis. Modern technologies in specialized meat and meat-wool sheep farming are based on the maximum utilization of the biological features of animals to achieve maximum economic results, quantitative, and qualitative indicators of productivity [3].

The efficiency of livestock production is influenced by numerous factors, with one of the most significant being the genetic potential of the animal. Since most significant economic indicators have a polygenic nature, meaning they are determined by many genes, genetic improvement of breeds is a rather lengthy process. Today, the application of marker selection, in addition to traditional breeding, maintenance, and feeding methods, can become a powerful tool in the intensification of the breeding process of breed formation. It is known that most productivity indicators are under the combined control of a significant number of genes. However, the use of genetic markers in improving the meat productivity of sheep compared to other types of farm animals remains a less developed area [4]. Nevertheless, the identification of genes associated with meat productivity can be promising, as traits determining the growth of bone, muscle, and adipose tissues are characterized by low heritability [5-6]. Major lamb producers, such as Australia and New Zealand, actively implement marker-oriented and genomic selection programs.

One of the most promising marker genes for the assessment and prediction of meat productivity is the myostatin gene (*MSTN*) [7]. The identification of genetic markers determining alleles of the myostatin gene associated with the level of meat productivity is a relevant scientific task with significant practical implications for the development of Russian sheep farming [3]. Myostatin is an inhibitor of skeletal muscle growth, and mutations in the coding region of the gene lead to an increase in muscle mass, thus making them potential markers of meat quality and growth traits [8]. Therefore, polymorphic variants of the myostatin gene can have phenotypic effects on in vivo quantitative traits in sheep of different breeds, representing external manifestations of internal processes controlled by the myostatin system. This property has generated interest in studying myostatin polymorphism in some sheep breeds. However, in Russian-bred sheep breeds, as well as in the use of foreign gene pools, research on myostatin gene polymorphism has been insufficient, thus emphasizing the significance of the present study.

2 The aim of the study

was to examine the phenotypic effect of genotypes of the polymorphic myostatin gene (*MSTN*) on live weight in sheep of the Kalmyk curly breed and their crosses with the Charollais and Dorper breeds to establish the possibility of genetic marking of productivity at an early age.

3 Materials and Methods

The study was conducted at the "ARL" farm (Republic of Kalmykia, Yashkulsky district). The farm employs a pasture-stall system of maintenance, wherein 285 days are dedicated to

the grazing period. Natural pasture grass, mainly consisting of wormwood, several types of grasses (such as couch grass and fescue), and saltwort, constitutes 75-80% of the sheep's annual diet. Additionally, depending on the physiological condition of the ewes, approximately 7-10% of concentrated feed and 10-17% of harvested roughage are utilized. Thus, their main diet consists of 3-4 kg of grass from cereal-wormwood pasture, 1.5 kg of cereal-legume hay, 0.25 kg of concentrated feed (50% barley, 40% maize, 10% sunflower meal), and 0.08 kg of mineral feed.

Sampling for genotyping and recording of performance indicators were carried out from animals of the Kalmyk curly breed (KC) with meat and wool direction of productivity, Charollais (C) with meat and wool direction of productivity, as well as crosses with Charollais and Dorper (D) with a meat direction of productivity. The research subjects included KC breeding rams (n=6), C breeding rams (n=2), KC ewes (n=40): 20 for purebred breeding, 20 for crossing with C rams; ½D×½KC ewes (n=40) for crossing with C rams. The number and gender distribution of the offspring obtained were: KC (n=26: ♂14 and ♀12); ½C×½KC (n=32: ♂12 and ♀20), ½C×¼D×¼KC (n=50: ♂16 and ♀34).

DNA extraction was performed from whole blood of the sheep using the DNA Extraction Kit "DNA-Extran-1" (Synthol, Moscow) according to the instructions provided by the manufacturer.

Animal genotyping was carried out using the PCR-RFLP method. A 337 bp exon 3 fragment of the *MSTN* gene was amplified using primers: F 5'-CCGGAGAGACTTTGGGCTTGA-3'; R 5'-TCATGAGCACCCACAGCGGTC-3' [13]. Amplification was performed using the CFX96 system (BioRad, USA) in a 20 µL volume, including 10 µL of 10x PCR buffer, 1 µL of 10 mM MgCl2 (Synthol, Moscow), 0.2 µL of SynTaq DNA polymerase 5 U/µL (Synthol, Moscow), 2 µL of a dNTP mixture (2.5 mM), 5.6 µL of bidistilled water, and 1.2 µL of DNA. The temperature-time parameters of amplification were as follows: initial denaturation at 95°C for 5 minutes, 40 cycles (95°C for 20 s, annealing at 62°C for 20 s, extension at 72°C for 30 s), final extension at 72°C for 5 minutes. The results were analyzed using the High-Resolution Melts (HRM) analysis technology, based on the identification of differences in melting curves (DNA dissociation) after PCR-RFLP using the Precision Melt AnalysisTM software. The visualization of the genotyping results is presented in Figure 1.

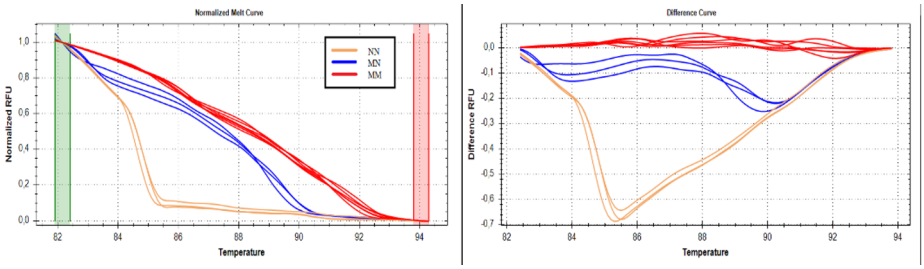

Fig. 1. Results of *MSTN* Gene HRM Analysis

The frequency of occurrence of alleles and genotypes, as well as the chi-square distribution criterion, were calculated using the Popgene program (Population Genetic Analysis 1.32).

Live weight of adult animals and offspring (at birth and at 4 months) of different genotypes was determined by weighing. The average daily gain was calculated accordingly. The obtained data were processed biometrically using the Microsoft Excel program. The significance of differences between the compared indicators among the groups was evaluated using the Student's t-test with a significance level of no less than $p<0.05$.

4 Results and Discussion

The characterization of the frequencies of the M and N alleles of *MSTN* among the purebred population of Kalmyk curly sheep and its crosses with the Charollais and Dorper breeds is provided in Table 1.

Table 1. Frequencies of the M and N alleles of the *MSTN* gene in Kalmyk curly sheep and its crosses with the Charollais and Dorper breeds.

Allele	Rams	Ewes	Young animals	Young rams	Young Ewes
	Breed affiliation				
	KC	KC	KC		
M	0,58±0,08	0,60±0,05	0,35±0,04	0,21±0,06	0,50±0,08
N	0,42±0,08	0,40±0,05	0,65±0,04	0,79±0,06	0,50±0,08
	C	KC	½Cx×½KC		
M	0,25±0,22	0,60±0,05	0,53±0,03	0,42±0,08	0,60±0,05
N	0,75±0,22	0,40±0,05	0,47±0,03	0,58±0,08	0,40±0,05
	C	½KCx½D	½C×¼D×¼KC		
M	0,25±0,22	0,55±0,02	0,36±0,02	0,44±0,06	0,32±0,03
N	0,75±0,22	0,45±0,02	0,64±0,02	0,56±0,06	0,68±0,03

It was found that the frequencies of the M and N alleles differ between the parents and offspring, both in purebred breeding and in crossbreeding. Specifically, among the Kalmyk curly breeding rams and ewes, the frequency of the M allele was 0.58 and 0.60, respectively, whereas in the purebred offspring, the frequency of the N allele increased to 0.79, with a decrease in the occurrence of the M allele to 0.21 among the lambs. Similar changes in allele frequencies were observed in the group of crossbred offspring when using the Charollais breed. While the frequency of the M allele was 0.25 in the Charollais rams and 0.60 in the purebred Kalmyk curly ewes, its concentration in the offspring ranged from 0.42 to 0.60, whereas the N allele in the parents was found with a frequency of 0.75 and 0.40, and in the offspring, its distribution ranged from 0.40 to 0.58. In the offspring obtained from crossbred ewes and Charollais rams, the alleles M and N were identified with nearly the same frequency as in the mothers.

The analysis of genotype distribution in the studied breeding variants and purebred breeding included an assessment of the correspondence of the observed genotype distribution to the theoretically expected distribution according to the Hardy-Weinberg equation (Table 2).

Table 2. Distribution of genotypes and evaluation of genetic equilibrium ($\chi 2$) in *MSTN* in Kalmyk curly sheep and their crosses with the Charollais and Dorper breeds. (%)

Genotype	Rams		Ewes		Young animals		Young rams		Young Ewes	
	Breed affiliation									
	KC		KC		KC					
	H	O	H	O	H	O	H	O	H	O
MM	50,00	34,0	30,0	36,0	7,7	12,0	0,0	04,6	16,7	25,0

MN	16,67	48,6	60,0	48,0	53,8	45,3	42,9	33,7	66,7	50,0
NN	33,33	17,4	10,0	16,0	38,5	42,8	57,1	61,7	16,7	25,0
χ^2	2,59		0,63		0,47		0,52		0,67	
	C		KC		½C×½KC					
MM	-	7,0	40,0	36,0	25,00	28,2	16,6	17,4	30,0	36,0
MN	50,0	37,0	40,0	48,0	56,25	49,8	50,0	48,6	60,0	48,0
NN	50,0	56,0	20,0	16,0	18,75	22,0	33,3	34,0	10,0	16,0
χ^2	0,22		0,28		0,27		0,00		0,63	
	C		½D×½KC		½C×¼D×¼KC					
MM	0,0	7,0	30,0	30,3	0,0	13,0	0,0	19,1	0,0	10,5
MN	50,0	37,0	50,0	49,5	72,0	46,1	87,5	49,2	64,7	43,8
NN	50,0	56,0	20,0	20,2	28,0	41,0	12,5	31,6	35,3	45,8
χ^2	0,22		0,001		7,91		4,84		3,89	

N - observed, O - theoretically expected values; deviation of N from O according to the Hardy-Weinberg law is significant at $\chi 2 \geq 3.84$.

The data obtained indicate that in practically all the studied groups of animals, except for the Kalmyk curly breeding rams and ewes, there was an excess of observed frequencies of heterozygotes compared to the expected ones according to the Hardy-Weinberg law. Specifically, among the Kalmyk curly breeding rams, the most common genotype was MM (50.0%), while the frequency of the MN genotype was only 16.67%. However, among the offspring (purebred ewes), there was an increase in the frequency of heterozygotes MN to 66.7%.

The analysis of genotype distribution among the crossbred three-breed offspring demonstrates a significant deviation of the observed genotype distribution from the theoretically expected equilibrium ($\chi 2=6.15$) due to the excess of heterozygotes (72.0% observed frequency compared to 46.1% expected).

Comparison of the live weight of rams of different genotypes showed that the Kalmyk curly breeding rams with the NN genotype tended to exceed the carriers of the MN and MM genotypes, partly explaining the distribution pattern of genotype frequencies in this group towards an increase in the observed frequency of animals carrying the NN genotype (33.33%) compared to the expected frequency (17.4%) (Table 3). Presumably, the high live weight of animals with the MM genotype determined the preferential selection of carriers of this genotype. Indirect confirmation of this is the fact that among the Charollais breeding rams, animals with the MM genotype were not identified. However, due to the small sample size, the interpretation of this observation as a regularity requires the expansion of the study with the involvement of a larger number of animals.

Among the purebred and crossbred ewes, there were no significant differences in live weight between animals of different genotypes in *MSTN* (Table 3). This circumstance is presumably explained by the fact that there is not such a strict selection among the ewes as

among the rams. Typically, individual animals with significant developmental deviations are excluded from breeding and not used for reproduction.

Table 3. Live weight of breeding rams and ewes of different genotypes according to the *MSTN* gene

Breed affiliation		For all genotypes, kg	Genotype		
			MM	MN	NN
Productive rams	KC	89,3±1,1	88,6±2,0	89,05±1,6	91,8±0,1
	C	80,3±1,1	-	79,2	81,4
Ewes	KC	61,9±0,50	62,0±0,6	61,7±0,8	61,4±0,7
	½D×½KC	62,9±0,31	63,3±0,1	62,6±0,6	62,9±1,0

The indicators of live weight at birth, at 4 months, and the average daily gain during this period in purebred and crossbred offspring of different genotypes according to *MSTN* are presented in Table 4.

Table 4. Live weight and average daily gain in purebred and crossbred offspring of different genotypes according to *MSTN*

Indicators	Breed affiliation								
	KC			½C×½KC			½C×¼D×¼ KC		
	Genotype								
	MM	MN	NN	MM	MN	NN	MM	MN	NN
Live mass, kg: at birth	4,0	3,8 ±0,3	4,1 ±0,2	3,6 ±0,2	3,7 ±0,2	3,7 ±0,5	–	3,8 ±0,1	4,2 ±0,2
4 months	40,1	38,6 ±1,5	39,3 ±0,3	36,8 ±1,4	37,9 ±1,1	39,5 ±1,5	–	39,5 ±0,5	40,5 ±0,6
Average daily increase, g	295,1	290,9 ±9,9	293,1 ±2,0	272,1	285,5 ±7,9	299,1 ±7,8	–	298,1 ±4,4	303,2 ±5,8

The research revealed that regardless of the mating combinations, the live weight of the offspring at birth of different *MSTN* genotypes did not differ. However, by the age of 4 months, there was a tendency for animals with the NN genotype to exceed those with the MM genotype, with this difference being particularly pronounced in the ½C × ½KC mating combination, amounting to 2.7 kg or 6.84%. Animals with the NN genotype also stood out in terms of average daily gain, with the highest figure observed in animals of the ½C × ¼D × ¼KC breed variant (303.2 g).

Our findings are consistent with those of Sahu A.R and colleagues (2019) [9], who identified polymorphism in the G5622C locus of exon 3 of *MSTN* in the Indian breed of Nilagiri sheep. However, Lenis-Valencia C. and colleagues (2019) [10] did not find such polymorphism in crossbred Colombian Creole wool × Pelibuey sheep; the gene was monomorphic (MM). Associative analysis in Nilagiri sheep did not reveal significant differences in live weight at birth, weaning, and live weight at 6, 9, and 12 months of age among different *MSTN* genotypes [9].

Osman N.M. and others (2021) [11] investigated a 386 bp fragment in the first intron of the *MSTN* gene and identified six single nucleotide polymorphisms in the breeds of Ossimi,

Rahmani, Najdi, and Barki. It was found that substitutions at positions c.18 G>T and c.241 C>T were significantly associated with birth weight and average daily gain up to 12 months of age. A significant association of the polymorphism at position c.*1232 in the first intron of the *MSTN* gene with live weight and daily gain up to two months of age was also observed in Kamensky sheep [12].

Han J et al. (2013) [13] studied genetic variations in the *MSTN* gene in purebred and crossbred New Zealand sheep. As a result, 28 nucleotide substitutions were identified in the segment from c.*1199 to c.*1813 of *MSTN*, including the well-described c.*1232G>A (*MSTN* g+6223G >A). Among them, 3 were located in the promoter region, 3 in the 5'UTR, 11 in intron 1, 5 in intron 2, and 5 in the 3'UTR, and 1 in exon 1 (c.101G>A). It was shown that the c.101G>A substitution leads to the amino acid substitution of glutamic acid (Glu) to glycine (Gly) at codon 34 and also affects muscle growth, similar to the impact of the g+6223G>A substitution.

The study of another variant of the *MSTN* gene - a 311 bp fragment of the intron 2 region - showed the presence of polymorphism in Marwari sheep with two genotypes, AA and AB, with frequencies of 0.86 and 0.14, respectively. However, this polymorphism did not have a significant effect on the live weight of the sheep [14].

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