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traits. The minimal live weight of adult rams of the steppe type is 78 kg and that of ewes is 56 kg. The natural wool is strong, 12-14 cm long Adult rams of the mountain type are characterized by 55 kg of live weight and ewes weigh 42 kg. The wool length is 10-12 cm.
The scouring yield is 50-60%. The birth rate of Tuvan sheep of all types is 100-110 lambs per 100 ewes (Yuldashbaev *et al.*, 2016).
One of the ways to intensify sheep breeding is to use genetic markers of productive traits to organize marker selection. Marker selection (MAS) is the use of DNA markers to improve the selection response in an animal population. Markers should be closely related to one or more target loci, which for the most part can be quantitative trait loci.
The discovery of polymorphic variants of various genes is a key moment in the start of breeding programs or work to conserve sheep breeds. Polymorphic variants of genes can be associated with different levels of productive traits.
One of the possible (potential) genetic markers of fecundity is the growth differentiation factor gene (GDF9) located on the fifth chromosome of sheep. This gene contains two exons and one intron (Bodensteiner *et al.*, 1999; Sadighi *et al.*, 2002: Hanrahan *et al.*, 2004).
The aim of this study is to detect polymorphic variants of the GDF9 gene to assess the possibility of its use in breeding programs and work on the conservation of the Tuvan short-fat tailed breed of sheep.

II. MATERIALS AND METHODS

Polymorphism of the studied gene was detected using the PCR-RFLP method. Blood samples for DNA analysis were taken from 250 sheep of Tuvan short-fat tailed breed of sheep belonging to two different intra-breed types: 106 samples of the mountain intra-breed type originated from State Unitary Enterprise “Malchyn” and 144 samples of the steppe intra-breed type originated from Municipal Unitary Enterprise "Despen". About 9 ml of blood per sample was collected in sterile tubes. Good preservation of blood samples was achieved with the help of K3-EDTA-sprayed tubes and sample freezing at -20º C.
Genomic DNA was isolated using commercial kits in accordance with the manufacturer's instructions.
To obtain amplicons of the GDF9 gene, the following pair of primers was used:
GDF9-F: 5'-GAAGACTGGTATGGGGAAATG-3’;
GDF9-R: 5’-CCAATCTGCTCCTACACACC T-3’.
The amplification reaction was carried out under the following conditions. 35 cycles: 94° C for 2 min., then 94° C for 30 s., 63° C for 40 s., 72° C for 30 s. and final elongation at 72° C for 4 min (Bahrami *et al.*, 2014; Kolosov *et al.*, 2015; Gorlov *et al.*, 2018). Fragments of GDF9 gene obtained in PCR were digested with *AspLEI* restriction endonuclease at 37° C for 12 hours. All initial amplicons and digested PCR products of GDF9 gene fragments were separated on a 2.0-3.0% agarose gel and visualized after staining with ethidium bromide in a gel documentation system.
The data generated by electrophoresis of *AspLEI* digested samples were used for estimating the frequency of different restriction fragment patterns.
The genotypes and allelic frequency were estimated by standard procedure.
Genotypes frequency was calculated according to following formula:

$$P_i = \frac{n_i}{N} \; ;$$

where: P_i is the ith genotype frequency;
n_i is a number of samples of the ith genotype;
N is a total number of samples of all genotypes.
Allelic frequency was calculated in the following way:

$$p_i = \frac{2n_{(homozygote)} + n_{(heterozygote)}}{2N} \; ;$$

where: p_i is the ith allele frequency;
n is a number of homozygotes of particular gene and heterozygotes, respectively;
N is a total number of individuals.

III. RESULTS

Amplified products of 462 bps fragments of GDF9 gene were obtained after amplification in the analyzed samples (Figure1).

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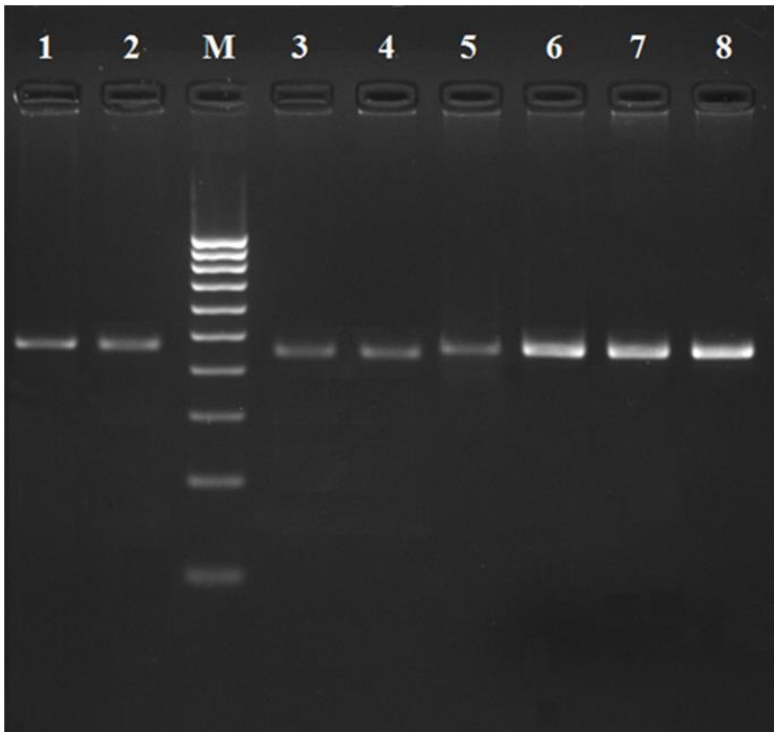

Figure 1. The electrophoretic analysis results of PCR products of GDF9 gene lane M – 100 bps DNA, lane 1-8 – PCR products. Viewed on 3.0% agarose gel.

C and D alleles of DGF9 gene were observed after digesting of PCR products by *AspLEI* restriction enzyme. Three genotypes presented by DNA-fragments of different size were established (Figure 2).

The *AspLEI* digestion of amplified loci of GDF9 gene produced fragments of 254, 156 and 52 bps for allele C. Allele D was identified as a 410 and 52 bps patterns. Homozygous genotype CC was characterized by 254, 156 and 52 bps bands. DD genotype contained 410 bps and 52 bps fragments. Heterozygous genotype CD consisted of 4 bands: 410, 254, 156 and 52 bps (Bahrami *et.al.,* 2014; Kolosov *et.al.*, 2015).

Fragments sized 52 bps were low-observable (Figure 2).

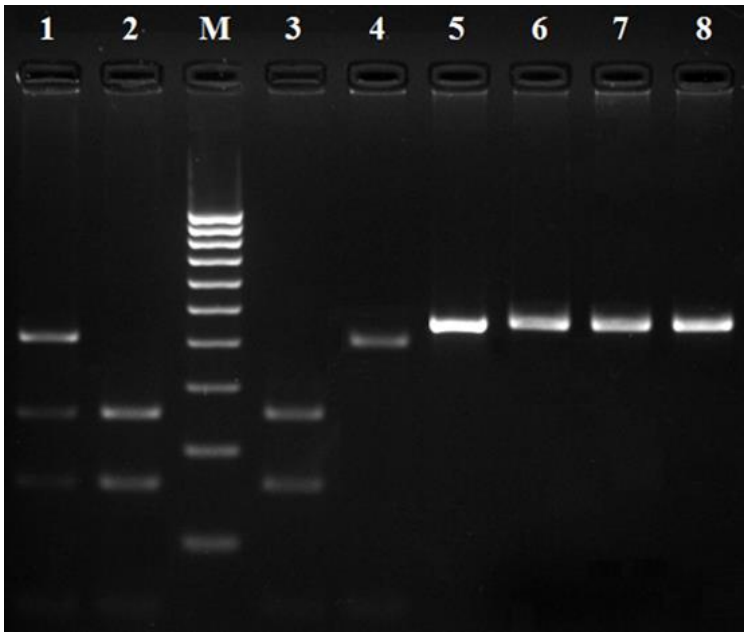

Figure 2. The electrophoretic analysis results of DNA pattern of amplicons after digestion with *AspLEI* restriction enzyme: lane M – 100 bps DNA, lane 1 - CD genotype, lanes 2, 3 - CC genotype, lane 4 - DD genotype, lane 5-8 – PCR products. Viewed on 3.0% agarose gel.

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The largest part of investigated populations of sheep was homozygous of C alleles of GDF9 gene (Table 1).

According to results of the investigation the largest part of animals in mountain type population was characterized by CC genotype and its frequency was 0.886. The frequency of CC genotype in steppe type population was 0.833. Heterozygous genotype CD was not so widespread in populations and had a frequency of occurrence equal to 0.114 and 0.160 in animals of mountain and steppe intra-breeds, respectively.

DD genotype was observed in steppe type sheep population only. Its frequency was the smallest in the result of the investigation (0.007).

Table 1. The genotypes frequency for GDF9 genes in Tuvan short-fat tailed sheep breed in SUE "Malchyn" (mountain type, n=106) and MUE "Despen" (steppe type, n=144)

Types of Tuvan sheep breed	Genotypes	The number of animals	Genotypes frequency
Mountain type	CC	94	0.886
	CD	12	0.114
	DD	0	0
Steppe type	CC	120	0.833
	CD	23	0.160
	DD	1	0.007

Table 2. The alleles frequency for GDF9 genes in Tuvan short-fat tailed sheep breed in SUE "Malchyn" (mountain type, n=106) and MUE "Despen" (steppe type, n=144)

Types of Tuvan sheep breed	Alleles	Allele frequency
Mountain type	C	0.94
	D	0.06
Steppe type	C	0.91
	D	0.09

C allele of GDF9 gene had the highest frequency in sheep of all bred types of Tuvan sheep (Table 2). The frequency of C allele in sheep population of steppe and mountain types was 0.91 and 0.94, respectively. This mark for D allele of GDF9 gene was 0.06 in mountain type sheep and 0.09 in steppe inter-breed type.

IV. DISCUSSION

The distribution of identified genotypes in Tuvan short-fat tailed sheep breed populations are in agreement with the polymorphism detected in ovine GDF9 gene in other local sheep breeds.

One of the previous experiments wild genotype (CC genotype in our investigation) in Hisari sheep was presented with a frequency of 93.64%. The frequency of heterozygous genotype (CD in our investigation) was 6.36% (Bahrami *et al.*, 2014).

The analysis of polymorphism for GDF9 gene in Baluchi sheep indicated all three possible genotypes: FecG+/FecG+ (CC genotype in our investigation) was 0.72, FecG+/FecG1 (CD in our investigation) was 0.20, FecG1/FecG1 (DD genotype in our investigation) was 0.08 (Moradband *et al.*, 2011).

The genotypes frequencies for GDF9 gene in Salsk and Volgograd sheep breeds had the similar character. The frequency of CC and CD genotype in Salsk breed was 88% and 12% respectively and those in Volgograd breed were 84% and 16%. Homozygous DD genotypes were not observed in the studied populations (Gorlov *et al.*, 2018).

In the other investigation Salsk sheep breed had a high frequency of CC genotype equal to 90%. The frequency of CD genotype was 10% (Kolosov *et al.*, 2015).

The frequencies of alleles and genotypes of GDF9 gene showed a higher level of polymorphism in the Romanov sheep population. CC genotype was detected (60.9%), DD genotype was not observed and the frequency of CD genotype was 39.1% (Kolosov *et al.*, 2015).

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In this way, the observed character of GDF9 genotypes distribution was typical in many sheep breeds population.

V. CONCLUSION

The characteristic of Tuvan short-fat tailed sheep populations by GDF9 gene is one of the steps in the implementation of the candidate genes approach in sheep breeding of the Tuva Republic and can be the basis of complex projects for conservation of local sheep breeds.

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