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MOLECULAR ANALYSIS OF OVINE MYOSTATIN GENE (MSTN) IN NORTHEAST BULGARIAN MERINO SHEEP BREED USING PCR-RFLP

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Abstract

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The purpose of this study was to identify genotypes of myostatin gene by PCR-RFLP method in 32 rams from Northeast Bulgarian Merino Sheep Breed. The animals are raised in Agricultural Experimental Station in Targovishte – Bulgaria. Genomic DNA was extracted from blood samples. The polymerase chain reaction was used to amplify 337 bp fragment of exon III of MSTN locus. The PCR products were digested by restriction enzyme *HaeIII* and the obtained patterns were visualized on 2% agarose gel stained with GelRed. All thirty two animals were monomorphic for the allele *m* and only genotype *mm*, respectively.

Key words: sheep, Northeast Bulgarian Merino breed, myostatin (MSTN) gene, PCR-RFLP method

Introduction

Knowledge of the genetic diversity in modern animal populations is essential to improve the breeds, for their adaptation to different environmental conditions and for obtaining of high-quality animal products, in particular meat. One of the genes which play a key role in the development of muscle is myostatin (MSTN) also called Growth and differentiation factors 8 (GDF8), which is studied in different species – ruminants and non- ruminants (Miranda et al., 2002; Stinckens et al., 2008; Alakilli et al., 2012). The protein encoded by this gene blocks the growth of skeletal muscle. Inhibition of myostatin expression leads to increased muscle mass, while the enhancement of myostatin level leads to degeneration processes, coupled with incomplete regeneration, extensive fibrosis and fatty replacement over time. In sheep myostatin affects the skeletal muscle development and may regulate adipogenesis (Liu et al., 2012). MSTN gene is located at the end of the long arm (2q32.2 locus) on a chromosome 2 of the sheep (*Ovis aries*) genome. The ovine myostatin gene consists of three exons and two introns (Bellige et al., 2005). In sheep MSTN gene were found polymorphisms associated with muscle hypertrophy (Kijas et al., 2007; Boman et al., 2010; Wang et al., 2015). Sheep carrying MSTN mutations are in widespread use in the sheep meat industry (Tellam et al., 2012).

In recent decades the demand for sheep meat and milk have been increased, while for wool is discontinued. Northeast Bulgarian Merino sheep (Nikolov et al., 2011) is fine fleece breed with good meat traits. Ewes weight 65–70 kg and rams – 110–120 kg, and fertility is within 125–135% (Tzonev, 2014). Very little information about gene polymorphism is currently available to compare different Bulgarian sheep breeds. The purpose of this study was to identify genotypes of myostatin gene in animals from Northeast Bulgarian Merino Sheep Breed.

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Materials and Methods

Sample collection and genomic DNA extraction

The investigation was carried out in the University of Forestry, in the Laboratory of Genetics. The material in present study involved thirty-two rams from Northeast Bulgarian Merino Sheep Breed, raised in the Agricultural Experimental Station in Targovishte – Bulgaria. Approximately 3 mL of peripheral blood was collected from *v. jugularis* in vacuum tubes, containing EDTA.

DNA was extracted by manual commercial kit for DNA purification according to the manufacturer's instruction (QIAamp DNA Blood Mini Kit Qiagen). The DNA concentration of each sample was determined by spectrophotometer *Biodrop*.

PCR amplification

PCR amplification of exon III fragment of sheep MSTN gene was carried out in total volume of 10 µl containing 4 µl DNA, 5 µl Red Taq Polymerase Master Mix (VWR), 0.4 µl of each primer (Bioneer) and 0.2 ddH_2O. The primer sequences were (Dehnavi et al., 2012) for forward primer: 5′- CCG GAG AGA CTT TGG GCT TGA -3′ and for reverse: 5′- TCA TGA GCA CCC ACA GCG GTC -3′. PCR reaction conditions was as follows: initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 sec, annealing at 58°C for 45 sec, extension at 72°C for 1 min and final extension at 72°C for 10 min.

Restriction Fragment Length Polymorphism (RFLP) analysis

The genotypes of the analyzed individuals were established using RFLP analysis. The digestion reaction was carried out in 10 µl final volume, containing 6 µl PCR product and 10 U/µl HaeIII enzyme (Bioneer). PCR products were incubated at 37°C for 15 h. The fragment size was determined using GeneRuler™ Ladder, 50 bp (Thermo) supplied with 1 ml 6xDNA Loading dye. The obtained PCR product and restriction fragments were tested on 2% agarose (Health care) gel in 1xTBE stained with GelRed (Biotium) and the bands were visualized on UV trans-illuminator.

Results and Discussion

The quality of extracted DNA was tested on 1% agarose gel stained with GelRed (Figure 1).

In our investigation a fragment of 337 bp of exon III of MSTN locus was amplified (Figure 2). The obtained PCR

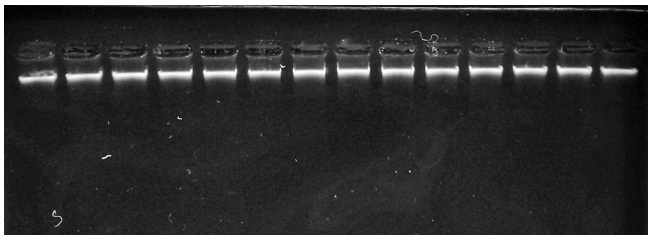

Fig. 1. Agarose electrophoresis for testing of extracted DNA samples

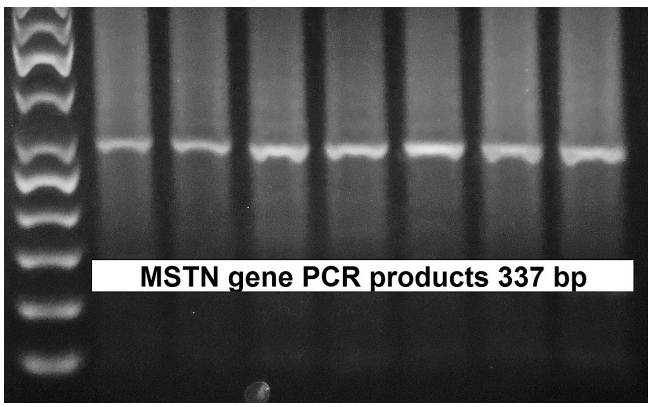

Fig. 2. Amplified 337 bp fragments of MSTN gene exon 3 in sheep

products were digested by *HaeIII* restriction enzyme. The enzyme digests allele m with formation of three fragments with length 131, 123, and 83 bp, but not digests allele M. The possible genotypes are MM (one fragment of 337 bp), Mm (four fragments – 337, 131, 123, and 83 bp) and mm (three fragments – 131, 123, and 83 bp).

Our findings show that all 32 samples carry genotype mm (Figure 3). As a result, all rams from studied herd of Northeast Bulgarian Merino Sheep Breed are monomorphic for the allele m with visualized three fragments with length 131, 123, and 83 bp.

The established outcome agrees with our previous study of other Bulgarian breed – Synthetic Population Milk sheep (Georgieva et al., 2015). Similar result was reported by Dehnavi et al. (2012) in Iranian Zel breed and by Azari et al. (2012) in 110 native Dalagh sheep in Iran. Elkorshy et al. (2013) also identified in animals from Egyptian breeds Rahmani, Barki, Ossimi and from Saudi breeds Najdi and Harri only the genotype mm – all animals were monomorphic for the m allele. The obtained survey results differ from these settled by Jamshidi et al. (2014) in Iranian Mehraban's sheep which discovered two genotypes Mm and mm with frequencies 0.053 and 0.947, respectively. Soufy et al. (2009) in Sanjabi sheep in Iran determined three genotypes – MM,

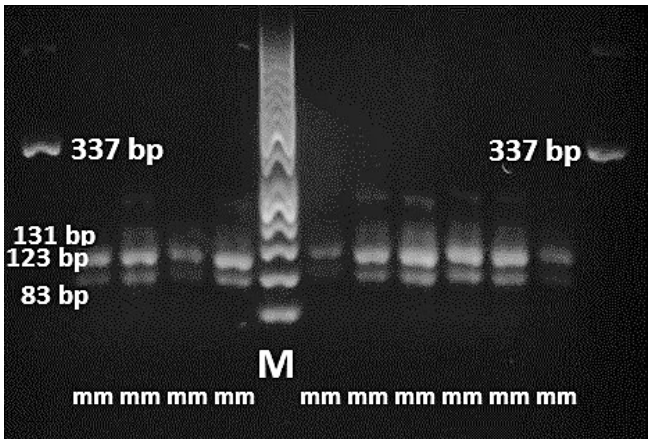

Fig. 3. Restriction fragments of MSTN gene in sheep digested with enzyme Hae III, observed on 2% agarose gel

Mm and mm with frequencies 2.00%, 1.33% and 96.70%, respectively and allele frequencies estimated 3.00% for allele M and 97.00% for allele m.

Conclusion

It may be concluded that MSTN gene is monomorphic for this herd of Northeast Bulgarian Merino sheep in Bulgaria. It was detected only the allele m and the genotype mm with frequency 1.00.

Acknowledgments

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