STUDY ON GENETIC POLYMORPHISM OF MYOSTATIN GENE IN MAGRA SHEEP

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ABSTRACT

Genetic variation in myostatin (*MSTN*) gene affects the body weight of sheep through regulation of muscle growth between individuals of sheep population. Variation in myostatin gene may yield higher body weight with more muscular growth and less fat deposition. Therefore the present study was undertaken in Magra sheep of Bikaner (Rajasthan) to detect the polymorphism in ovine *MSTN* exon 2 gene through polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) genotyping. Genomic DNA was extracted from whole blood of 67 unrelated animals through spin column method as per manufacturer's protocol. The quality and concentration of extracted genomic DNA was checked on 0.8% agarose gel and Nano Drop spectrophotometer, respectively. The exon 2 region of *MSTN* gene was amplified using caprine *MSTN* primers designed from GenBank (Accession No. DQ167575). The amplified 375 bp region of sheep *MSTN* gene was digested for 3 hours with 10 units of *Hae* III restriction enzyme at 37ºC. The genetic variability in exon 2 region of *MSTN* gene in Magra sheep was assessed on 8% polyacrylamide gel electrophoresis. Two different genotypic pattern 'Mm' and 'mm' were detected for the locus under study. Pattern 'Mm' was detected in seven animals whereas the pattern 'mm' was observed in sixty Magra sheep. The genotypic frequency of 'Mm' and 'mm' genotype were found to 0.10 and 0.90, respectively whereas the gene frequency of 'm' and 'M' allele were observed to be 0.947 and .053, respectively. The result showed the suitability of PCR-RFLP for detection of *MSTN* variant animals to evaluate its role in body weight and muscular growth.

***Key words*:** Sheep, myostatin, PCR-RFLP

Introduction

Sheep plays an important role in rural economy of Rajasthan through gainful employment to large number of illiterate and socially backward farmers (Choudhary *et al*., 2012). The body weight is one of the major indicators of health and productivity in sheep (Zaffer *et al*., 2015). The economics of sheep production is greatly affected by the growth and body weight of lambs (Singh *et al*., 2012) that ultimately depends on the muscular development. Myostatin (*MSTN*) or *GDF8* (growth and differentiation factor 8) gene is considered as candidate gene with functional and positional role in the regulation of muscular growth in different parts of the body (Tahmoorespur *et al*., 2011). The *MSTN* gene functions as a negative regulator of skeletal muscle growth in mammals (Peng *et al*., 2013). The *MSTN* gene is a member of the transforming growth factor â superfamily that normally acts to limit skeletal muscle mass through regulation of number and the growth of muscle fibres (McPherron *et al*., 1997). The *MSTN* gene of sheep is located on chromosome 2 (Archibald *et al*., 2010) and molecular analysis of the *MSTN* gene in different species has shown that it consists of three exons and two introns (Kurkute *et al*., 2011).

The myostatin gene in various species is found to be associated with increased muscle mass in a number of species. Natural mutations that decrease the amounts of myostatin and/or inhibit its function have been identified in human, cattle and sheep breeds (Boman *et al*., 2009). Quantitative trait loci (QTL) studies showed that myostatin gene affect the muscular development and muscle depth (Zhang *et al*., 2012) in New Zealand Texel sheep, UK Texel and Charollais sheep (Hickford *et al*., 2009). Mutations within myostatin gene have lead to muscular hypertrophy in Belgian Blue and Piedmontese cattle in which the animals had less bone, less fat, and 20% more muscle on an average (Kobolak and Gocza, 2002). The gene is found to be highly conserved however Zhou *et al*. (2008) identified a mutation in exon 2 region of *MSTN* gene.

Simple molecular technique like polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) (Saikia *et al*., 2015) is a useful tool to detect polymorphism at genetic level. Studies on the polymorphism of *MSTN* gene in Magra sheep through PCR RFLP are not available. Identification of polymorphism in myostatin exon-2 gene of Magra sheep through PCR-RFLP would be useful in finding better animal in terms of body weight and to assist the future selection programmes, especially marker-assistant selection for economic traits. Thus the present study was conducted in Magra sheep to identify the different genotypes of the myostatin exon-2 genes through PCR-RFLP in order to detect effective alleles that may influence the body weight of Magra sheep.

Materials and Methods

Animals (N=67) belonging to Magra sheep breed were selected randomly from different herds of sheep located at different regions of Bikaner district, Rajasthan. About 2 ml blood samples were collected from unrelated animals in the vacutainers containing EDTA as an anticoagulant. The genomic DNA was extracted by using spin column method as per standard protocols (Sambrook *et al*., 1989). The quality and the concentration of DNA were checked on 0.8%

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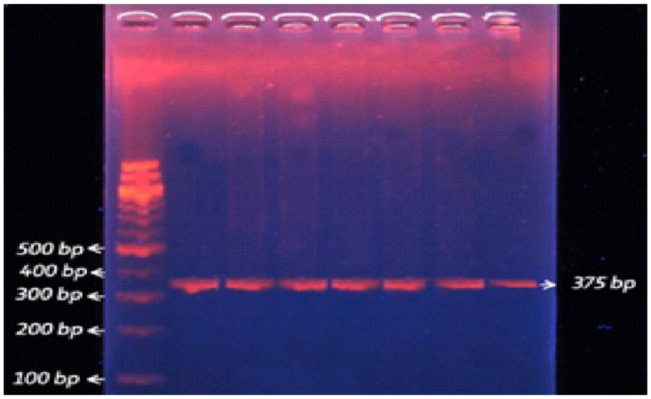

Fig.1: PCR amplification of myostatin (*MSTN*) exon-2 gene of Magra sheep. Lane 1: Molecular weight marker. Lane 2-8: PCR amplicons of myostatin (*MSTN*) exon-2 gene

agarose and Nano Drop Spectrophotometer, respectively. A primer pair (F5'AAAAACCCAAATGTTGCTTCTTTA3'; R5'CAGTCCTTCTTCTCCTGGTCTGG3') for the amplification of myostatin exon-2 gene was designed from homolog regions of goat (GenBank accession number DQ167575) to amplify the 375 bp fragment of exon-2 region of *MSTN* gene. Amplification reactions for each fragment was done by using the following constituents: in a final volume of 25 µl containing 5X PCR buffer, 1 unit of *Taq* DNA polymerase, 0.2 mM each of dNTPs, 1.5 mM $MgCl_2$, 75 pMol of each primer and 100 ng of template DNA. Amplification was performed in a thermal cycler with the following program; after an initial denaturation step at 95°C for 5 min, 35 cycles were programmed as follows: 94 °C for 30s, 54°C for 60s, 72°C for 60s and final extension at 72°C for 10 min. The amplified DNA fragments were stained and visualized on 1.5% agarose gel under Gel Documentation System (Fig. 1).

The restriction digestion of each amplified product (6 µl) of 375 bp of *MSTN* exon-2 gene was carried out for 3 hours with 10 units of restriction enzyme *Hae* III at 37ºC. Then the enzyme was inactivated by increasing the incubation temperature to 80°C for 20 minutes. The genetic variability in exon-2 region of *MSTN* gene in Magra sheep was assessed on 8% polyacrylamide gel electrophoresis with 100 bp DNA ladder in a gel documentation system. The statistical analysis for calculation of gene and genotypic frequencies were carried out using PopGene32 (ver. 1.32) (Yeh *et al*., 2000).

Results and Discussion

The polymorphism in ovine *MSTN* exon-2 gene of Magra sheep was detected by PCR-RFLP technique in the present study. The 375 bp fragment of exon 2 of *MSTN* gene was amplified and digested with the restriction enzyme *Hae* III. The *Hae III* digests the 'm' allele, but not 'M' allele. The digestion of the m allele produced two fragments of 88 and 287 bp. Two different genotypic pattern 'Mm' and 'mm' were detected for the locus under study (Fig. 2). Pattern 'Mm' was detected in seven Magra sheep whereas the pattern 'mm' was observed in sixty animals. As a result, the polymorphic nature of 'm' allele was observed in the present study. The frequency of 'm' and 'M' allele was found to be 0.947 and 0.053, respectively (Table 1). The genotypic frequency of "mm" and "Mm" genotype was observed to be 0.90 and 0.10, respectively. The genotype

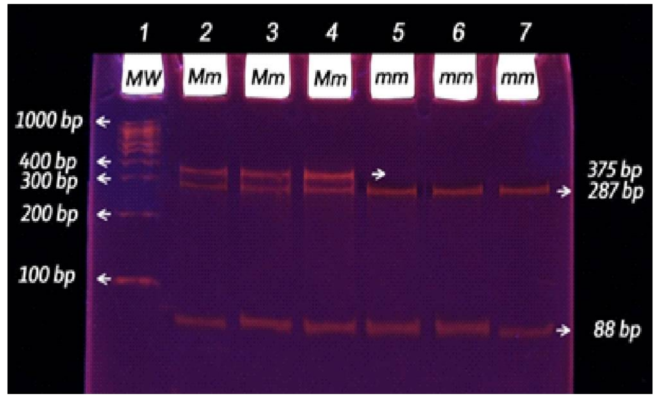

Fig. 2: RLFP polymorphism of myostatin (*MSTN*) exon-2 gene in Magra sheep. Lane 1: Molecular weight marker (1Kb ladder). Lane 2-4: Genotypic pattern (mm). Lane 5-7: Genotypic pattern (mm)

"MM" was not observed in any of the animal studied. The investigated population of Magra sheep showed a low degree of genotypic variability for the *MSTN* exon-2 gene.

The physiological regulation of muscle growth in animals is under the control of multiple genes. The *MSTN* gene has been considered as an important candidate gene for growth and development of domestic animals due to its key role in muscle growth (Miranda *et al*., 2002). Sheep, as one of the key livestock species, has good adaptability, wide geographic distribution, and very abundant breed resources. The information on *MSTN* variant individuals could also be utilized for the evaluation of animals in meat production.

The results of the present study are in agreement with Soufy *et al*. (2009) who also observed polymorphism in *MSTN* gene in Sanjabi sheep. Similar results were also observed in Iranian Baluchi sheep by Ansary *et al*. (2008). The two genotype observed in the present study were in agreement with the genotypes observed by Jamshidi *et al*. (2014) who observed lower frequency (0.053) of heterozygous genotype and higher frequency (0.947) of 'mm' genotype. Lazar *et al*. (2016) observed relatively very high frequency (0.833) of 'Mm' genotype in Teleorman Black Head lambs in contrast to low frequency (0.10) observed for Magra sheep in the present study. However, the results obtained in this study is in contrast with Dehnavi *et al*. (2012) who observed only "mm" genotype in all the samples in Iranian Zel sheep breed. Azari *et al*. (2012), Georgieva *et al*. (2015) and Dimitrova *et al*. (2016) also observed the monomorphic nature of myostatin gene in Dalagh, Synthetic Bulgarian and Bulgarian Merino sheep, respectively. The inconsistency in results of different workers may be ascribed to breed differences, population and sampling size, environmental factors, mating strategies, geographical position effect and frequency distribution of genetic variants.

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Table 1: Gene and genotypic frequency of *MSTN* exon 2 gene in Marwari sheep

Genotypic Frequency		Gene Frequency	
		m	M
MM	0.00	0.947	0.053
Mm	0.10		
mm	0.90		

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