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Study of Polymorphism in exon 3 region of leptin Gene in Harnali sheep

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Abstract
Leptin hormone primarily expresses in the adipose tissues and has significant importance in regulating various functions like growth, puberty, reproduction and milk production in both animals and humans. The objective of the present study was to determine the polymorphism of Leptin (LEP) gene in Harnali sheep. For analyzing Leptin gene polymorphism, polymerase chain reaction- restriction fragment length polymorphism method was used. A total of 110 Harnali sheep were selected and the genomic DNA was isolated by phenol-chloroform extraction method. The PCR product (463 bp) of the Leptin gene was screened. For detection of a particular SNP in coding region of the Leptin gene, the PCR product was digested with *Cail1* restriction enzyme for detection of SNP. The digested products were resolved on 2.5% agarose gel. All studied samples had only 463 bp after digestion with *Cail1* restriction and resolved into monomorphic pattern BB in Harnali sheep. The results indicated highly conserved sequence of Leptin gene in Harnali sheep.

Keywords: Harnali sheep, leptin gene, polymorphism, PCR-RLFP

Introduction

Sheep with its multi-facet utility for meat, milk, skin, wool and manure, form a vital part of rural economy in most of the areas of the country particularly in the arid, semi-arid and mountainous areas. India is rich source of sheep genetic resources having 43 breeds. As per recent records of 20th census (2019), India stands at number three in world sheep population with 74.26 million sheep. Harnali sheep is a new synthetic strain which has been evolved by cross breeding for superior carpet wool, better growth and adaptability. The crossbreds having 62.5 per cent exotic inheritance from Russian Merino and Corriedale and 37.5 percent from local Nali breed were mated inter se for several generations for stable performance. Harnali population has now become stable (Sehrawat, 2005) [26] and stability is one of the most desirable properties of a genotype to be released as a breed for wider utilization.

Now a days sheep are reared on a large scale especially for meat production and the increased productivity is reached by selection for more number of offspring, better growth rate and increased muscling, such that higher number of offspring reach market weight more rapidly and with a higher meat yield (Dwyer and Bunger, 2012) [7]. Sheep industry places a strong emphasis on selection for rapid lamb growth and high meat yield. This can also reduce labour inputs and animal health costs (Golding *et al.*, 2008 [10]; Judson *et al.*, 2009 [15]). Therefore, breeding for optimal birth weight and increased weight gains, is a major factor in sheep breeding programmes. Growth rate is related to muscle protein accretion, this being the difference between the rates of muscle protein synthesis and degradation. The higher the protein accretion at a set rate of degradation, the higher the growth rate of the animal (Therkildsen and Oksbjerg, 2009) [30]. As a result of the revolution in molecular biology, it is feasible to determine the genetic markers with great association with many aspects of DNA structure especially of the genes with major effect on the economic traits. Such genes for growth traits have also been identified.

Leptin gene (LEP) is associated with the important traits in animal breeding. The Leptin gene was detected by positional cloning techniques in 1994. This gene consists of three exons and two introns (Barzehkar *et al.,* 2009) [4]. Leptin gene produces leptin, a 167 amino acid protein which is synthesized by adipose tissue and is involved in regulation of food intake, energy balance, fertility and immune functions (Fruhbeck *et al*., 1998) [9]. Various research findings have indicated that leptin plays an important role in the regulation of growth, development and feed conversion efficiency (Yang *et al*., 2007) [32].

Further, it is highly correlated with body weight and adiposity. In livestock variation in Leptin gene has been characterized in cattle and pig but scanty information is available in Ovine. First attempt for the identification of LEP (Exon 3) gene variation was done in Iranian Makoei sheep (Hashemi *et al*. 2011) [13]. Hence, the present study was designed to investigate SNPs in exon 3 region of LEP genes in Harnali sheep.

Materials and Methods

A. Sampling

All the procedures have been conducted in accordance with the guidelines laid down by the Institutional Ethics Committee. The blood for the present study was collected from 110 Harnali animals maintained in the Department of Animal Genetics and Breeding, Lala Lajpat Rai University of Veterinary and Animal sciences, Hisar. 5ml of blood was aseptically collected from the jugular vein in vacutainer tube containing EDTA (2.7%). The tube was rolled gently in between palms to ensure proper mixing of blood and EDTA. The samples were suitably labelled and were stored at -20 ˚C till the isolation of genomic DNA. DNA was isolated from blood using standard phenol-chloroform method as described in Sambrook and Russell (2001) [25] with minor modifications.

B. DNA amplification and genotyping

Primers were designed using the Oligo software, based on published sequence information (Gen Bank No.: HE605296.1) to amplify a 463bp fragment including exon 3 of Leptin gene. The forward and reverse primers were F: 5′-TGTTGTCCCCTTCCTCCTG-3′ and R: 5′-CCCACATAGGCTCTCTTCTGC-3, respectively. PCR was performed in a final volume of 45 µl containing 50-100 ng DNA template, 0.25 µM of each primer, 18 µl Master Mix (2X PCR Master Mix Red-Mg Cl2:1.5 mM) and 22.2 µl distilled water. The PCR was programmed as follows: an initial denaturation step at 95 °C for 5 min, followed by 30 cycles of 95 °C for 1 min, 63 °C for 1 min, and 72 °C for 1 min. A final extension step was performed at 72 °C for 5 min. Electrophoresis of the amplicons was carried out in 1% agarose gels containing Green Viewer in 0.5x TBE buffer, and the gels were visualized under ultraviolet light. Suitable restriction enzyme for RFLP method was found by web cutter program. The PCR products were digested by *Cail 1* (*Thermo fisher*) restriction enzyme in order to detect polymorphism. The digestion of amplicon was performed at 37 °C for 10 hours using 5 U enzyme and 8 µl PCR product. Digestion products were separated in 4% agarose gel for 70 min at 70 V and stained with ethidium bromide.

Results and Discussion

Quality of extracted DNA was assessed by 2.5% agarose gel electrophoresis (Fig. 1). The 463bp fragment comprising exon 3 region of LEP gene was successfully amplified. The locus specific clear dark bands were visualized in all samples (Fig. 2). For detection of allelic variation in the amplified exonic regions of LEP, the PCR products were further processed for RFLP. The PCR product was digested with *Cail 1* restriction enzyme for detection of SNP. The digested products were resolved on 2.5% agarose gel, revealed uniform PCR-RFLP band pattern for LEP exon 3 in all DNA samples (Fig. 3). The studied samples were found homozygous BB genotype. The absence of polymorphism indicated the probable lack of mutation or presence of high degree of conservation of Leptin gene in Harnali sheep. Several polymorphisms have been reported across different livestock species for Leptin gene. Zhou *et al*. (2009) [33] reported four SNPs in exon 3 of the ovine LEP out of which three SNPs were non-synonymous in Kermani sheep. Polymorphism in the exon 3 region of leptin a gene was also studied in Iranian Makoei sheep and Five SSCP patterns were identified by (Hashemi *et al*., 2011). Singh *et al.,* (2009) [28] reported five major haplotypes in exon 2 region and six major haplotypes in intron 2 region of LEP gene in Barbari and Jamunapari goat breeds of India. Three SNPs were detected in the Exon 3 of LEP gene in Poll Dorsets, Suffolk, Texels and Tan sheep (Li *et al*., 2008) [17]. Tahmoorespur *et al*. (2010) [29] observed significant association of three genotypes of LEP with additive EBVs for weaning weight at 90 days in Baluchi and Kermani sheep and it was concluded that LEP gene polymorphism might be the one of the important genetic factor influencing growth traits. Some workers have reported five genotypes for Exon 3 of LEP gene in Makoei sheep of Iran (Hajihosseinlo *et al*., 2012) [12]. Genotyping of Assaf, Awassi and Dorper breeds of sheep in the Exon 3 of LEP gene resulted in three synonymous and three nonsynonymous mutations (Reicher *et al*., 2011)[24]. Lara *et al*. (2012) [16] reported polymorphism in exon 3 region of LEP gene of native, commercial and crossbred sheep. The sequencing of LEP exon 3 of Nilagiri revealed two SNPs as 16973 G>A (SNP-L1) and 17476 C>T (SNP-L2). Shojaei *et al*. (2010) [27] suggested that polymorphism in Leptin gene loci may be used as a selective marker to improve growth traits in future.

The findings of monomorphic exon 3 of ovine LEP gene in the present study are in agreement with those of Gregorio *et al*. (2014) [11] who observed monomorphic pattern in the genomic sequence of exon-3 of ovine LEP. The results are also in agreement with Cauveri *et al*. (2014) [6] that studied polymorphism in exon 3 of leptin gene in Nilagiri sheep. Nilagiri sheep had only one SNP (SNPL1) that is mono-morphic (AA genotype). Qureshi *et al.* (2015) [23] also reported monomorphic pattern at position A2262T, C2256G, -273 ^{0}C in intron 2 and A3201G at exon 2 sequence of Leptin gene in three sheep breeds of Pakistan using PCR RFLP technique. However, they observed polymorphism at position C1467T and A3050- in intron 2 of Ovine LEP. In an earlier attempt by Li *et al.* (2008) [17] did not find polymorphism in exon 2 using PCR-SSCP protocol in Poll Dorset, Suffolk, Texel and Tan sheep. Study of polymorphism in exon 2 of LEP gene have been done in Dorset and Suffolk breeds (Boucher *et al.,* 2006) [5] and Shal, Zandi and Zel breeds (Barzhekar *et al*., 2009) [4] but no polymorphism were detected by them in this region. Meena *et al*. (2016) [21] reported three SNPs in exon 3 of LEP gene in Malpura sheep breed using PCR-RFLP technique. The T387G locus was found polymorphic while A316C and A271G loci were monomorphic. Maitra *et al.* (2019) [19] found monomorphic pattern with presence of only T allele in Leptin gene in Barbari, Ganjam, Sirohi, Black Bengal and Osmanabadi whereas found polymorphism in Leptin gene in Beetal and Malabari goat breeds with (CT) heterozygote frequency of 0.33. The monomorphic nature of Leptin gene in the present study indicates the lack of mutation in LEP gene.

Conclusion

The present study revealed that Leptin gene was monomorphic at exon 3 locus in the screened samples of Harnali Sheep. This shows that Harnali sheep have no

variability in amplified exon 3 region of Leptin gene. All animals were found to be monomorphic with BB pattern. So, in Harnali sheep particular amplified region is highly conserved. This might be due to the relatively small population of Harnali sheep in which the Leptin gene might have more protected genetic structure. This monomorphism may be a breed specific characteristic. Since present study has formulated the results based on a relatively small sample, further studies are required to study SNPs in large samples to establish the role of Leptin gene in growth. Further research on other candidate growth related genes can be targetted for development of a suitable marker in sheep.

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Fig 1: Quality checking of the genomic DNA

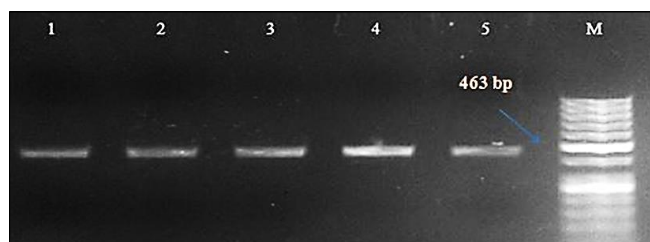

Fig 2: PCR amplicons of Leptin gene in Harnali sheep

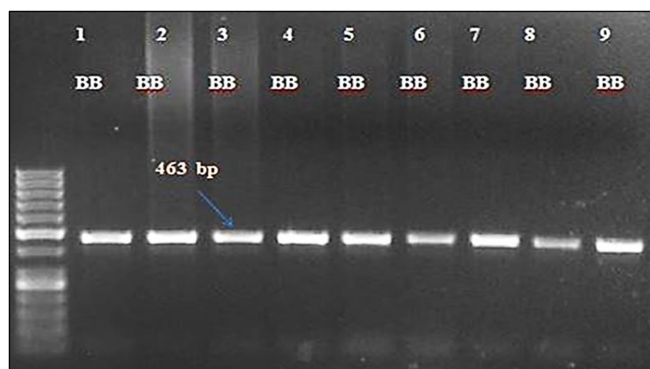

Fig 3: PCR-RFLP genotypes of Leptin gene using *Cail 1* RE in Harnali sheep

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