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NUCLEOTIDE VARIATIONS IN LEPTIN (*LEP*) GENE – A COMPARISON BETWEEN THE NILAGIRI, MERINO AND DORSET x NILAGIRI SHEEP

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Abstract: *LEP* gene is one of the potential genes that is involved intricately in the metabolism and growth of animals. The characterization of *LEP* gene as compared with the exotic breeds would help us understand the genetic diversity of indigenous breeds. There were five variations between the indigenous (Nilagiri), exotic (Merino) and one crossbred (Dorset x Nilagiri) sheep. In Intron 1 at 13774 bp a 'GTT' segment which was present in two copies in the reference sequence was found as a single copy in Nilagiri. In the intronic regions that were amplified along with Exon 2, the transitions 13893 T>C (SNP-L1) in Intron 1, 14074 A>G (SNP-L2) and 14090 G>A (SNP-L3) in Intron 2 were identified. For SNP-L1, Nilagiri and Dorset x Nilagiri had only CC genotypes while Merino had both *T* and *C* alleles. The SNP-L3 was found in Merino and Dorset x Nilagiri breeds while Nilagiri breed did not vary from the reference sequence. Analysis of the sequenced regions from the amplified region of Exon 3 revealed one variation 16973 G>A (SNP-L4) which was fixed with A allele in all the breeds studied here as compared to G in the reference sequence.
Keywords: Sheep, Leptin gene, indigenous breeds, Merino, SNPs.

Introduction

Sheep make a valuable contribution to the livelihood of economically weaker sections of the society and sheep with its utility for meat, wool, skin and manure form an important component of rural economy. As per the 19th Livestock Census (2012), the sheep population in India is 65.06 million and India has a rich diversity of sheep genetic resources with 42 recognised breeds of sheep. The demand for meat is ever growing and the mutton production of the state is 15 thousand tonnes which is five per cent of the total mutton production of the country (Report, 2013). Leptin, a 16-kDa protein is synthesized by adipose tissue and involved in regulation of feed intake, energy balance, fertility and immune functions and has major impact on performance and well being of livestock species. To identify genetic marker for growth *LEP* gene is gaining much importance and has the potential to act as candidate gene for growth in sheep. The Nilagiri sheep, native to the Nilgiris of Tamil Nadu is said to have evolved during 19th century and contains unknown levels of inheritance of Coimbatore,

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Tasmanian Merino, Cheviot and South Down breeds of sheep (Rao *et al.,* 1960). Comparison of Nilagiri with purebred Merino and Nilagiri x Dorset crossbred would reveal the variations in these three groups.

Materials and Methods

Blood samples were collected from Nilagiri, Merino and Dorset x Nilagiri sheep (10 nos. each) and DNA was isolated. Five primers to amplify the entire *LEP* gene based on the sequence from NCBI (Accession No. NC_019461 and Gene ID 443534). After standardisation of annealing temperature PCR was performed the product was sent for sequencing. Sequence data were analysed using the SeqMan program of LASERGENE software (DNASTAR Inc., USA) and comparisons made.

Results and Discussion

There were five variations between these three genetic groups. In Intron 1 at 13774 bp a 'GTT' segment which was present in two copies in the reference sequence was found as a single copy in Nilagiri. In the intronic regions that were amplified along with Exon 2, the transitions 13893 T>C (SNP-L1) in Intron 1, 14074 A>G (SNP-L2) and 14090 G>A (SNP-L3) in Intron 2 were identified. For SNP-L1, the Nilagiri and Dorset x Nilagiri had only CC genotypes while Merino had both *T* and *C* alleles. The SNP-L3 was found in Merino and Dorset x Nilagiri breeds while Nilagiri did not vary from the reference sequence. Analysis of the sequenced regions from the amplified region of Exon 3 revealed one variation 16973 G>A (SNP-L4) which was fixed with A allele in all the breeds studied here as compared to G in the reference sequence.

The SNP-L2 has been reported in other exotic breeds with conflicting reports on the effect on feed intake, efficiency and growth. In an earlier attempt by Li *et al*. (2008) to detect the SNPs of Exon 2 using PCR-SSCP protocol in 358 sheep including Poll Dorset, Suffolk, Texel and Tan sheep, no polymorphism was found in Exon 2 region in any of the four breeds but four SNPs were detected in parts of Intron 2. Partial characterisation of Exon 2 of *LEP* gene have been done in Dorset and Suffolk breeds (Boucher *et al*., 2006) and Shal, Zandi and Zel breeds (Barzhekar *et al*., 2009) but no polymorphism was detected by them in this region. This mutation had been reported earlier in Dorset and Suffolk sheep breeds by Boucher *et al*. (2006) in which the frequency of the *A* allele which they named as wild type (WT) was 0.87 and 0.93 respectively.

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Table 1. Variations identified in the Exon 2 fragment of *LEP* gene compared with the exotic breed

Locus (Position in bp)	Reference	Merino	Dorset x Nilagiri	Nilagiri
13774	GTT (2 copies)	2 copies	one copy (62 %) two copies (38 %)	one copy
13893 T>C (SNP-L1)	T	TT/TC/CC	C	C
14013 C>T (SNP-L2)	C	-	-	All CC
14074 A>G (SNP-L3)	A	AA/AG/GG	AA/AG	A
14090 G>A (SNP-L4)	G	GG/GA/AA	G	G

Figure 1. Chromatogram showing one copy of 'GTT' in Nilagiri and two copies in Merino breed of sheep

Merino

Nilagiri