STUDY OF LEPTIN GENE POLYMORPHISM IN MEHSANA BUFFALOES (*Bubalus bubalis*)

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ABSTRACT

Leptin is a hormone which is mainly produced in adipose tissue and secreted in to blood stream as a 16 kDa protein. It plays a key role in the regulation of food intake, energy expenditure, fertility and immune functions. Leptin binds to a receptor mainly localized on neuropeptide Y-neurons, which results in reduction of feed intake and increase of energy expenditure. Neuropeptide Y is also involved in the control of reproductive functions. This study was aimed to reveal PCR-RFLP pattern of the leptin (exon 2) locus in the Mehsana breed of buffaloes. Fragments of leptin 331 bp and OLR1-288bp were amplified by PCR, and subsequently RFLP study was carried out to identify genotypes of the animals with *HphI* restriction enzyme. Further representative samples were cloned and in vector, and after sequencing, sequence variation in nucleotide sequences of leptin was analysed.

Keywords: PCR-RFLP, OLR1 gene, leptin gene, polymorphism, *Bubalus bubalis*

INTRODUCTION

Buffaloes constitute 35% percent of the total bovine population in India, but they contribute more than 55% to the total milk production. That's why Indian buffaloes have a major contribution to making India one of the higher milk producing countries in the world (Kumar *et al.,* 2007). The Mehsana is one of the best milch breeds of buffalo in India and is found in the northern part of the Gujarat State (Gupta, 1997). Current technologies enable scientists to improve the accuracy and efficiency of traditional selection by applying genetic markers. This can be done through MAS and analysis of candidate genes. The candidate gene approach consists of the study of different genes potentially involved in a physiological process (e.g., milk protein synthesis, milk fat synthesis) and identification of the allele responsible for a desired phenotype. The leptin gene is considered as a potential QTL, influencing milk performance and reproduction traits in cattle (Buchanan *et al.,* 2002).

Leptin is a hormone which is mainly produced in adipose tissue and secreted into the blood stream as a 16 kDa protein. It plays a key role in the regulation of food intake, energy expenditure, fertility and immune functions (Fruhbeck, 2001). Leptin binds to a receptor mainly localized on neuropeptide Y- neurons, which results in reduction of feed intake and increase of energy expenditure. Neuropeptide Y is also involved in the control of reproductive functions (Magni *et al.,* 2000). As very scanty information is available on leptin gene

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polymorphism in Mehsana buffaloes (*Bubalus bubalis*), the present study was under taken to reveal PCR-RFLP pattern of the leptin (exon 2) locus in the Mehasana breed of buffaloes.

MATERIALS AND METHODS

Buffalo population, sampling and DNA extraction

To analyze the status of leptin/*HphI* locus polymorphism, blood samples were collected randomly from 60 unrelated buffaloes of the Mehsana breed registered under progeny testing programme of the Dudhsagar Research and Development Association, Mehsana, Gujarat state. DNA was extracted using standard protocol by the phenol:chloroform extraction procedure (Sambrook *et al*., 1989).

Molecular genotyping

The primer pair reported by Hageman *et al*. (2000) **(F:** 5'GGGAAGGGCAGAAAG ATAG3' **R:**5'TGGCAGACTGTTGAGGATC3') was used to amplify the region corresponding to leptin exons 2 and the RFLP site The HphI site was targeted.

PCR was carried out in a final reaction volume of 25 µl. Amplification cycling conditions for leptin involved initial denaturation 94°C for 10 minutes, followed by 35 cycles at 94°C for 1 minute, 51°C for 45 seconds and 72°C for 1 minute with a final extension at 72°C for 10 minutes.

For the PCR-RFLP analysis, 10 µl of each PCR amplified product was digested with 5 units of the *Hph1* (GGTGA$(N)_8$↓) in a 30 µl total reaction and incubated in a water bath at 37°C for 16 h. The digestion products were separated by electrophoresis on a 2% agarose gel in 0.5% TBE buffer.

Cloning and sequencing

PCR products from a representative sample (331bp of Leptin exon 2) were purified and cloned in pTZ57R/T vector (InsT/Aclone™ kit). Ligated recombinant vector was transformed in competent *E.coli* (DH5-α) cells. Recombinant plasmids were extracted and used for cycle sequencing and subjected to automated DNA sequencing on ABI PRISM® 310 Genetic Analyzer (Applied Biosystems, USA). Sequencing was carried out using BigDye® Terminator v3.1 Cycle sequencing kit (Applied Biosystems, USA). Sequence data obtained was analyzed *in silico* by employing software tools viz. NCBI BLAST, SeqScape and ClustalW to access the genetic variation.

RESULTS AND DISCUSSION

On screening the Leptin/*HphI* in 60 Mahsana buffaloes, all the samples showed an identical restriction pattern with the absence of restriction site producing 331 bp fragment only (Figure 1). All the animals revealed only one genotype AA and monomorphic pattern indicating allele A fixed in Mehsana buffalo.

The molecular basis of Leptin/*HphI* polymorphism is mutation G>T in recognition site of *HphI* restriction enzyme. Recognition sequence of *HphI* is 5'-G **G** T G A$(N)_8$^-3' is found to be mutated to→5'-G **T** T G A $(N)_8$ -3' in the Mehsana buffalo.

The C to T transition results in RFLP by *Hph*I restriction enzymes generating a band pattern consisting of a single fragment of 331 bp (CC) (absence of site) and two fragments of 311 bp + 20 bp(TT) (a cut at nt position 311) for the wild and the mutant type alleles, respectively.

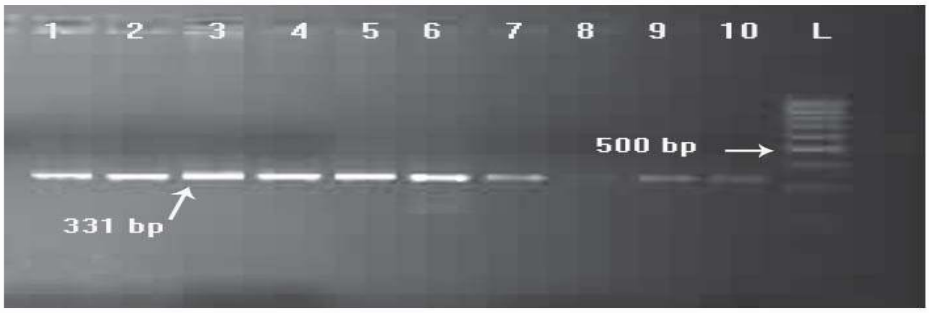

Figure 1. Leptin /HphI PCR –RFLP of leptin exon 2.

Lane 1-9 : Leptin/ HphI digest showing single fragment of 331 bp showing absence of site in the fragment.

Lane 10 : 331 bp PCR product.

Lane 11 : 100 bp.

Table 1. *Bubalus bubalis* Leptin Exon 2 - Blastn in GenBank + EMBL + DDBJ with *Bos taurus* and *Bubalus bubalis*.

Sr. No.	Accession	Description	Location/ Source	Max Indent
1	U50365.1	*Bos taurus* leptin (obese) gene, complete cds	(1-330) 2800-3130	97%
2	AJ512639.1	*Bos taurus* partial ob gene for leptin, exon 3	(1-330) 21-351	97%
3	AY731089.1	*Bos taurus* leptin (obese) gene, obese-A allele, exon 3 and partial cds	(3-330) 1-329	97%
4	AY534919.1	*Bos taurus* leptin (obese) gene, exon 3 and partial cds	(3-330) 1-329	97%
5	AF536174.1	*Bos taurus* leptin gene, exon 2 and partial cds	(239-330) 1-92	96%
6	Y11369.1	*Bos taurus* obese gene	(1-330) 1563-1893	97%
7	DQ831143.1	*Bubalus bubalis* breed Nili Ravi leptin gene, exon 3 and partial Cds	(3-330) 866-1193	98%
8	AY495587.1	*Bubalus bubalis* leptin gene, exons 2, 3 and complete cds	(3-330) 2898-3227	98%
9	DQ831142.1	*Bubalus bubalis* breed Surti leptin gene, exon 3 and partial cds	(3-330) 865-1192	98%
10	AY338973.2	*Bubalus bubalis* breed Murrah leptin (obese) gene, exon 3 and partial cds	(3-330) 1-328	98%

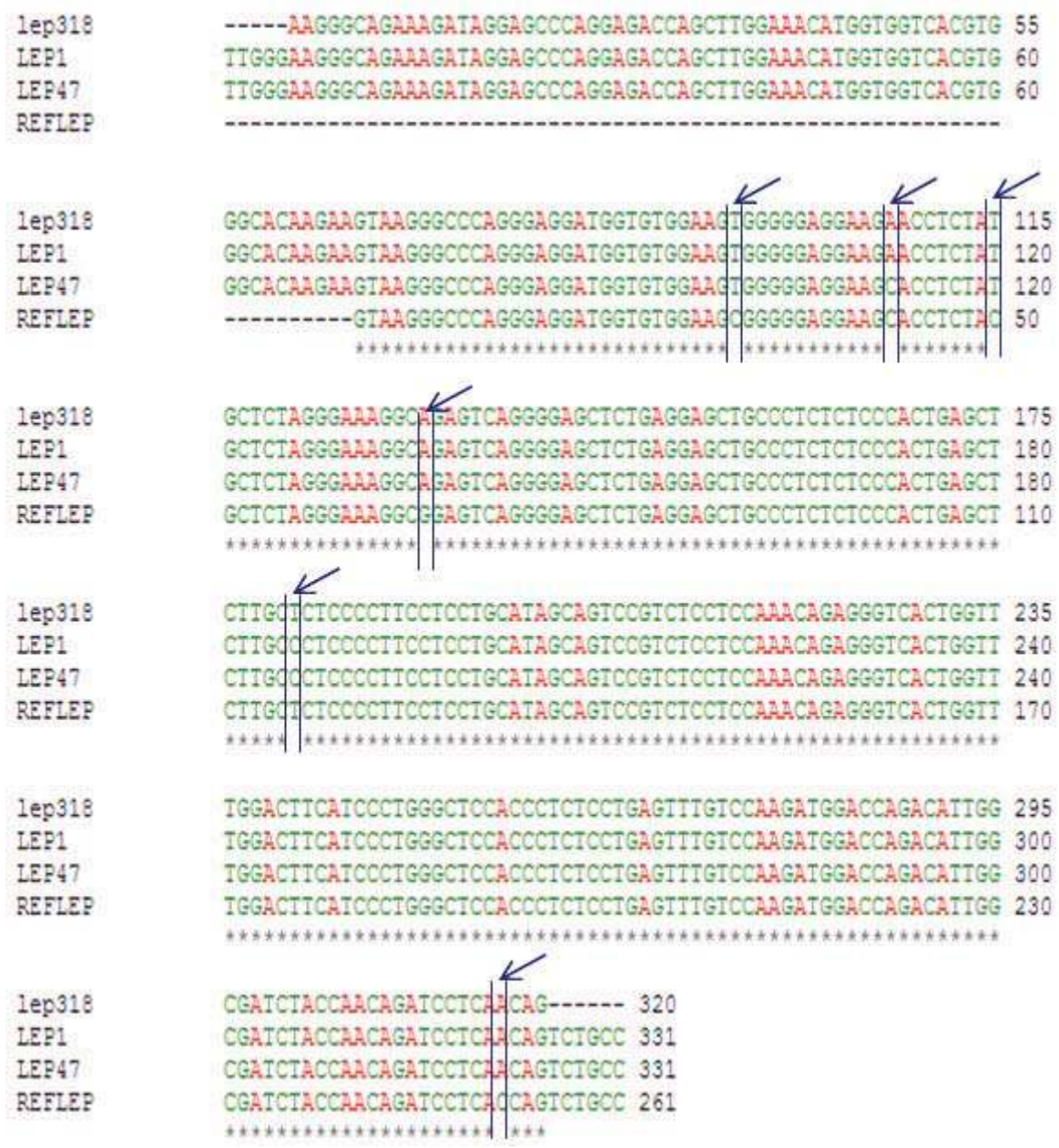

Figure 2. Clustal W results of Leptin exon 2 *Bos taurus*.

Hageman *et al*., (2000) reported allelic variation C>T and A>G transition in exon 2 causing an alanine (r) > valine and a glutamine(r) > arginine substitution.

As there is no polymorphism, association analysis with production traits is not warranted .

The results obtained for buffaloes suggest a species difference with respect to *HphI* polymorphism in the leptin gene.

The results of the present study are not accordance with previous reports in cattle. Buchanan *et al*. (2002) reported C to T transition in exon 2 of leptin that encodes an Arg25Cys substitution (position four of the secreted peptide) associated with body fat deposition in beef cattle. Buchanan *et al*.(2003) reported that this same genetic variant was also present in six dairy breeds, viz. Holstein, Ayrshire, Brown Swiss, Canadiene, Guernsey and Jersey, and compared lactation performance data using a mixed model. Animals homozygous for the T allele produced more milk (1.5 kg/d vs. CC animals) and had higher somatic cell count linear scores, without significantly affecting milk fat or protein percent over the entire lactation. The increase in milk yield was most prominent in the first 100 d of lactation (2.44 kg/d), declining to 1.74 kg/d between 101 and 200 d in lactation. They opined that the milk yield advantage, observed in cows homozygous for the T allele, could represent a major economic advantage to dairy producers. In Holstein animals allele frequency for T and C was 0.46 and 0.54 respectively.

A consensus sequence of 331 bp leptin exon 2 was obtained by assembling the forward and reverse compliment sequence using Seq Scape software programme. The consensus sequences were then aligned with known sequences for leptin exon 2 in GenBank using NCBI BLAST and ClustalW programmes. The nucleotide sequence alignment of leptin with published sequences revealed 96% to 99% homology (Table 1).

The nucleotide sequence variation between cattle and buffalo was observed at six nucleotide positions, i.e. 100,112,119,135,185,321 (Figure 2).

Leptin (exon 2) nucleotide sequences were submitted to Genbank of NCBI database, accession number GQ385228 .

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