IDENTIFICATION OF GENETIC POLYMORPHISM IN TWO EXONIC CODING REGION OF LEPTIN GENE AMONG INDIGENOUS AND CROSSBRED CATTLE

Umesh Singh*, Sushil Kumar, Indrajit Ganguly, G.K. Gaur, Jagadeesan K., Sunil Kumar, Sandeep Mann and Rani Singh

Molecular Genetics Laboratory,
Central Institute for Research on cattle, Meerut Cantt- 250 001, India

Received: 13-02-2013
Accepted: 13-09-2013

ABSTRACT

Leptin gene has its role in appetite, metabolism, growth and milk production in cattle. PCR-RFLP method was carried out to detect the polymorphism in leptin gene among Frieswal, Ongole and Sahiwal cattle. Frieswal population under study revealed that 11%, 51% and 38% are TT, CT, and CC respectively genotypes with respect to exon 2. But the same population had 22% TT, 51% CT and 27% CC genotype for first SNP in exon 3; 4% TT, 38% CT and 58% CC genotype for second SNP in exon 3. Ongole population revealed only CT genotype with respect to exon 2, but it showed TT genotype for first SNP and CC genotype for second SNP in exon 3. Sahiwal population had 4% TT, 88% CT and 8% CC genotype (exon 2), respectively. Whereas the same population revealed 4% TT, 55% CT and 41% CC genotype for first SNP in exon 3 and 4% CT and 96% CC genotype for second SNP in exon 3. Sahiwal population had no TT genotype for second SNP in exon 3.

Key words: Frieswal, Leptin gene, Ongole, PCR-RFLP, Polymorphism, Sahiwal cattle.

INTRODUCTION

Leptin is a 167 amino acid or 16 kDa polypeptide, which is synthesized predominantly in the adipose tissue involved in the growth and metabolism and plays a crucial role in the regulation of feed intake, energy balance, fertility, milk production, growth and immune functions (Lien et al., 1997; Blache et al., 2000; Liefers et al., 2002; Chilliard et al., 2005; Nkrumah et al., 2005) Nucleotide polymorphism is responsible for genetic variability of livestock population and their characterization which may be helpful to identify possible hybridization and ancient evolutionary linkage (Choudhary et al., 2005). Instead of using lengthy and expensive progeny-testing, molecular genetics techniques are quite cheaper and resulted in direct genotyping for candidate genes using polymerase chain reaction. The total length of leptin gene is 3897 bp in Bosindicusand 4067bp (Tellam, 2004) in Bos taurus cattle. The bovine leptin gene has been mapped to chromosome 4 (Stone et al., 1996; Pomp et al., 1997). It consists of three exons and two introns and only two exons are translated into the proteins (He et al., 1995). The objective of our present work is to distinguish the allelic variation of leptin gene coding exons (exon 2 & 3) among Frieswal cross (HF X Sahiwal) and indigenous breeds (Ongole and Sahiwal) of cattle.

MATERIALS AND METHODS

The present study was conducted on Frieswal, Ongole and Sahiwal animals to identify polymorphism in the coding region of leptin gene. Blood samples were collected and genomic DNA were isolated from blood using standard phenol chloroform extraction method. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was used to screen the DNA polymorphisms of the leptin gene. Two regions in exon 3 (317bp and 331bp) and one region in exon 2 (94bp) of leptin gene were amplified. Amplification of the desired leptin gene fragments was performed with published primer pairs (Table 1). PCR was performed using template of approximately 100 ng of genomic DNA in a final reaction volume of 25 µl containing 1X PCR buffer (Sigma Aldrich), 1.5 mM Mgcl2 (Sigma Aldrich), 200 µM dNTPs (Sigma

*Corresponding author’s e-mail: usinghas@gamil.com.
TABLE 1: Primer Sequences for three exons of Leptin gene with their lengths and product sizes.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer Sequence</th>
<th>Length</th>
<th>Product size</th>
<th>References</th>
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<tbody>
<tr>
<td>Exon 2</td>
<td>F 5' ATG GGC TGT GGA CCC CTG TAT C 3'</td>
<td>22</td>
<td>94 bp</td>
<td>Haegeman et al., 2000</td>
</tr>
<tr>
<td></td>
<td>R 5' TGG TGT CAT CCT GGA CCT TCC 3'</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon 3.1</td>
<td>F 5' CAA GAT GGA CCA GAC ATT CG 3'</td>
<td>20</td>
<td>317 bp</td>
<td>Buchanan et al., 2002</td>
</tr>
<tr>
<td></td>
<td>R 5' CTG GAC TTT GGG AAG AGA GG 3'</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon 3.2</td>
<td>F 5' GGG AAG GGC AGA AAG ATA G 3'</td>
<td>19</td>
<td>331 bp</td>
<td>Lagonigro et al., 2003</td>
</tr>
<tr>
<td></td>
<td>R 5' TGG CAG ACT GTT GAG GAT C 3'</td>
<td>19</td>
<td></td>
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Aldrich), 0.5 µM of each primer and 1 U Taq DNA polymerase (NEB). The primer sequences are mentioned in the table 1 for all three regions. Initial denaturation for 5 minutes at 94°C followed by 35 cycles of 94°C (30 s), variable annealing temperature (30 s), 72°C (30 s) and a final extension at 72°C for 10 min. Annealing temperature were 60 °C for exon 2, 58 °C for one region of exon 3 and 54 °C for another region of exon 3.

The PCR products were isolated and verified by preparative gel electrophoresis using a 1.5% agarose gel with ethidium bromide for 60 min and visualized under UV trans illuminator and Gel Documentation System (Alphalmager EP, USA). The restriction digestion was carried out in a final volume of 15 µl following manufacturer’s instructions. The PCR products for each sample was digested overnight at 37°C with 8 U of restriction enzyme BspEI (NEB) for exon 2 and 10 U of restriction enzymes i.e. NruI and HphI (NEB) for exon 3.1 and exon 3.2, respectively. The Digested products were separated in horizontal gel electrophoresis using 2.5% agarose gel. Digested fragments’ size were estimated by comparing them against DNA ladder (Low Molecular weight ladder for exon 2 and 2-log DNA ladder for both regions of exon 3). Gene (allele) and genotype frequencies were calculated as per Falconer and Mackey (1996).

RESULTS AND DISCUSSION

Three polymorphisms were identified in the bovine leptin gene in the present study. These results showed three genotypes in each of the region during study of leptin gene. In the exon 2, fig.1,three genotypes were observed in Frieswal crossbred heifers. Buchanan et al. (2002) described a cytosine (C) to thymine (T) substitution (C→T substitution) in exon 2 of the leptin gene of Bos taurus breeds suggesting the existence of C and T allele and therefore, it should be CC, TT and CT genotypes. This substitution encodes an amino acid change of an arginine to cystine in bovine leptin gene. It was found in our study, a 94 bp PCR product of exon 2 leptin gene, after restriction digestion with BspEI in 126 Frieswal heifers, three digestion patterns were identified indicating three genotypes: an intact 94 bp fragment as TT; 75 and 19 bp fragments as CC and 94, 75 & 19 bp fragment as CT genotypes. Konfortov et al., (1999) also reported this mutation in both B. taurus and its crossbreds with B. indicus. In BspEI-RFLP genotypes as mentioned in Table 2, a total of 14 TT, 63 CT and 49 CC animals were identified. The frequency of T allele was estimated at 0.36.
For the region 317 bp PCR fragment three digestion patterns with NruI were found in the leptin exon 3 region in Frieswal and Ongole breeds. Three genotypes were found as shown in fig.2; an intact 317 bp fragment as TT genotype, 297 and 20 bp as CC and 317, 297 and 20 bp as TC genotype. All of three genotypes were observed in Golpayegani, Najdi, Sarabi and Sistani by Aslaminejad et al., (2010). However Nassiry et al., (2008) in Golpayegani and Choudhary et al. (2005) in Hariana, Sahiwal, Gir and Nimari cattle did not detect TT genotypes. However they reported comparative high TT genotype frequencies (0.30) in Jersey cattle, supporting our study in which frequency of TT genotype is 0.22 in Frieswal breed having allele frequency of T allele (0.48) lower than C allele.

The 331 bp PCR products digested with HphI revealed uncut fragment of 331bp as CC genotype, cut fragments of 311 and 20 bp as TT genotype and 331, 311 & 20 bp fragments as CT genotype in animals of all three breeds under study shown in fig.3. The analyzed polymorphic sites are situated within the third exon i.e. C/T transition which resulted in the Alanine to Valine (A59V) change in the secreted protein.

Gene and genotype frequencies were calculated for the polymorphic study in all the three breeds (Table 2). The TT genotype frequency was found to be comparatively low in Frieswal crossbred. In Ongole and Sahiwal indigenous breeds, T and C allele frequency was near about same in the Exon 2 whereas in Exon3.2, T allele frequency was very low with respect to C allele frequency. Similar results were reported by Choudhary et al. (2005) in case of KpnI-RFLP of Lepin exon 2 in various crossbreds and Holstein Friesian cattle. The T allele frequency was comparatively low in crossbred cattle population. Our findings are similar to those of Choudhary et al. (2005), who reported C and T allele frequency as 0.82 and 0.18 respectively in crossbreds. Konfortov et al. (1999) also found that T allele frequency was 0.41 in Taurine cattle.

Statistical analysis by Kulig and Kmie (2009) has revealed that A59V polymorphism in Limousin cattle affected the body weight at 210 days of age (P
and the average daily gain between 3 and 210 days of age ($P \leq 0.01$) and the average daily gain between 3 and 210 days of age ($P \leq 0.05$) with T as a desirable allele. Similarly, Nkrumah et al. (2005) found that individuals with T allele in beef steers were characterized by a significantly higher daily gain compared to individuals homozygous for C allele. In another study of crossbred beef steers, Nkrumah et al. (2005) found association between R4C polymorphism and the rate of gain in ultrasound back fat. Hphl polymorphism had a significant effect on milk and protein yield (Madeja et al., 2004). Animals with TT genotype had approximately 2X higher estimated breeding values for milk and protein yield. (Dandapat et al., 2009) reported that leptin genotypes have significant effect on growth traits, first lactation milk yield, second lactation milk yield and average daily milk yield during first lactation and days in milk in first lactation. Hphl is a possible marker for milk production traits. The results suggested that if breeding programmes are based upon the A59V polymorphism, TT genotype might contribute to improve body weight in Frieswal cattle. However, further research on association between the leptin genotype and growth traits in crossbred cattle are necessary before using the results in selection programmes. For finding the evolutionary relationships, leptin is a suitable and informative marker system. The data generated for Frieswal cattle in this study may be utilized for characterizing the possible genetic relationships of cattle among Frieswal and other countries breeds. As per our knowledge this is the first study of polymorphism in exon 2 and exon 3 of leptin gene in Frieswal crossbred, Ongole and Sahiwal indigenous cattle breeds.

**ACKNOWLEDGEMENT**

The authors are thankful to the Director, CIRC, Meerut for providing the necessary facilities to carry out this work. The authors are also thankful to the Director, Frieswal Project and Officer Incharge, Military Farm, Meerut for giving permission to collect the blood samples.
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