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Genetic Polymorphism at the Leptin Gene in Iranian Holstein Cattle by PCR-RFLP

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Abstract: Leptin is a protein involved intricately in the growth and metabolism of animals and which plays an important role in the regulation of feed intake, energy metabolism, growth and reproduction of cattle. We used the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique to screen for DNA polymorphisms of the leptin gene in 112 Iranian Holstein cattle (*Bos taurus*) in Karaj Animal Breeding Center. PCR was carried out between exon 2 (intron 2). A strategy employing polymerase chain reaction was used to amplify a 422 bp from semen DNA. Digestion of polymerase chain reaction products with Sau3AI revealed two alleles: allele A was 390, 32 fragments and allele B was 303, 88, 32 (only 303 fragment visible on the gel). Three patterns were observed and frequencies were 60.71, 37.5 and 1.79% for AA, AB and BB, respectively. This polymorphism could be further evaluated for marker-assisted selection and developed PCR methodology would expedite screening for large numbers of animals required for such studies.

Key words: Leptin, poly morphism, RFLP, DNA, chain reaction, Iran

INTRODUCTION

Variations at DNA level contribute to the genetic characterization of livestock populations and this may help to identify possible hybridization events as well as past evolutionary trends (Choudhary *et al*., 2005). Until recently, directly selection of bulls for specific alleles has been limited, mainly because of the lengthy and costly progeny-testing procedures required. However, molecular genetics techniques are currently available that allow direct genotyping for candidate genes using PCR (Houseknecht *et al*., 1998; Matarese *et al*., 2002). Leptin is a 16 kDa polypeptide hormone which is synthesized predominantly in the adipose tissue and affects a number of processes in the body. It is involved in maintaining the energy equilibrium by controlling food intake and energy expenditure as well as in regulating reproductive functions and immune response (Kulig and Kmiec, 2009). In cattle, the leptin gene is located on chromosome 4. It consists of three exons and two introns of this gene. Only two exons are translated into the protein. The coding region of the leptin gene (501 nucleotides in length) is contained in exons 2 and 3 which are separated by an intron of approximately 2 kb. The leptin gene promoter region spans approximately 3 kb (Zadworney and Kuhnlein, 1990). Leptin treatment of animals has been shown to cause a decrease in feed intake, body weight loss, fat deposit, weight loss and increase in energy metabolism. Therefore, leptin not only causes reduced feed intake but also the potential body weight losses are enhanced due to an increased metabolic rate (Lindersoon *et al*., 1998). The aim of this study was to investigate the frequency of leptin gene polymorphism in Iranian Holstein bulls breed.

MATERIALS AND METHODS

Semen samples were randomly collected from 112 Iranian Holstein cattle (*Bos taurus*) in Karaj Animal Breeding Center. Genomic DNA was isolated from semen samples with DNP™ Kit (CinnaGen, Iran) according to the producer's instructions. Genotype analyses were performed using the PCR-RFLP method. Amplified region is located in the intron between two exons of leptin. The genomic bovine leptin sequences which consist of three exons was obtained from GeneBank (accession No. U50365). Sequence of primers and PCR reaction described by Liefers *et al*. (2002). Forward (5'-TGGAGTGGCTTGTTATTTTCTTCT-3') and reverse(5'GTCCCCGCTTCTGGCTACCTAACT-3')primers were used in the amplification. The PCR reaction mixture (total of 25.0 μL) contained 50 ng of genomic DNA, 2.5 μL of 10× PCR buffer, 1.5 μL of $MgCl_2$ (2.5 mmol L^{-1}), 5 pmol μL^{-1} of each primer (2.5 mmol L^{-1}), 2.5 μL of

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dNTP (2 mmol L^{-1}), 0.125 μL of Taq DNA polymerase (5 U μL^{-1}). PCR conditions were at 95°C for 5 min followed by 30 cycles of 94°C for 30 sec, 62°C for 40 sec and 72°C for 40 sec. After 30 cycles, reactions were completed by an extension at 72°C for 7 min.

The PCR product for each sample was digested with 10 units of Sau3AI at 37°C overnight. One RFLP in the intron between two exons of the bovine leptin gene was detected. There were two sau3AI sites in 422 bp fragments. The digested AA PCR product exhibited two fragments of 390 and 32 bp. For the BB genotype exhibited 303, 88 and 32 bp (only 303 bp fragment was visible).

RESULTS AND DISCUSSION

Presence of A and B alleles of the leptin gene for Iranian Holstein bulls was observed in this study. Figure 1 shows the restriction patterns of the three genotypes AA, AB and BB for 112 Iranian holstein bulls (*Bos taurus*). The Result shows that, the most frequent genotype for leptin gene was AA. So that the frequency of the A allele was 79.75 and for B allele was 20.25 in Iranian Holstein bulls. The number of individuals with different genotypes and allele frequencies for this polymorphism of leptin gene in Holstein bulls are shown in Table 1.

Genetic characterization to assess the existing biodiversity and differences among the important cattle breeds is an essential prerequisite to facilitate the conservation program in an effective and meaningful way. More recently, an array of new markers has been developed to carry out the genetic variation studies at DNA level (Nassiry *et al*., 2008).

It is now generally accepted that leptin may be a strong candidate gene for economically important production traits such as back fat thickness, feed intake and reproduction function. Recent evidence suggests new key roles for leptin during pregnancy.

Lagonigro *et al*. (2003) suggested an association between leptin and feed intake. Jiang and John (1999) analyzed genetic polymorphisms in the leptin gene and their association with fatness in four pig breeds, polymorphic locus of 3,649 bp in the leptin gene was possibly related with back fat thickness of pig. Xi *et al*. (2000) showed that the RFLP band types were significantly different in fat-type and lean-type pigs. There was a 4.3 kb band in all lean-type pigs and a 3.5 kb band in all fat-type pigs (Xi *et al*., 2000). Liefers *et al*., 2002) reported that heifers with the Sau3AI-AB genotype produce 1.32 kg day^{-1} more milk and consume

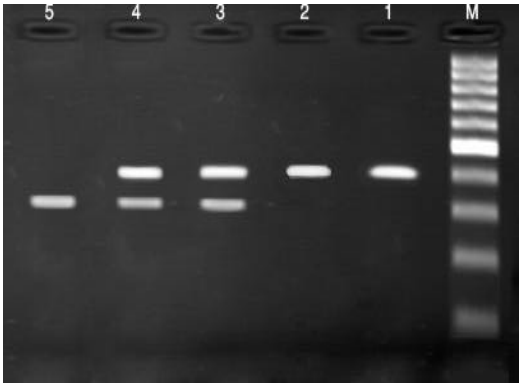

Fig. 1: Genotyping for Bovine leptin gene. Lane M is a marker of molecular weight (GeneRuler, 100 bp). Lane 1 and 2 are AA genotype (390 and 32 bp size), Lane 3 and 4 are AB genotype (390, 303, 88 and 32 bp size) and Lane 5 is BB genotype (303, 88 and 32 bp size)

Table 1: Genotypes and allele frequency of bovine leptin gene

Characteriazation	Frequency (%)
Genotype	
AA (n = 68)	60.71
AB (n = 42)	37.50
BB (n = 2)	1.79
Allele	
A	79.75
B	20.25

0.73 kg day^{-1} more food compared with the Sau3AI-AA genotype. In one study in China association of polymorphisms of leptin gene with body weight and body sizes indexes were evaluated. The results of that study showed that the allele B might be associated with better growth traits.

The association of the leptin polymorphism with growth traits of Chinese indigenous cattle in this study suggests its feasibility as a molecular breeding marker and cows with genotype BB had remarkable growth and some of them which had better performance could be used for the breeding of new breeds of beef cattle (Yang *et al*., 2007).

CONCLUSION

The result of the study show that the survey of B allele frequencies (Desirable allele) in Karaj animal breeding center and comparison with other strains in same studies (Javanmard *et al*., 2008) indicated that Karaj animal breeding bull station were smaller than native bulls station. Other related projects will require to evaluate relationships between this polymorphism with milk production and reproduction and feed intakes traits.

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