African Journal of Biotechnology Vol. 7 (15), pp. 2685-2687, 4 August, 2008
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB07.782
ISSN 1684–5315 © 2008 Academic Journals

Short Communication

The diversity of *leptin* gene in Iranian native, Holstein and Brown Swiss cattle

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Accepted 16 February, 2008

This study describes genetic variability in the *leptin* in Iranian native, Brown Swiss and Holstein cattle (*Bos Indicus and Bos Taurus*). This is the first study of genetic polymorphism of the *leptin* gene in Iranian native cattle. We examined exon 2 of the leptin gene from 587 individuals in six different populations of Iranian native cattles (86 Sarabi, 66 Taleshi, 94 Sistani, 76 Golpayegani, 104 Brown Swiss and 161 Holstein cattle) using PCR-RFLP method. Analysis of the frequencies of the various alleles in each breed indicated that allele *C* in Sarabi, Taleshi, Sistani, Golpayegani, Brown Swiss and Holstein cattle with 68, 55, 69, 71, 55 and 57% value were the most frequent alleles. Observed heterozygosities were highest in Golpayegani (57.89%). These new data suggest that allele frequencies of leptin differ between the various Iranian cattle breeds.

Key words: *Leptin*, PCR-RFLP, Iranian native cattle.

INTRODUCTION

Genetic characterization to assess the existing bio-diversity 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 (Bradley et al., 1996; MacHugh et al., 1998). Among these, one of the candidate genes for marker assistant selection is leptin. Leptin is a 16-kDa protein that is synthesized by adipose tissue and is involved in regulation of feed intake, energy balance, fertility and immune functions (Fruhbeck et al., 1998). Plasma leptin levels in cattle and sheep increase linearly with increased body fat mass and with increased energy balance (Blache et al., 2000; Ehrhardt et al., 2000). Leptin gene expressed in a variety of tissues including adipose tissue, placenta, mammary glands, skeletal muscles, gastric mucosa, brain and pituitary glands. It seems that leptin has a large effect in coordinating whole body energy metabolism and may be classified as a "metabolism modifier" (Houseknecht et al., 1998). It has been shown that leptin gene influences milk performance in cattle (Liefers et al., 2002) and reproduction in beef cattle (Almeida et al., 2003). In exon 2, three RFLP were described: *Cla* I (an A/T substitution resulting in an amino acid change from tyrosine to phenylalanine) (Lagonigro et al., 2003), *Kpn* 2I (*Bsp*13I) (a C/T substitution resulting in an amino acid change from arginine to cysteine) (Buchanan et al., 2002) and *Sau* 3AI (Pomp et al., 1997). The cysteine/arginine change in exon 2 (*Bsp*13I) is a non-conserved substitution and is more likely to alter the functioning of the leptin hormone (Buchanan et al., 2002). Variability within and among populations of a certain species is directly related to the interplay between effective population size, time of divergence and the intensity of selective pressure at a particular locus.

Results indicate that the leptin TT genotype is associated with increased milk and protein yield, without changing yield of milk fat. That is, all dairy breeds tested showed the presence of the favorable T allele (Buchanan et al., 2002).

The present study, using PCR-RFLP method, was designed to determine the allelic frequencies of exon 2 of the *Leptin* gene of a total of 587 individuals belonging to six distinct of cattle, namely, Sarabi, Taleshi, Sistani, Golpayegani, Brown Swiss and Holstein. These Iranian

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Table 1. Frequencies and hetrozygosities of *Leptin* alleles for Iranian cattle breeds.

Leptin alleles	Sarabi (N=86)	Taleshi (N=66)	Sistani (N=94)	Golpayegani (N=76)	Holstein (N=161)	Brown Swiss (N=104)
Allele C	0.68	0.55	0.69	0.71	0.57	0.55
Allele T	0.32	0.45	0.31	0.29	0.43	0.45
$H^{observed}$	50.57	36.36	44.68	57.89	52.80	51.5
$H^{expected}$	43.90	49.97	42.89	41.41	49.22	48.5

N = Number of individuals; H = Hetrozygosity rate.

breeds are found at distinct geographical areas in Iran; Golpayegani (Golpayegan region, Esfahan state, center of Iran), Taleshi (Talesh city, Guilan province, north of Iran), Sarabi (Eastern Azerbaijan state, Northwest of Iran), Sistani (South east of Iran), Holstein and Brown Swiss (North east of Iran: Moghan farm and Razavi farm, respectively).

MATERIALS AND METHODS

Animals and DNA extraction

In total 587 individuals, obtained from six different breeds, were examined for the distribution of *Leptin* alleles including 86 Sarabi cattle, 66 Taleshi cattle, 94 Siatani, 104 Brown Swiss, 161 Holstein cattle and 76 Golpayegani cattle. Blood samples were collected in 0.5% EDTA and DNA was extracted from 100 µl of blood according to Boom et al. (1989) method.

Amplification of *Leptin* exon 2

1 µl of DNA was amplified in a total volume of 25 µl PCR mix using the Biometra T Personal Ver: 1.11 thermocycler. The PCR mix contained: 2.5 µl PCR buffer 10-X (200 mM $(NH_4)_2SO_4$, 0.1 mM Tween 20%, 750 mM Tris-HCl pH = 8.8), 2.5 mM $MgCl_2$, 200 µM dNTPs, and 10 pM from each primer, 1 U *Taq* DNA polymerase and 11 µl ddH2O. The thermal cycling profile consist of an initial denaturation step of 5 min at 94°C, followed by 35 cycles 45 s at 94°C, 45 s at 58°C, 45 s at 72°C and final extension step of 10 min at 72°C. A 94 bp fragment from exon II of the bovine leptin gene was amplified using the following primers; LeptF: 5'-ATGCGCTGTGGACCCCTGTATC-3' and LeptR: 5'-TGGTGTCATCCTGGACCTTCC-3'. Products of amplification were recognized by electrophoresis on 1.5% agarose gel stained with etithium bromide.

RFLP typing

5 µl of PCR products were incubated for 5 h at 50°C with three units of *Bsp* 13I restriction enzyme. The C allele was cleaved into two fragments of 75 and 19 bp, while the T allele remained uncut at 94 bp (Buchanan et al., 2002). Restriction fragments were revealed by gel electrophoresis on 8% acrylamide gel and visualized with silver staining.

Statistical analysis

Allele frequencies (*f*) were obtained by using Arlequine ver. 2.000 (Schneider et al., 2000). The observed frequencies of heterozygotes ($H^{observed}$), were obtained directly by diving the number of heterozygous individuals by the total number of individuals. The expected frequencies of heterozygote ($H^{expected}$) were calculated by using Arlequine ver. 2.000 (Schneider et al., 2000). The observed heterozygosity (H^{obs}) and expected heterozygosity (H^{exp}) were calculated with the same program.

RESULTS AND DISCUSSION

Distributions of alleles of Leptin in the groups of studied animals are presented in Table 1. The spectrum of the most frequent alleles was different in six breeds. The genotypes of 587 individuals from six different Iranian populations of cattle were determined for exon 2 of the *Leptin* allele by PCR-RFLP typing (Table 1). Observed frequency of heterozygotes and expected frequency of hemozygotes were computed to assess genetic variability in the six Iranian native cattle breeds. The values are shown in Table 1. Observed heterozygotes were the highest in Golpayegani (57.89%).

To avoid further loss of important gene/gene-pool and preserve maximum amount of genetic diversity, an objective breed classification based on genetic uniqueness is of priority (Hall and Bradely, 1995). In cattle, analysis of allelic variation at *Leptin* loci could potentially be used to evaluate temporal changes in genetic diversity. There are no studies of *Leptin* (Exon II) genotyping in the Iranian important cattle breeds. Our results showed three genotypes (CC, CT and TT) for leptin exon II gene. The CC and CT genotypes were observed in six breeds whereas the TT genotype was observed only in the Taleshi, Sistani, and Holstein cattle breeds. Similar results were reported by Choudhary et al. (2005), who did not detect TT genotype in the Hariana, Sahiwal, Gir and Nimari cattle breeds, but reported comparatively high TT genotype frequency (0.30) in Jersey cattle.

For the allelic frequencies, the frequency of T allele was lower than C allele in six cattle breeds (Table 1). Our results agree with Buchanan et al. (2002) who had reported C allele frequencies of 0.66 in Charolias cattle and 0.68 in Simmental cattle and Madeja et al. (2004) who found frequency of 0.66 in Polish Black and White cattle. Buchanan et al. (2003) reported 0.55 for C allele frequency in Brown Swiss cattle. This result provides evidence that breeds of *B. Taurus* and *B. Indicus* can be

clearly differentiated. The Golpayegani showed the maximum mean observed heterozygosity while the Taleshi population showed the minimum H^{obs}. The heterozygote deficiency observed in the Taleshi might be explained by inbreeding due to small number of reproducers and genetic drift.

For finding the evolutionary relationships among closely populations, *Leptin* is a suitable and informative marker system. The diversity data generated for Iranian native cattle may be utilized for characterizing the genetic relationships with *B. indicus* and *B. Taurus* from other countries as well. This study is the first using *Leptin* polymorphism to understand genetic diversity of native cattle breeds in Iran. Very little information is currently available to compare different cattle populations from Iran. Although we have used only six breeds, the present study may be regarded as the beginning of attempts to understand the genetic diversity of local cattle breeds in Iran. Further investigations including more native Iranian cattle breeds would be useful to clarify their recent origin and relationships between them. In addition, our analysis showed that breeds can be differentiated using *Leptin* variability.

ACKNOWLEDGEMENT

This investigation was supported by grants from Agricultural College, Ferdowsi University of Mashhad, Iran.

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