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Polymorphism in Exon 3 of Leptin Gene in Iranian Native Cattle Breeds

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Abstract

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*This study describes genetic variability of the leptin gene in four Iranian native cattle breeds. A 317 bp fragment of exon 3 of the leptin gene from 332 individuals in four different populations of Iranian cows including Golpayegani (**N**=92), Najdi (**N**=54), Sarabi (**N**=82) and Sistani (**N**=104) was amplified and PCR products digested with **NruI** restriction enzyme. Allele C in Golpayegani, Najdi, Sarabi and Sistani breeds were 75, 78, 59 and 52 per cent, respectively. Observed heterozygosities were 0.29, 0.37, 0.68 and 0.92 for Golpayegani, Najdi, Sarabi and Sistani cattle, respectively. This study suggests that allele frequencies of leptin differed among Iranian cattle breeds.*

Key words: Polymorphism, leptin, exon 3, PCR-RFLP, Iranian native cattle.

Introduction

An array of new markers has been developed to carry out the genetic variation studies at DNA level. Among these, one of the candidate genes for marker assisted selection (MAS) is leptin (Fruhbeck *et al.,* 1998). Leptin is a 16-kDa protein that circulates in the serum in free and bound forms and functions as a lipostatic signal (Geary *et al.,* 2003). In farm animals, control and prediction of fatness is of a high economic interest. The exaggerated adipose tissue development in farm animals negatively affects whole body metabolism and meat quality (Taouis *et al.,* 2001). Leptin polymorphisms have significant effect on alterations in energy balance, milk production, fertility traits and live weight (Buchanan *et al.,* 2002; Lagonigro *et al.,* 2003; Almeida *et al.,* 2003; Nkrumah *et al.,* 2005). 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 (Vivek *et al.,* 2005). In ruminants, leptin receptor expression seems to be affected by high and low nutrition levels (Chilliard *et al.,* 2005) and blood leptin concentrations seem to interfere in luteinizing hormone secretion (Kadokawa *et al.,* 2006) and to stimulate growth hormone release (Nonaka *et al.,* 2006).

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In cattle, the leptin gene is located on Chr 4 (Stone *et al*., 1996) and consists of three exons. The last two exons contain the coding sequence and are separated from the promoter and first exon by a large intron of 14 kb (He *et al*., 1995; Gong *et al*., 1996).

There are few articles on exon 3 polymorphism of leptin gene and no study has been carried out in Iranian ative cattle breeds. The objective of this study was to investigate genetic variations in the exon 3 of leptin gene in Iranian native cows.

Materials and Methods

A total of 332 individuals, obtained from four different breeds, including Golpayegani (*N*=92), Najdi (*N*=54), Sarabi (*N*=82) and Sistani (*N*=104) cows, were examined for the distribution of leptin alleles. Blood samples were collected in 0.5% EDTA and DNA was extracted from 100 µl of blood according to guanidium thiocyanate-silica gel method (Boom *et al*., 1990). Quality and quantity of DNA were measured by spectrophotometer by taking the optical density at wavelength of 260 and 280 nm, respectively.

One 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 10X [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 double distilled water. A 317 bp fragment from exon 3 of the bovine leptin gene was amplified, using the primers described by Lagonigro *et al*. (2003).

For RFLP analysis, 5 µl of PCR products were digested with 5 U of *Nru*I (New England Biolabs, Hitchin, UK) at 37C for at least 4 h and final incubation for 30 min. The digested DNA fragments were separated by electrophoresis in 8% non denatured polyacrylamide gel and visualized with silver staining. These produced an undigested fragment of 317 bp for the T allele and two fragments of 297 bp and 20 bp for the C allele (Lagonigro *et al*., 2003).

The frequencies of genotypes, alleles, mean expected and observed heterozygosities and χ^2 test were calculated using PopGene32 software (ver. 1.31).

Results and Discussion

The spectrums of the most frequent alleles were different in four breeds (Fig. 1). Observed heterozygotes were highest in Sistani (92%) breed (Table 1). 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. In cattle, analysis of allelic variation at leptin loci could potentially be used to evaluate temporal changes in genetic diversity.

These results showed three genotypes (CC, CT and TT) for exon 3 of gene leptin. All of three genotypes were observed in Golpayegani, Najdi, Sarabi and Sistani. However, Nassiry *et al*. (2008) reported only CC and CT genotypes in exon 2 for Golpayegani and Sarabi. Also Choudhary *et al*. (2005) did not detect TT genotype in the Hariana, Sahiwal, Gir and Nimari cattle breeds. However, they reported comparatively high TT genotype frequency (0.30) in Jersey cattle. For the allelic frequencies, the frequency of T allele

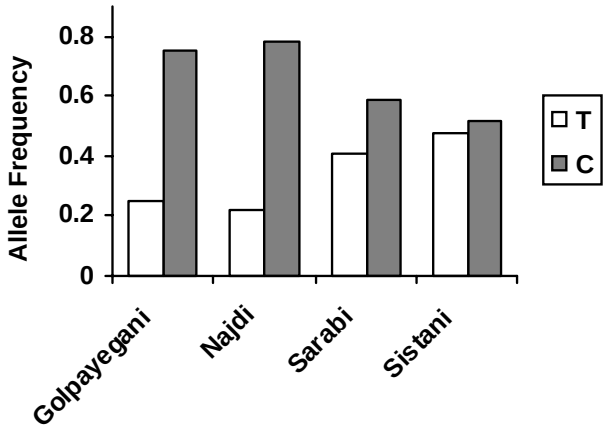

Fig. 1. Allelic frequencies of the leptin exon 3 for four Iranian native cattle Breeds

Table 1
Observed, expected, average heterozygosities and χ^2 test and genotype frequencies of leptin exon 3 for the Iranian native cattle breeds

Breed	Observed hetrozygosity	Epected hetrozygosity	Average hetrozygosity	χ^2	Genotype frequencies		
					TT	TC	CC
Golpayegani	0.293	0.371	0.37	4.137*	0.1	0.3	0.6
Najdi	0.37	0.35	0.35	0.212^{ns}	0.04	0.37	0.59
Sarabi	0.68	0.49	0.48	13.17**	0.07	0.69	0.24
Sistani	0.92	0.50	0.50	74**	0.02	0.92	0.06

*significant ($p<0.05$); **significant ($p<0.01$).

was lower than C allele in all of four cattle breeds (Fig. 1).

The heterozygote deficiency observed in the Golpayegani and Najdi might be explained by inbreeding due to small number of reproducers for these breeds and possibly the genetic drift. For finding the evolutionary relationships among closed and inbred populations, leptin is a suitable and informative marker system. The diversity data generated for Iranian native cattle in this study may be utilized for characterizing the possible genetic relationships of cattle among Iranian and other countries breeds. This is the first study of polymorphism in exon 3 of leptin gene in Iranian native cattle breeds.

Very little information is currently available to compare different cattle populations in Iran. Further investigations including more Iranian native cattle breeds would be useful to clarify their origin and any possible relationships among them.

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