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A missense mutation in the bovine leptin gene in Iranian Taleshi cattle

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Introduction Candidate gene approaches facilitate discovering and localizing causative genes for quantitative traits and polymorphisms within selected candidate genes can be tested for association with variationin the quantitative trait (Campbell et al., 2003). Leptin is a 16-kDa protein that is synthesized and secreted predominantly by white adipocytes tissue and its expression is regulated by body fatness and energy balance. Leptin gene expressed in a variety of tissues including adipose tissue, placenta, mammary glands, skeletal muscles, gastric mucosa, brain and pituitary glands. In cattle, the leptin gene is located on chromosome 4 and consists of three exons .Several studies shown the association between polymorphism at the leptin gene and feed intake, energy balance, fertility and immune functions. It has been shown that leptin gene influences milk performance in cattle and reproduction in beef cattle(Liefers et al., 2002). Buchanan et al. (2002) described a cytosine (C) to thymine (T) substitution in exon 2 of the leptin gene of the *B. taurus* breeds, suggesting the existence CC, TT and CT genotypes. The aim of this study was to identify the single nucleotide polymorphism of exon 2 in Iranian Taleshi cattle.

Materials and methods Blood samples were collected from 64 Taleshi cattle from Talesh city in Guilan province, Iran. DNA was extracted from 100 micro litters whole blood by Guanidinium Thiucianate-silica gel method. Quality and quantity of extracted DNA were measured by Biometra UV photometer. One µl DNA (50 ng) was amplified in a total volume of 20 µl PCR mix using the Biometra T Personal thermocycler Ver: 1.11. The PCR mixture contained: 2.5 µl PCR buffer 10-X (200 mM $(NH_4)_2SO_4$, 0.1 mM 750 mM Tris-HCl pH=8.8), 2.5 mM $MgCl_2$, 2 mM dNTPs, 2 µl mixture of primers (10 pM from each primer), 1 u *Taq* DNA polymerase and 12 µl ddH2O. Samples were amplified for 34 cycles at the following program: initial denaturation step of 4 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 8 min at 72 ° C. The sequences of primers (AF120500 accession number) were: 5'ATGCGCTGTGGACCCCTGTATC 3' and 5'- TGGTGTCATCCTGGACCTTCC–3', that amplified a 94 bp fragment from the exon 2 of bovine leptin gene. Products of amplification were recognized by electrophoresis on 2% agarose gel stained with etithium bromide. The PCR products were digested with 3 units of *Bsp*13I enzyme for 6 hours in 50º C. Digestion fragments were revealed by 3% agarose gel stained with etithium bromide. The frequencies of alleles and genotypes were calculated by Popgene 1.32 software.

Results Restriction Fragment length polymorphism (RFLP) analysis revealed two different alleles: allele T was a 94bp fragment and C was two fragments of 75 and 19 bp. The allele frequencies of T and C were 0.45 and 0.55, respectively. Three genotypes of TT, TC and CC were found with the respective frequencies of 0.27, 0.36 and 0.37. The observed, expected and average heterozygosity were 0.359, 0.498 and 0.494, respectively (Table1). The χ^2 test ($P<0.05$) confirmed that Hardy-Weinberg equilibrium existed in this population.

Table1.The allelic and genotypic frequencies, observed, expected, Nei's expected and average heterozygosity and $\chi 2$ test for the exon 2 of the leptin gene.

allelic frequencies		genotypic frequencies			Obs_Het	Exp_Het*	Nei**	Ave_Het	$\chi 2$
T	C	TT	TC	CC					
0.45	0.55	0.27	0.36	0.37	0.359	0.498	0.494	0.494	5.03

* Expected homozygosity and heterozygosity were computed using Levene (1949)
** Nei's (1973) expected heterozygosity

Conclusions The results of this study showed that there was large variability in this breed. This variability Could be regarded as a useful tool for selection programming, mainly marker associated selection between different genotypes of the presented loci.

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