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GENETIC POLYMORPHISM OF LEPTIN GENE USING PCR-RFLP METHOD IN THREE DIFFERENT POPULATIONS

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ABSTRACT

This investigation was conducted to identify polymorphism of the leptin gene in Holstein cows, Mazandarani native cattle and buffaloes by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (*PCR-RFLP*). The frequencies of A and B alleles were 0.4 and 0. 6 - in Holstein cows, 0.56 and 0.44 - in Mazandarani native cattle, 0.61 and 0.35 in buffaloes, respectively. The heterozygote genotype (AB) had the highest frequency in all three populations: 0.73, 0.622 and 0.509 in Holstein cows, Mazandarani cattle and buffaloes, respectively. Chi-square test showed that Mazandarani cattle and buffaloes were in Hardy-Weinberg equilibrium but Holstein cows were not in equilibrium.

Key words: leptin gene; Holstein; Mazandarani native cattle; buffaloes; PCR-RFLP

INTRODUCTION

Leptin is a hormone which is produced in white adipose tissue and secreted into the bloodstream as a 16 KD protein. It plays important roles in control of body weight, feed intake, energy expenditure, immune function and reproduction (Frunhbeck *et al*., 1998). It has been shown that leptin gene influences milk performance in cattle (Liefers *et al*., 2002; Madeja *et al*., 2004). In cattle, this gene is located on chromosome 4 and consists of three exons (Pomp *et al*., 1997). Several polymorphic studies on the bovine leptin gene have been reported (Pomp *et al*., 1997; Lien *et al*., 1997; Oprzadek *et al*., 2003; Choudhary *et al*., 2005; Yang *et al*., 2007) and some researchers have studied polymorphism of this gene in buffaloes (Javanmard *et al*., 2005; Orru *et al*., 2007; Daungjinda *et al*., 2008).

The aim of present study was to investigate distribution of the allelic and genotypic frequencies of this gene in three genetically different populations [including Holstein cows (*bos taurus*), Mazandarani native cattle (*bos indicus*) and river buffaloes (*bubalus bubalis*)] and test for Hardy-Weinberg equilibrium.

MATERIAL AND METHODS

A total of 158 animals of Holstein cows (n = 52), from Behin Talise farm in Golestan Province, north of Iran, Mazandarani native cattle (n = 53), and river buffaloes (n =53) were genotyped for leptin gene.

The blood samples were collected randomly. The blood samples were placed into tubes containing 0.5M *EDTA* and were stored at -20°C until *DNA* extraction. *DNA* of Holstein cows and Mazandarani native cattle was extracted using salting out extraction protocol (Miller *et al.*, 1988), but for buffaloes, *DNA* was extracted by *DNA* extraction kit. Spectrophotometry and 1 % agarose gel electrophoresis methods were used to determine quality of the extracted *DNA*.

The 522 bp fragment from intron 2 and exon 3 of the leptin gene was amplified using primers suggested by Lien *et al.* (1997). The primer sequences were as follows:

Leptin-F (5´- GTC TGG AGG CAA AGG GCA GAG T-3´)
Leptin-R (5´-CCA CCA CCT CTG TGG AGT AG-3´)

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The PCR program was 94°C for 5 min, followed by 35 cycles of 94°C for 30s, 62°C for 30s and 72°C for 1 min. The final step prolonged 5 min at 72°C.

PCR was carried out using Personal Cycler™ amplificator (Biometra, Germany) and PCR Master Kit (CinnaGen Inc. Iran). The Kit contained master mix including 0.04 u/µl *Taq DNA Polymerase*, PCR Buffer, 3mM $MgCl_2$ and 0.04 mM of each *dNTP*. In each reaction, 7.5 µl master mix, 1 µl of DNA solution (50 to 100 ng/ µl), 3 µl primers (5 pmol/ µl) and some deionized water up to final volume of 15 µl were used. The fragments were separated on a 1.5 % agarose gel by electrophoresis.

The PCR products were digested using *BsaAI* (*Ppu21I*) restriction enzyme at 30°C for 16 hours. Each digestion contained 5 µl PCR products, 2 µl Buffer 10X, 5 U (0.5 µl) restriction enzyme and 9 µl deionized water. The fragments were separated on 2.5 % agarose gels by electrophoresis.

Determination of gene and genotype frequencies and χ^2 test were carried out using POP Gene 1.31 software (Yeh *et al*., 1997).

RESULTS AND DISCUSSION

The extracted *DNA* by both methods showed a good quality and PCR resulted in clear bands. *PCR-RFLP* results revealed two alleles (A and B) and three genotypes for leptin locus (AA: 522 bp band; BB: 441 bp and 81 bp bands; AB: 522, 441, and 81 bp bands) (Fig 1). Genotypic and allelic frequencies in three investigated groups of animals are presented in Table 1.

Hardy-Weinberg equilibrium was found in Mazandarani native cattle, and buffaloes ($P < 0.05$), but the allele frequencies of the leptin gene in Holstein cows did not follow Hardy-Weinberg's law of Equilibrium ($P > 0.05$) (Table 1).

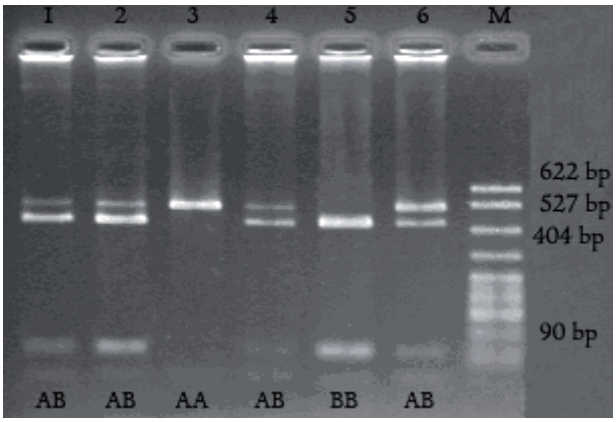

Fig. 1: Results of PCR products of the leptin gene digested by *BsaAI* on 2.5 % agarose gel, stained by ethidium bromide, Lanes 1-6= Digested PCR products, M= DNA size marker: pBR322/ *MspI*

Table 1: Genotypic and allelic frequencies of the leptin gene and Chi-square test results in Holstein cows, Mazandarani native cattle, and Mazandarani buffaloes

Gene	Animal	Allele Frequency		Genotype Frequency			χ^2
		A	B	A	A	AB	BB
LEP	Holstein cows	0.4	0.6	0.038	0.73	0.23	13.43
	Mazandarani native cattle	0.56	0.44	0.245	0.622	0.132	3.36*
	Mazandarani buffaloes	0.65	0.35	0.396	0.509	0.094	0.66*

*($P < 0.05$)

The study showed different allele frequencies of studied polymorphism in different populations. The heterozygote genotype (AB) had the highest frequency in all populations as Holstein cows and buffaloes showed the maximum and minimum level of heterozygosity, respectively. Mazandarani cattle in this regard occupied middle position. This result may be concerned to applied mating strategies in these groups. In Holstein cattle extensively used artificial insemination with different sires (semen) of course leads to more heterozygosity. Inversely, lack of planned mating programs and closed mating systems, using a few sires, are probably the main result of a low level of heterozygosity (especially in buffaloes).

Frequency of the A allele was higher than B in Mazandarani native cattle (0.56) and buffaloes (0.65) compared to Holstein cows (0.4). Current results are in agreement with previous studies by Choudhary *et*

al. (2005), who analyzed this gene with BsaAI enzyme and reported higher frequency of the B allele (0.82) in Holstein Friesian cattle. However, Yang *et al.* (2007) and Pomp *et al.* (1997) determined that the frequency of the B allele was lower than the A allele in Holstein cattle. Also, Javanmard *et al.* (2005) analyzed Lep-Sau3AI and reported higher frequency for the B allele in Mazandarani native cattle, and buffaloes. In addition, they revealed in other Iranian native cattle that the frequency of A allele was higher in Sarabi and Golpayegani breeds and B allele was higher in Sistani, Taleshi and Dashtiyari breeds. Also, several researchers like: Choudhary *et al.*, 2005 and Pomp et al., 1997, who works on Lep-BsaAI and Lep-Sau3A, respectively and Yang *et al.*, 2007; Daungjinda *et al.*, 2008 and Oprzadek *et al*., 2003 studied this gene in other breeds and observed the same or opposite results like our results in present study.

It must be pointed out that, frequency of any allele can be altered simply by mating strategies in different herds based on economical and population demands. Unfortunately, phenotypic records were not available in our study to indicate desired allele and genotype. Genotyping the superior cow did not elucidate the situation. Without any doubt, increasing number of investigated animals, considering role of other major genes and physiological status of animals simultaneously will perfectly explain more facts in this regard.

In the current study, the distribution of the genotype frequencies in Mazandarani native cattle, and river buffaloes were in the Hardy-Weinberg equilibrium. But χ^2 test did not confirm the Hardy-Weinberg equilibrium in Holstein cows. This results maybe due to selection plans on the leptin gene in Holstein cows, whereas there is no any special selection program for this gene in native cattle and buffaloes.

CONCLUSION

The goal of this study was to determine genetic polymorphism of the leptin gene in three different populations. These results revealed that polymorphism was detected in all studied herds and showed that *PCR-RFLP* is an appropriate tool for detecting genetic polymorphism. This study also opens interesting aspects for future selection programs, especially marker assisted selection.

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