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Comparison of DNA Polymorphism of Bovine Pituitary-Specific Transcription Factor and Leptin Gene Between Iranian *Bos indicus* and *Bos taurus* Cattle Using PCR-RFLP

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Abstract: 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. The leptin and Pit-1 are attractive candidate genes for production and reproduction traits in cattle. A total of 247 animals from four breeds from two species of Iranian cattle populations in include *Bos taurus* (Sarabi, Golpayegani) and *Bos indicus* (Sistani, Taleshi) were genotyped for the Pit-1 HinfI and leptin Sau3AI polymorphisms by the Polymerase Chain Reaction and Restriction Fragment Length Polymorphism (PCR-RFLP). The genotype and gene frequencies for each group were determined and shown to be quite variable among the breeds. The allele B for the leptin gene and allele A for the Pit-1 gene were investigated high frequency in *Bos indicus*. Candidate gene approach may be a useful method to measure of genetic distance for cross breeding program between taurin and indicine cattle.

Key words: Pit-1, leptin, *Bos taurus*, *Bos indicus*, PCR-RFLP, polymorphism

INTRODUCTION

The assumption in animal breeding of genetic variation of quantitative traits caused by simultaneous segregation of many genes each with small effect (so called infinitesimal model) is considered in animal breeding context very often. However it is doubtful that all gene influencing trait all have small effect and it is possible that a few genes may accountant for relatively large proportion of the genetic variation, these loci are known as Quantitative Traits Loci (QTL). Candidate genes are chose for study on basis known relationship in biochemical or physiological processes.

Candidate gene approach may be a useful method to measure of genetic distance for cross breeding program between taurin and indicine cattle. Pit-1 gene has been identified as the pituitary specific transcription factor that regulates the expression of the Growth Hormone (GH) and Prolactin (PRL) genes in the anterior pituitary (Tuggle *et al*., 1993). Renaville *et al*. (1997) showed that the A allele (especially the AB genotype) may have an effect on milk yield and its components. Leptin is a 16 KDa proteins synthesized by adipose tissue (Hossner, 1998) and it is involved in the regulation of feed intake (Lagonigro *et al*., 2003), energy balance, fertility and immune function (Lieferes *et al*., 2002). Leptin gene consists of three exons and two introns. Liefers *et al*. (2002) reported that heifers with the Sau3AI-AB genotype produce 1.32 kg day^{-1} more milk and consume 0.73 kg day^{-1} more food compared to those with the AA genotype.

They also suggested that the B allele may have a role in improving milk production without negative energy balance and low fertility. Buchanan *et al*. (2003) reported that the T allele of the bovine leptin gene resulted in the production of more milk and higher somatic cell count but did not have a significant effect on milk fat or protein during lactation. Lagonigro *et al*. (2003) reported an association between five single nucleotide polymorphisms within the leptin gene and feed intake as well as fat traits. Almeida *et al*. (2003) found two alleles responsible for increasing the calving interval thus, they

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suggested selection against these carriers could improve calving interval at least 2 months. The main objective of the present investigation was to comparison of genetic variations in the Pit-1 and leptin gene of the two Iranian *Bos indicus*, *Bos taurus* cattle.

MATERIALS AND METHODS

Experimental material: A total of 247 of *Bos taurus* cattle (Sarabi, n = 82 and Golpayegani, n = 57) and *Bos indicus* cattle (Sistani, n = 38 and Taleshi, n = 70) were used in this study. The populations were located at the Shabestar, Sarab, Delijan, Golpayegan, Zehak, Talash and Mazandaran Jahad-e-Keshavarzi animal breeding stations (Fig. 1).

DNA extraction: DNA was extracted from blood samples from each animal using the method of Boom *et al*. (1989). To an aliquot of 100 μL thawed blood, 400 μL of lysis buffer (Guanidin Thiocyanate, 20 mM; EDTA, 20 mM; Tris-HCl, 10 mM; Triton X_{100}, 40 g L^{-1}; DTT, 10 g L^{-1}) was added, the mixture was vortexed and incubated at 65°C for 5 min. The cells were resuspended in 20 μL of nuclease solution (4 g Silica gel, 100 mL Guanidine solution) and spun for 10 Sec at 12,000× g. The pellet was resuspended in 200 μL of lysis buffer. The white blood cell suspension was then added to 400 μL of saline buffer (1M NaCl, 10 mM Tris-HCL, 1M KCl and 20 mM EDTA), the mixture was vortexed and then spun for 10 Sec at 5,000× g.

The DNA was precipitated with 45-55 μL of extra gene solution (10% Ion Exchange Resin:, 0.02% Orange G color, 0.01% Triton X_{100}) and then incubated at 65°C for 3-5 min. Protein was then precipitated by centrifugation (3 min at 1000× g) and the upper layer containing the DNA was transferred to another tube. Relative purity of the DNA was determined using a spectrophotometer based on absorbances at 260 and 280 nm.

PCR-RFLP analysis: The sequences of the forward and reverse primers for the amplification of the Pit-1 gene were:

Pit-1F 5′-GAGCCTACATGAGACAAGCATC-3′
Pit-1R 5′-AAATGTACAATGTGCCTTCTGA-3′

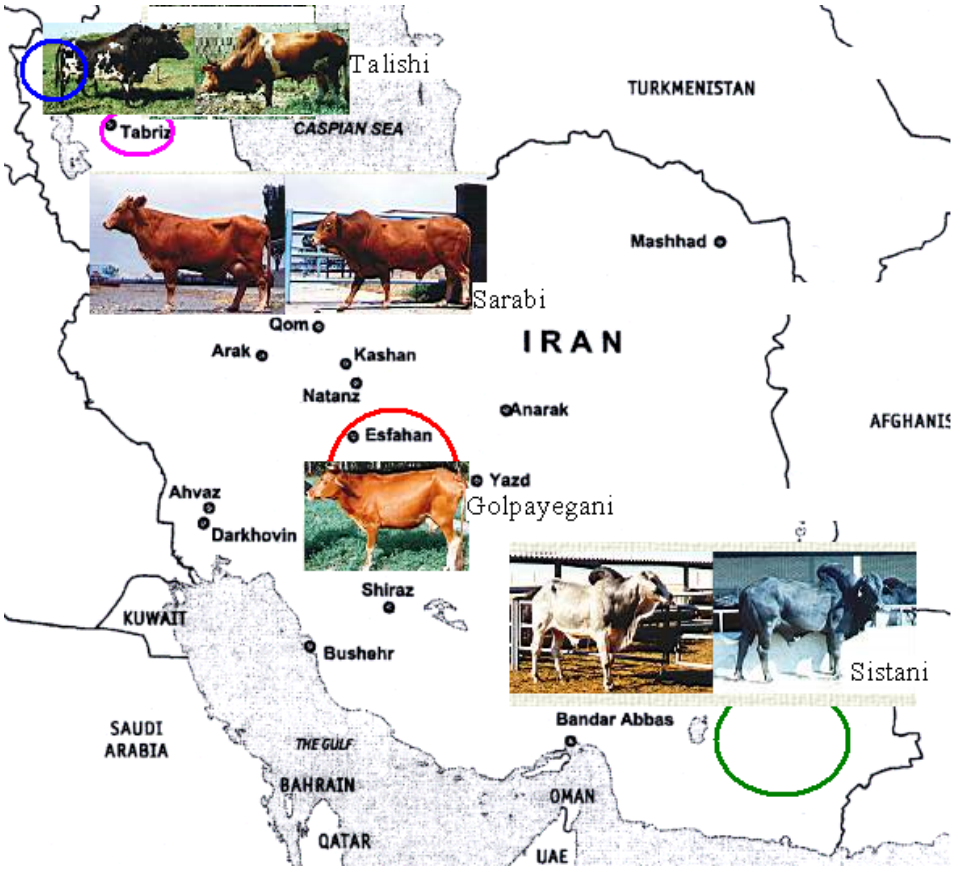

Fig. 1: Location of animal breeding stations

The polymerase chain reaction for the Pit-1 gene pair primers was performed in 25 μL reaction mixtures containing 1.5 mM $MgCl_2$, 200 μM of each dNTPs, 0.3 μM of each primers, 1× PCR buffer, 1 U Taq polymerase (Cinagen, Iran) and 100 ng of genomic DNA template. The DNA thermal cycler was (Perkin Elmer 9700). Thermal cycling conditions included: an initial denaturation step at 95°C for 2 min followed by 30 cycles of 95°C for 45 sec, 60°C for 1 min and 72°C for 1 min and a final extension at 72°C for 3 min. The PCR products were digested with 10 U of HinfI (Gibco BRL, life Technologies, USA) at 37°C for at least 14 h.

One part of second intron of leptin gene was amplified using the following primers:

LepF: 5′-TGGAGTGGCTTGTTATTTTCTTCT-3′
LepR: 5′-GTCCCCGCTTCTGGCTACCTAACT-3′

PCR reaction was performed using 0.5 U Taq polymerase and 50 ng of DNA. Thermal cycling conditions were as follows initial denaturation at 94°C for 2 min followed by 35 cycles of 94, 55 and 72°C (each for 1 min) and a final extension at 72°C for 15 min. The PCR products were digested overnight at 37°C with 10 U of Sau3AI (Roche, Germany). The Restriction fragments from the above PCR reactions were electrophoresed on 2% agarose gels and stained with ethidium bromide.

Statistical analysis: The data was analyzed using the POPGENE software version 1.31 (Yeh *et al*., 1999). Observed and expected hetrozygosity calulated by using Nei (1978) formula.

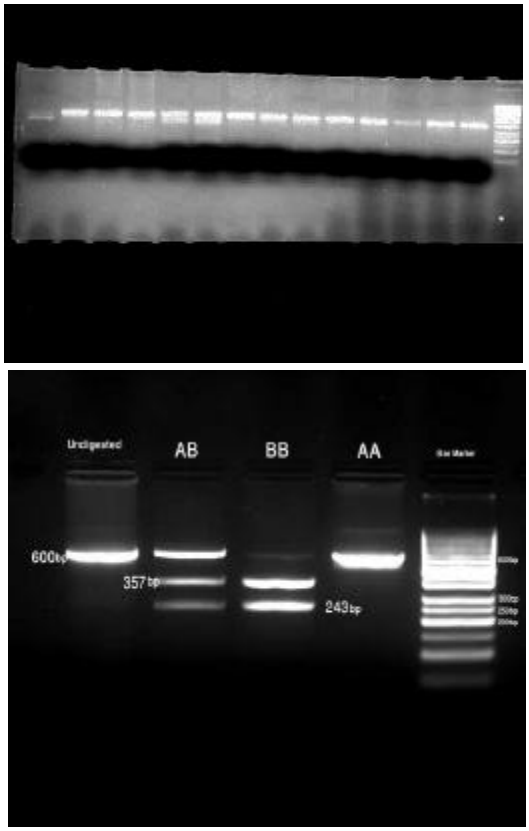

Fig. 2: Sau3AI and HinfI polymorphism at bovine Leptin (A) and Pit-1 (B) genes. Electrophoretic patterns of three genotypes separated on 2% agarose gel for each gene. Molecular weight of marker is SM037 from Fermentas (SM037 from MB1 fragments)

RESULTS AND DISCUSSION

There was one polymorphic HinfI site in the 600 bp PCR product within exon 6 of Pit-1 gene. The digested PCR product exhibited one fragment of 600 bp for AA genotype and for the BB genotype, the 600 bp fragment was two fragments of 357 and 243 bp. Figure 1 shows the restriction pattern of three genotypes AA, AB and BB upon digestion of Pit-1 HinfI. The 442bp fragment of the leptin gene also contained two restriction sites of Sau3AI. The AB PCR product was three fragments of 303, 88 and 32 bp (bands not detected on the gel). Sequence analysis of Sau3AI site in the leptin gene revealed a mutation at position 1180 that was a C-T transition. Figure 2 shows the restriction pattern of the three genotypes of the leptin gene. Allele and genotype frequencies and the average heterozygosity in four Iranian cattle populations for the Pit-1 and leptin loci were resulted (Table 1). Although, the B allele is favorable for the leptin gene (Pomp *et al*., 1997; Almeida *et al*., 2003) and A allele for the Pit-1 gene (Sabour *et al*., 1996), AB genotype is preferred by both the candidate genes (Woollard *et al*., 1994; Renaville *et al*., 1997; Pomp *et al*., 1997; Almeida *et al*., 2003). The highest B allele frequency for the leptin gene (0.87) and A allele for the Pit-1 genes were estimated in Sistani cattle. However, the highest AB genotype frequency was found in Taleshi and Sarabi for the leptin and Pit-1 genes, respectively.

A main goal of the animal breeder is to select superior animals for breeding. Screening favorable alleles for selection at the DNA level provides an ideal tool for marker-assisted selection. RFLP polymorphism within the bovine Pit-1 gene was first detected with HinfI nuclease by Woollard *et al*. (1994).

Sabour *et al*. (1996) showed that allele A in Pit-1 locus positively affected milk production traits in Friesian cattle. This allele (frequency of 0.18) showed a significant superiority over allele B for milk and milk protein yields and body conformation traits within Italian Holstein Friesian cattle. The allele and genotype frequencies are variable among different studied populations and also

Table 1: Distribution of Leptin and Pit-1 genotype and allele frequencies in Iranian (Sarabi, n = 82 and Golpayegani, n = 57) and *Bos indicus* cattle (Sistani n = 38 and Taleshi, n = 70)

		Leptin gene					Pit-1gene				
		Genotype frequencies			Allele frequencies		Genotype frequencies			Allele frequencies	
Population	No. of sample	AA	AB	BB	A	B	AA	AB	BB	A	B
Sarabi	82	0.56	0.37	0.07	0.75	0.25	0.45	0.34	0.21	0.62	0.38
Golpayegani	57	0.54	0.35	0.11	0.72	0.28	0.61	0.26	0.14	0.75	0.25
Sistani	38	0.21	0.26	0.53	0.35	0.65	0.84	0.16	0.000	0.92	0.78
Talishi	70	0.10	0.75	0.15	0.47	0.53	0.61	0.31	0.08	0.77	0.23

the favorable allele and genotype frequencies in world cattle populations. The Pit-1 A allele frequencies have been estimated to be 0.45 in Angus; 0.26 in Holstein; 0.21 in Herford; 0.28 in Gelbvieh; 0.1 in Brahman; 0.25 in Polish and 0.95 in Gry cattle (Zwierzchowski *et al*., 2001). Pomp *et al*. (1997) have reported leptin B allele frequencies of 0.3 in Limousine; 0.21 in Simmental; 0.28 in Gelbvieh; 0.29 in Holstein; 0.5 in Hereford, 0.27 in Angus, 1.0 in Brahman and 0.4 in Branguse. Therefore, breeding strategies could be designed for introgression of the A allele for Pit-1 and B allele for leptin from Iranian *Bos indicus* and *Bos taurus*. Therefore, it is suggested that crossbreeding should be done between these populations and/or with exotic breeds to increase the frequency of the favorable genotype.

CONCLUSION

In conclusion, the possible association between molecular polymorphisms within these candidate genes and economic traits for the studied populations should be further investigated.

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