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Relationship Between Leptin Gene Polymorphism with Economical Traits in Iranian Sistani and Brown Swiss Cows

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Abstract: The aim of this study was to investigate leptin gene polymorphism in Sistani cows and brown swiss cows and relationship between it's polymorphism with economical traits in them. Blood samples were collected from Sistani cows and Brown Swiss cows. DNA was extracted from the blood samples and then it was amplified by Polymerase Chain Reaction (PCR) technique to amplify a 422 bp DNA fragment. Digestion of amplicons with BstMB1 was done on PCR product. Genotype frequencies were 0.77, 0.22, 0.01 for AA, AB and BB, respectively in Sistani cows and 0.64, 0.35 and 0.01 for AA, AB and BB, respectively in Brown Swiss cows. The populations were in Hardy-Weinberg equilibrium with respect to Leptin gene. The AB genotype had a significant effect on growth traits including weight at 9 and 12 months and age at successful insemination than the other genotypes in Sistani cows. AB genotype also had a significant effect on milk production, days open and milking days compared with other genotypes in Brown Swiss cows.

Key words: Leptin, RFLP-PCR, economical traits, Sistani cows, Brown Swiss cows, genotype

INTRODUCTION

Leptin is a 16 kd protein that is synthesized by adipose tissue and is involved in regulation of feed intake, energy balance, fertility, reproduction, milk production as well as immune functions (Houseknecht *et al*., 1998; Fruhbeck , 2001). The expression and secretion of leptin is highly correlated with body fat mass and adipocyte size (Fruhbeck *et al*., 1998; Hossner, 1998). In cattle, the leptin gene is located on chromosome 4 and consists of 3 exons and 2 introns of which only 2 exons are translated into protein.

Several polymorphims had been reported in the bovine leptin gene but only four of them changed the amino acid sequence in the encoding polypeptide (Konfortov *et al*., 1999; Haegeman *et al*., 2000; Buchanan *et al*., 2002; Lagonigro *et al*., 2003; Liefers *et al*., 2003). Recently, the promoter region has been sequenced and twenty SNPs (single nucleotide polymorphisms) had been found (Liefers, 2004). Wilkins and Davey (1997) reported restriction fragment length polymorphisms of the bovine leptin gene. Polymorphisms in the bovine leptin gene had been described and an association with fat deposition in beef cattle was reported (Pomp *et al*., 1997). Lagonigro *et al*. (2003) reported association of five SNP bovine leptin gene with feed intake and fat-related traits. Individuals with genotype A/T at exon 2 had 19% greater mean feed intake than individuals with genotype AA. Liefers *et al*. (2002) reported that heifers with the AB genotype produced 1.32 kg day^{-1} more milk and consumed 0.73 kg day^{-1}more dry matter compared with the AA genotype.

They suggested that B allele could yield a higher milk yield without negative affect on energy balance and fertility. Block *et al*. (2001) showed that leptin is negatively correlated with the amount of non esterified fatty acids which reflects the amount of fat mobilization. As leptin could play an important role in the processes occurring during the lactation period in dairy cows. The aim of this study was to estimate the frequency of leptin gene and relationships between its polymorphism with variations in growth and age at successful insemination in an Iranian native cattle, Sistani and milk production, days open and milking days in Brown Swiss cattle.

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MATERIALS AND METHODS

Animals and DNA extraction: This study took place at biotechnology laboratory in Ferdowsi University of Mashhad, Iran, 2008. Blood samples were collected from 103 Iranian Sistani cattle and 104 from Brown Swiss cattle randomly. They were obtained from Animal Breeding Station in Zahak district of Zabol city and Animal Breeding Station in Torogh and Telgerd of Mashhad city, respectively. Blood was collected on K_2EDTA tubes and stored either at -20°C for few weeks. DNA extraction was done on the blood samples using guanidium thiocyanate-silica gel (Boom *et al*., 1989). DNA concentration was calculated by spectrophotometry by taking the optical density at 280 nm.

PCR and PCR-RFLP condition: Amplified region is located in the intron 2 (422 bp) between two exons of leptin. The genomic bovine leptin sequence which consists of three exons was obtained from Gene Bank (accession number U50365). The following primers were used for amplification of the product:

Forward: 5'-CTGGAGTGGCTTGTTATTTTCTTCT-3'
Reverse: 5'-GTCCCCGCTTCTGGCTACCTAACT-3'

Amplification reaction was carried out with 100 ng of DNA in a 25 μL total volume containing 1× PCR buffer; 2.5 mM $MgCl_2$; dNTPs, 100 μM of each; 0.5 μM of each primer and 1 unit of Taq DNA polymerase. The thermal cycling profile consist of an initial denaturation step of 3 min at 94°C followed by 35 cycles 45 sec at 94°C, 45 sec at 60°C, 45 sec at 72°C and final extension step of 10 min at 72°C. Electrophoresis was carried out on 2% agarose gel with 5 μL of PCR product. Then PCR products were digested with 5 U of BstMB1 enzyme for 6 h at 65°C. Restriction fragments were revealed by gel electrophoresis on 8% acrylamide gel and visualized with silver staining. pUC 19 and M100 bp were used as molecular weight markers. Genotypic and alleleic frequencies of leptin gene were analyzed using the Popgen 1.32 computer program.

Statistical analysis: The growth and age at conception data were analyzed by analyzing Standard Least Square within mixed models using JMP software (version 4.0.4; SAS Institute Inc, NC. USA). The model contained the effects of genotype and sires nested in the genotype. The milk production during 4 months of lactation and also cumulative milk production in the first 60 and 100 days of lactation were analyzed by analyzing Standard Least Square within mixed models using JMP software (version 4.0.4; SAS Institute Inc, NC. USA). The model contained the effects of genotype, sires nested in the genotype, age and month of parturition. Milking days was analyzed using a model containing the effects of genotype, sires nested in the genotype and interval between two consequent calving. Finally, the model for analyzing the days open data contained the effects of genotype, sires nested in the genotype, interval between parturition and second insemination and number of inseminations per pregnancy (Moussavi *et al*., 2006).

RESULTS AND DISCUSSION

Allele frequency: One RFLP site in the intron 2 of the bovine leptin gene was detected. There were two BstMB1 sites in 422 bp fragments. The AA genotype PCR product exhibited two fragments of 390 and 32 bp. The BB genotype exhibited three fragments 302, 88 and 32 bp. The AB genotype exhibited four fragments 390, 302, 88 and 32. Figure 1 shows the restriction patterns of the three genotypes AA, AB and BB. Chi-square test are shown in Table 1. The frequency of allele A was 0.88 and 0.82 and for allele B was 0.12 and 0.18 in Sistani cows and Brown Swiss cows, respectively. There was only one animal with the BB genotype which was excluded from the analysis. Chi-square test shown populations were in Hardy-Weinberg equilibrium with respect to leptin gene.

Effect of leptin gene polymorphism on growth: The least squares mean, standard deviation for weight at 9 and 12 months in Sistani cows were shown in Table 2. Cattles with AB genotype had higher weight at 9 and 12 months of age than the AA genotype ($p<0.01$).

Effect of leptin gene polymorphism on age at conception: The effects of leptin genotypes on age at conception in Sistani cows are shown in Table 2. In the age at insemination induce pregenancy, significant differences were found between genotypes. The AB genotype demonstrated the highest values for age at conception ($p<0.05$).

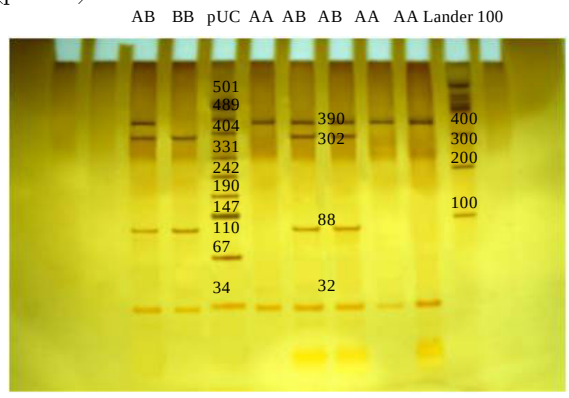

Fig. 1: Restriction patterns of the three genotypes AA, AB and BB on 8% acrylamide gel

Table 1: The genotypes, allele frequencies, Chi square and number of individual with different genotype of BstMB1 polymorphisms in the intron 2 region of bovine leptin gene in Sistani and Brown Swiss cows

		Genotype frequency			Allele frequency		
Breeds	No. of animal	AA	AB	BB	A	B	Chi square test
Sistani	103	0.77	0.22	0.01	0.88	0.12	0.188367^{NS}
Brown Swiss	104	0.64	0.35	0.01	0.82	0.18	2.63^{NS}

NS = Non Significant, $p<0.05$, df = 2

Table 2: Effect of animal genotype on growth and age at conception traits in Sistani cows

Traits	AA	AB
Weight at 9 month (kg)	117.80±0.99[a]	133.85±1.630[b]
Weight at 12 month (kg)	140.28±1.19[a]	155.80±1.740[b]
Age at conception (day)	1117.98±9.44[a]	1154.78±15.07[b]

Values are shown as LSM±SE; LSM = Least Squares Mean, SE = Standard Error, [a]The value traits in AA genotype, [b]The value traits in AB genotype

Table 3: Effect of animal genotype on milk production traits

Traits	AA	AB
1 month lactation (day)	20.04±4.0200[a]	33.70±5.380[b]
2 month lactation (day)	29.20±3.3700[a]	39.12±3.580[b]
3 month lactation (day)	23.55±3.6500[a]	33.66±2.910[b]
4 month lactation (day)	20.76±3.0300[a]	28.93±2.420[b]
Cumulative milk production in the first 60 days of lactation (kg)	1457.74±196.03[a]	2242.24±262.41[b]
Cumulative milk production in the first 100 days of lactation (kg)	2419.52±295.26[a]	3415.53±236.18[b]

Values are shown as LSM±SE; LSM = Least Squares Mean, SE = Standard Error, [a]the value traits in AA genotype, [b]the value traits in AB genotype

Effect of leptin gene polymorphism on milk production: The least squares mean, standard deviation for milk production during 4 months of lactation and cumulative milk production in the first 60 and 100 days of lactation in Brown Swiss cows were shown in Table 3. Milk production during 5-9 months of lactation did not affect by animal genotype. While milk production during 4 month of lactation ($p<0.05$) and cumulative milk production in the first 60 and 100 days of lactation ($p<0.01$) affected by animal genotype. The genotype AB demonstrated the highest values for milk production ($p<0.05$).

Effects of leptin gene polymorphism on days open and milk days: The effects of leptin genotype on days open and milking days in Brown Swiss cows is shown in Table 4. Significant differences in the days open and milk days were found in between genotypes. The genotype AB demonstrated the highest values for days open ($p<0.01$) and milk days ($p<0.05$). The present study showed allelic frequencies of 0.82 for A allele in Brown Swiss cows and 0.88 for A allele in Sistani cows. Similar results were reported by Liefers *et al.* (2002) in Holstein cows and Moussavi *et al.* (2006) in Iranian Holstein cows. The frequency of the A allele was reported 0.85 and 0.95, respectively in the Liefers *et al.* (2002) and Moussavi *et al.* (2006) reports. Association between polymorphism of leptin gene and growth, reproduction and milk production have indicated in several studies. For example, Hale *et al.* (1998) suggested that the leptin gene may be useful as a marker of carcass traits in Angus cattle. Oprzadek *et al.* (2003) reported that AA genotype had the highest weight of carcass. Liefers *et al.* (2002) reported that AB genotype had higher milk production and body weight in comparison with AA genotype. Also, the AB genotype had higher feed intake than the AA genotype and produced 1.32 kg day^{-1} more milk and consumed 0.37 kg day^{-1} more food compared with the AA genotype.

Table 4: Effect of animal genotype on days open and milk days

Traits	AA	AB
Days open	161.37±16.83[a]	134.71±17.01[b]
Milk days	323.07±12.04[a]	283.70±15.61[b]

Values are shown as LSM±SE; LSM = Least Squares Mean, SE = Standard Error, [a]The value traits in AA genotype, [b]The value traits in AB genotype

In the present study, the association between the leptin genotypes and growth, age at conception and milk production in Sistani cows and the association between the genotypes and milk production, days open and milking days in Brown Swiss cows were studied. The results showed that the AB genotype had best effects on growth, age at conception, milk production, days open and milking days. Similarly, Moussavi *et al.* (2006) evaluated the association of genetic differences in the bovine leptin gene and milk yield and reproduction in Iranian Holstein cows. They were detected a significant association between the AB genotype and 305 day milk yield ($p<0.05$). Also they had indicated that AB genotype leptin locus had a trend to better reproduction performance than the homozygous. The results of our and other studies demonstrate that the B allele can yield a higher milk production with a trend to better reproduction performance. In the study, AB genotype had positive role in cattle growth, age at conception, milk production, days open and milking days. There are significant differences between the genotypes AA and AB in milk production during 4 months of lactation and cumulative milk production in the first 60 and 100 days of lactation ($p<0.05$) and there were significant differences between the genotype AA and AB on days open ($p<0.01$) and milking days ($p<0.05$).

CONCLUSION

It seems that B allele is the favorable allele in economical traits in cattle. In addition to the region of leptin gene in this study, some research was carried out on another region of leptin gene. Nkrumah *et al*. (2005) reported association between SNP in the 5′ untranslated promoter region of the bovine leptin gene with serum leptin concentration, growth, body weight, feed intake and carcass merit in hybrid cattle and it showed that animals with the TT genotype show 48 and 39% increases in serum leptin concentration and 39 and 31 increases in backfat thickness and 13 and 9% increases in marbling score compared with CC and CT genotypes, respectively.

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