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Genetic Polymorphism at the Candidate Gene in Iranian Sistani Cattle (*Bos indicus*)

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Abstract: The genotypes for Leptin, Kappa-Casein, Calpastatin and BoLA-DRB3 loci were determined by polymerase chain reaction and restriction enzyme digestion method in native Iranian breed cattle, Sistani. Blood samples were collected from Sistani Breeding Station located in Zehak, Zabol in Iran. The extraction of genomic DNA was based on Guanidin Thiocyanate-Silica gel method. After PCR reaction, amplicons were digested with appropriate restriction enzymes. The Calpastatin locus had 3 genotypes with frequencies of 0.62, 0.29 and 0.09 for MM, MN and NN, respectively; κ-Casein and Leptin had 3 genotypes with frequencies of 0.27, 0.57 and 0.16 for κ-Casein, 0.77, 0.22 and 0.01 for Leptin for AA, AB and BB genotypes, respectively. For *BoLA-DRB3* we identified 19 alleles, that DRB3. 2*8 had the highest allelic frequency (22.4%) and DRB3. 2*3, *29, *37 and *51 had the lowest allelic frequency (1%). One of the 19 alleles had a new pattern. Average heterozygosity values for all loci were low. χ^2-test did not confirm the Hardy-Weinberg equilibrium for Leptin and Calpastatin in this population. These data provide evidence that Iranian's Sistani breed have a good genetic variability, which opens interesting prospects for future selection programs, especially marker-assistant selection.

Key words: Sistani cattle, candidate genes, RFLP, polymorphism

INTRODUCTION

Iranian Sistani cattle is a special breed that shows special features of adaptation to rustic environments, within the last few years it has become a biotype of great interest for the meat production (Tavakkolian, 2000). Marker-Assisted Selection (MAS) is one of the new methods that improve accuracy and progress of selection in genetic resource (Dekkers, 1998). Among specific genes that may affect important economically traits in cattle in MAS method, the Leptin, κ-Casein, Calpastatin and BoLA-DRB3 loci had been extensively studied.

In cattle, the leptin gene is located on chromosome 4 (Pomp *et al*., 1997; Stone *et al*., 1996) and consists of three exons. Several polymorphisms in this gene have been found (Madeja *et al*., 2004). Liefers *et al*. (2002) reported that heifers with the AB genotype produce 1.32 kg day^{-1} more milk and consume 0.73 kg day^{-1} more food compared with the AA genotype. They suggested that B allele could yield a higher milk production without negatively affecting energy balance and fertility.

Milk protein polymorphisms have effect on the milk yield and processing properties of its products. Caseins are the major constituents of total milk proteins (Kemenes *et al*., 1999). In bovidea, casein genes are located in chromosome 6 (Ferretti *et al*., 1990) and 2 polymorphisms of A and B were found for the Kappa-casein (CSN3) gene. Association between CSN3 variants and milk production has been reported by many researchers (Lien and Rogne, 1993). Lien and Rogne (1993) found a significant effect of κ-casein genotype on milk production ($p<0.001$) and reported that cows of the BB genotype producing 173 kg less milk than AA cows. It has been demonstrated that B variant of CSN3 is associated with a higher protein content, better quality of crud and increased yield of cheese.

Many genes are involved in meat production. Among them, calpastatin is the major constituents of post marten tenderization. Many studies suggested the calpastatin gene (CAST) as a candidate gene for meat quality (Palmer *et al*., 1998; Koohmaraie *et al*., 1995). Palmer *et al*. (1998) have described a two alleles system of polymorphic

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variant (M and N) in a region of the ovine CAST (exon and intron regions from a portion of the first repetitive domain) by PCR-RFLP method. Digestion with restriction endonucleases *Msp*I and *Nco*I differentiated alleles M and N. Polymorphism of CAST has been detected in several cattle breeds (Cockett *et al*., 1995; Palmer *et al*., 1998; Chung *et al*., 2001).

Major Histocompatibility Complex (MHC) genes, also called bovine lymphocyte antigen (BoLA), have received attention because of high degree of genetic polymorphism and their association with host immunity. The BoLA genes are located on the short arm of bovine chromosome 23. BoLA-DRB3.2 considered suitable marker system for genetic diversity studies owing to their abundance in the mammalian genome. By means of PCR (for amplification of exon 2 of the gene) followed by analysis of Restriction Fragment Length Polymorphism (RFLP), it is possible to identify the BoLA -DRB3 alleles (53 alleles) (Takeshima *et al*., 2002; Van Eijk *et al*., 1992).

Awareness of the values of genetic resource in livestock has estimated the study of the genetic diversity of native breeds. However, most of such studies have been done on European cattle breeds and very little information is available concerning the genetic polymorphism in native cattle breeds of Iran. The objective of this study was to describe the genetic variability in the Leptin, κ-Casein, Calpastatin and BoLA-DRB3 loci in Sistani native cattle (*B. indicus*) breeds from Iran.

MATERIALS AND METHODS

DNA sampling: Blood samples (n = 100) were collected from an Iranian Sistani herd in Zehak Research Station located at Zabol (southeast of Iran). Whole blood (100 μL) was used as source of DNA, which was extracted by the modified Guanidine Isothiocyanate-Silica gel method (Boom *et al*., 1989).

Amplification: Amplification reactions for the Lept., CSN3, CAST and BoLA DRB3.2 genes were carried out with 100 ng of DNA (5 μL) in a 25 μL total volume containing 1× PCR buffer; 2.5 mM $MgCl_2$; dNTPs, 100 μM of each; 10 pM of each primer (Table 1) and 1 U of *Taq* DNA polymerase. The thermal cycling profile was an initial denaturation step of 4 min at 94°C followed by 35 cycles 45 s at 94°C, 45 s at specific annealing temperature (Table 1), 45 s at 72°C and final extension step of 10 min at 72°C. Hemi-nested PCR was used for the amplification of the second exon (284 bp) of the BoLA-DRB3 gene. The first PCR stage was performed in a final volume of 20 μL containing 40 ng of template DNA, 10 pM of each primers, Subsequently, 2 μL of the first-stage PCR product was used as template DNA for the second-stage PCR in a final volume of 20 μL containing 10 pM of each primers, 1 U *Taq* DNA polymerase and the remaining components in the concentrations stated above. The initial denaturation (94°C for 2 min), was followed by 25 cycles of denaturation (94°C for 40 sec) and annealing-extension (65°C for 30 sec) and a final extension (72°C for 5 min).

Table 1: Characteristics of primers used in this study to amplify respective loci

Locus	Forward primer	Reverse primer	AT
Leptin	GGAGTGGCTTG TTATTTTCTTCT	GTCCCCGCTTCT GGCTACCTAACT	60
CSN3	TATCATTTATGG CCATTCCACCA	CTTCTTTGATG TCTCCTTAGAGTT	56
CAST	CTTGTCATCCG ACTTCACCT	TCTCTTTTCTCTT TGGGTGG A	61
BoLA first stage	ATCCTCTCTCT GCAGCACATTTCC	TTTAATTCGCGC TCACCTCGCCGCT	65°C
BoLA second stage	ATCCTCTCTCTG CAGCACATTTCC	TCGCCGCTGCAC AGTGAAACTCTC	65°C

Table 2: Restriction enzymes and reaction condition for RFLP analysis

Locus	Enzymes	Time (h)	Digested fragments length	Optimum temperature (°C)
Leptin	*BstMB*I	6	390, 303, 88 and 32	65
CSN3	*Hind*III	3	228, 135 and 95	37
CAST	*Msp*I	4	622, 336 and 286	37

The concentration and purity of the obtained DNA were assessed by spectrophotometer and electrophoresis in 1% agarose gels, respectively.

RFLP analysis: For RFLP analysis, 5 μL of PCR products were incubated with 5 units of respective restriction enzymes for definite time (Table 2). To examine the nucleotide sequence variability at the DRB3.2 locus, three restriction enzymes, *Rsa*I (GT↓AC), *Hae*III (GG↓CC) and *Bst*YI (Pu↓GATC↓Py) were chosen based on their ability to cut DNA in this exon. Digestion products of leptin, CNS3 and DRB3.2 were separated by electrophoresis on 8% non-denaturant polyacrylamid gel and visualized by silver staining. For the calpastatin, Restriction fragments were revealed electrophoresis on 2% agarose gel stained with etithium bromide.

Statistical analysis: Genotypic and allelic frequencies, Nei's heterozygosity value and χ^2-test for all loci were analyzed using the PopGen32 software (V. 1.31). A χ^2-test was performed to verify if genotype frequencies agreed with Hardy-Weinberg expectations. Average heterozygosity (h) was employed to estimate genetic diversity within the population (Nei and Li, 1979).

RESULTS

Leptin: The results showed that there were two *Bst*MBI cutting sites in 422 bp fragment of leptin gene. The AA

Table 3: Allelic and genotypic frequencies for Leptin, κ-casein and *Calpastatin loci* in Iranian Sistani cattle

	Genotypic frequency*			Allelic frequency**			
locus	1	2	3	4	5	χ^2	Average heterozygosity
Leptin	0.77	0.22	0.01	0.88	0.12	0.19^{ns}	0.21
CSN3	0.27	0.57	0.16	0.55	0.45	7.96*	0.49
Calpastatin	0.62	0.29	0.09	0.76	0.24	3.42^{ns}	0.36

*: The 1, 2 and 3 are the respective AA, AB and BB genotypes for Leptin and CSN3 and MM, MN and NN genotypes for calpastatin, **: The 4 and 5 are the respective A and B alleles for Leptin and CSN3 and M and N alleles for calpastatin, ns: non significant

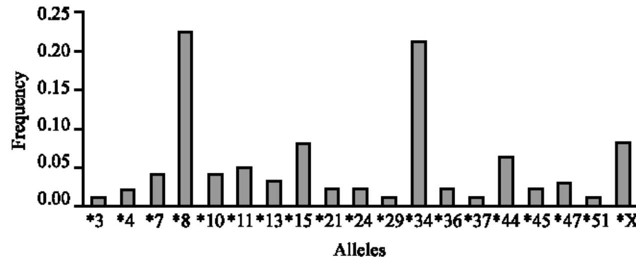

Fig. 1: Allelic frequency of BoLA-DRB3.2 in Sistani cattle breed, *: Names of DRB3.2 alleles

genotype pattern exhibited two fragments of 390 and 32 bp (not detected on the gel), BB genotype pattern exhibited 303, 88 and 32 bp and AB genotype exhibited 390, 303, 88 and 32 (Fig. 1). Present results confirmed transversion mutation (GAC⇒GAT) in the leptin gene. The allelic frequencies for A and B alleles were 0.88 and 0.12, respectively. The genotypes AA, AB and BB were observed with respective frequencies of 0.77, 0.22 and 0.01 (Table 3).

CSN3: The *Taq*1 and *Hind*3 digestions of the 228 bp fragment of exon 4 from CSN3 gene revealed two alleles with respective allelic frequencies of A = 0.25 and B = 0.75. The A allele had no restriction site for *Taq*I and *Hind*3, whereas B allele charachterized by the presence of two fragments of 135 and 95 bp. Genotypic frequencies were 0.27, 0.57 and 0.16 for AA, AB and BB, respectively. Frequencies of alleles A and B were 0.55 and 0.45, respectively (Table 3).

Calpastatin: Digestion of the 622 bp fragment from CAST locus with restriction endonuclease *Msp*1 differentiates two M and N alleles. The *Msp*1 enzyme did not digest the allele N amplicon and allele M digestion exhibited two fragments of 336 and 286 bp. The allelic frequencies were 0.764 and 0.236 for M and N, respectively. The genotypic frequencies for MM, MN and NN were 0.62, 0.29 and 0.09, respectively (Table 1).

BoLA-DRB3: Figure 1 shows the patterns of digesting DRB3 284bp fragment with *Rsa*1, *Hae*3 and *Bst*Y1. Of all the restriction patterns described in the literature (24 patterns for RsaI, 10 for *Hae*3 and 5 for BstYI) only 14, 5 and 5 patterns were identified for *Rsa*1, *Hae*3 and *Bst*YI, respectively (Table 1). In the present study, 19 alleles of the 53 previously reported BoLA-DRB3.2 alleles were detected (Fig. 1). As shown in Fig. 1, 70% of the cumulative gene frequencies corresponded to the six most common alleles, which are defined as variants with gene frequencies higher than 5% (DRB3.2 number 8, 11, 15, 34, 44 and new pattern of eac). Four alleles exhibited gene frequencies of 1% (DRB3.2*3, *29, *37 and *51). Digestion of PCR products from four animals showed a new combination of endonucleases patterns. By using *Rsa*1 and *Hae*3, these samples produced patterns of le and bc, respectively. According to PCR-RFLP patterns published by BoLA Nomenclature Committee (http://www.projects.roslin.ac.uk/bola/bolacom.html) expected alleles must be #7 (ecc) for 1 allele and #20 (lbb) or #34 (lab) for another one that the later must be finalized with *Bst*YI digestion. Surprisingly when we digested these samples with *Bst*YI, it revealed that they have a new digestion pattern (aa) rather than what expected (ac or bc). Therefore, the first allele for these animals is a new pattern (eac) that has not been published yet and the second allele was #20.

DISCUSSION

It was the first study using polymorphism of Leptin, κ-Casein, Calpastatin and BoLA-DRB3 loci to understanding genetic polymorphism of Sistani cattle in Iran. Therefore the present study may be regarded as the beginning of attempts to understand the genetic variability of native cattle breeds in Iran.

Leptin: The genotypes AA, AB and BB were observed with respective frequencies of 0.77, 0.22 and 0.01, respectively (Table 3). Liefers *et al.* (2002) in Holstein cattle breed observed similar results. They observed the AA, AB and BB genotypes with respective frequencies of 0.813, 0.185 and 0.002. Similar with our results only one animal with BB genotype was observed in this study. Moussavi *et al.* (2006) investigated similar region of leptin gene in Iranian Holstein cattle. They observed the AA and AB genotypes with respective frequencies of 0.86 and 0.14. The BB genotypes not observed in this study that confirmed our results with low BB genotypic frequencies. In contrast Javanmard *et al.* (2005) reported genotypic frequencies of 0.32, 0.43 and 0.25 for AA, AB and BB in Sarabi cattle breed, respectively. Madeja *et al.* (2004) investigated three restriction fragment length polymorphisms (RFLP): *Hph*I, *Kpn*2I and *Sau*3AI in the leptin gene in Polish Black and White cattle. They reported the allelic frequencies of 0.54 (C) and 0.46 (T) for *Kpn*2I; 0.66 (C) and 0.34 (T) for *Hph*I and 0.86 (A), 0.11 (B) and 0.03 (C) for *Sau*3AI. The polymorphisms of leptin gene were reported by several studies. Buchanan *et al.* (2002) reported a missense mutation in the bovine leptin gene and described cytosine (C) to thymine (T) substitution in exon 2 of the leptin gene of the *B. taurus* breeds. Pomp *et al.* (1997) using PCR-based polymorphism showed a rare polymorphism in the leptin gene. They reported that the *Sau3A*I restriction enzyme digested the PCR product four times instead of three times.

CSN3: κ-Casein A allele frequencies of Sistani cattle in this study are lower than the frequency reported in Iranian Holstein by Nassiry *et al.* (2003), but similar to frequency in German Black and White reported by Freyer *et al.* (1999). A rare polymorphism at an additional *Sau*3AI restriction site (the 690-bp fragment was digested into two fragments of approximately 470 and 220 bp) was observed in cattle from Simmental, Gelbvieh and Angus breeds. Present result did not show this polymorphism in Iranian Holstein dairy cows which is in agreement with other studies (Pomp *et al.*, 1997). Prinzenberg *et al.* (1997) was developed PCR-RFLP method to detecting CSN3 alleles (CSN3A, CSN3B, CSN3C, CSN3E, CSN3F and CSN3G). In addition to RFLP, polymorphisms of the CSN studied using other methods. Barroso *et al.* (1999) investigated CSN polymorphisms in various breeds of cattle using SSCP method. They detected four variants (A, B, C and D) in this gene and each variant yielded patterns clearly distinguishable from the others. The results showed that the frequency of B allele in Sistani cows is high. Therefore, there is a probability of selection the better quality for higher protein content and cheese yield.

Calpastatin: A *Taq*I Restriction Fragment Length Polymorphism was identified in the Bovine Calpastatin Gene (Cockett *et al.*, 1995) and three alleles (1, 2 and 3) were resulted. Chung *et al.* (2001) detected a genetic polymorphism in intron 6 of bovine Calpastatin gene by PCR-RFLP in purebred Angus cattle. Two alleles of A and B were observed with 0.75 and 0.25 respective frequencies. Recently, Schenkel *et al.* (2006) were identified a single nucleotide polymorphisms (SNP) in the CAST gene (G to C substitution) and genotyped on crossbred commercially fed heifers, steers and bulls from beef feedlots and steers. The allele frequencies of CAST C and G were 0.63 and 0.27, respectively. Chung *et al.* (1999) were identified two alleles (A and B), resulting in three genotypes (AA, AB and BB) using SSCP method in purebred Angus cattle. Allelic frequencies for A and B were 0.29 and 0.71, respectively.

BoLA-DRB3: Comparison between the present results with the allele spectrum and the gene frequencies profile previously reported in Bos taurus showed that 4 alleles (DRB3.2*47, *34, *45 and *51) were found in Sistani and Zebu Brahman Cattle. The most frequently detected BoLA alleles of Kenya Boran cattle, Caracu and Saavedreo Creole was DRB3.2*8. In present study, the same results were observed with Sistani cows. In present study with Iranian Sistani meat breed, alleles DRB3.2*27, *33, *42, *27, *18, *31,*5, *35, *6, *20, *16, *2, *6 were not observed. In contrast, allele *8, which had a frequency of 0.70, 2.94 and 3.45 on Gir breed, Curraleiro and Argentinean Creole (Machado *et al.*, 2005), respectively, was the most frequent allele (0.22) in our study. Similarly, Miretti *et al.* (2001) showed that BoLA-DRB3.2*8 accounted for 23.07% of the alleles in a population of Caracu cows in Brazil. The four most frequently alleles in Gir cows were BoLA-DRB3.2*16, *20, *2 and *29 and these accounted for about 51.7% of the alleles in this population (Machado *et al.*, 2005). BoLA-DRB3.2 alleles number 6,*2 and *27 were found in Kenya Borau cattle, not observed in Sistani cattle. The three least frequently isolated alleles in Golpayegani (Mosafer and Nassiry, 2005) and Sistani were BoLA-DRB3.2*4,*3 and *24. DRB3.2*7 Allele, which was a low frequency allele in Sistani cattle is the most common in Ayrshire cattle. More significant distinctions have been found between Iranian Sistani cattle and Bos taurus breeds studied. Polymorphism of the BoLA-DRB3 gene has been reported in the studies of Holstein-Friesian (Nassiry *et al.*, 2005); Jersey (Gilliespie *et al.*, 1999); Japanese Black, Japanese Shorthorn and Japanese Jersey cattle (Takeshima *et al.*, 2002) and Russian Ayrshire and Russian Black Pied cattle breeds (Udina *et al.*, 1998).

The Sistani cattle breed represented excellent source of biological information for studies on genetic characterization. This breed has been kept in closed station with no selection for these loci; therefore, we observed a low degree of genetic variability for the Leptin, κ-Casein and *Calpastatin loci*. This may be explained by conservation and breeding methods that have been carried out. Here, a few sires were selected and used for breeding. With respect to low effective number of population the inbreeding rate is high and so heterozigosity and genetic variability is low. For this problem it seems that in this station must be used the sires from other population. Although low variables were found for the those loci, in the other hand, these data provided an evidence some that Iranian's Sistani cattle had a good polymorphism for Leptin, κ-Casein and *Calpastatin loci*, which opens interesting prospects for future selection programs, especially marker-assistant selection between different genotypes of different locus and reproduction, milk traits, gain and meat traits. The present study may be regarded as the beginning of attempts to understand the genetic variability of Iranian native cattle breeds.

REFERENCES

Barroso, A., S. Dunner and J. Canon, 1999. A multiplex PCR-SSCP test to genotype bovine betacasein alleles A1, A2, A3, B and C. Anim. Genet., 30: 322-323.

Boom, R., C.J.A. Sol and M.M.M. Salimans, 1989. Rapid and simple method for purification of nucleic acids. J. Clin. Microbiol., 28: 495-503.

Buchanan, F.C., C.J. Fitzsimmons, A.G. Van Kessel, T.D. Thue, D.C. Winkelman Sim and S.M. Schmutz, 2002. Association of a missense mutation in the bovine leptin gene with carcass fat content and leptin mRNA levels. Genet. Sel. Evol., 34: 105-116.

Chung, H.Y., M.E. Davis and H.C. Hines, 1999. Effect of the calpain proteolysis and calpain genotype on meat tenderness of Angus Bulls. J. Anim. Sci., 77 (Suppl. 1): 31.

Chung, H.Y., M.S. Davis and H.C. Hines, 2001. Effect of calpain and calpastatin genotypes on growth of Angus Bulls. Research and reviews: Beef and sheep. Ohio State University Extension Research Bulletin: Special Circular, pp: 181-191.

Cockett, N.E., S.P. Jackson, G.D. Snowder and M. Georges, 1995. Rapid communication: A *TaqI* restriction fragment length polymorphism in the bovine calpastatin gene. J. Anim. Sci., 73 (Suppl. 1): 3790.

Dekkers, J.C.M., 1998. Optimizing strategies for selection on major genes. 47th Annual National Breeders Roundtable Proceedings. May 7-8, Missouri, America.

Ferretti, L., P. Leone and V. Sgaramella, 1990. Long range restriction analysis of the bovine casein genes. Nucleic Acids Res., 18: 6829-6833.

Freyer, G., Z. Liu, G. Erhardt and L. Panicke, 1999. Casein polymorphism and relation between milk production traits. J. Anim. Breed. Genet., 116: 87-97.

Gilliespie, B.E., B.M. Jayarao, H.H. Dowlen and S.P. Oliver, 1999. Analysis and frequency of bovine lymphocyte antigen DRB3.2 alleles in Jersey cows. J. Dairy Sci., 82: 2049-2053.

Javanmard, Elasi-Zarringabayi, Gharadaghi, Nassiry and Javadmanash, 2005. Molecular analysis of the bovin leptin gene in Iranian Sarabi cattle. 56th Annual Meeting of the European Association for Animal Production, Uppsala.

Kemenes, P.A., L.C.A. Reginato, A.J.M. Rosa, I.U. Parker, G.A. Razook, L.A. Figueiredo, N.A. Silva, M.A.L. Etchegaray and L.L. Coutinho, 1999. κ-casein, β-lactoglobulin na growth hormone allele frequencies and geneticdistances in Nelore, Gyr, guzerá, Caracu, Charolais, Canchin and Santa Gertrudis cattle. Genet. Mol. Biol., 22: 539-541.

Koohmaraie, M., S.D. Shackelford, T.L. Wheeler, S.M. Longergan and M.E. Doumit, 1995. A muscle hypertrophy condition in lamb (callipyge): Characterization of effects on muscle growth and meat quality traits. J. Anim. Sci., 73: 3596.

Liefers, S.C., R.F. Veerkamp and T. Vander Lene, 2002. Association between leptin gene polymorphism and production, live weight energy balance, feed intake and fertility in Holstein heifers. J. Dairy Sci., 85: 1633-1638.

Lien, S. and S. Rogne, 1993. Bovine casein haplotypes: Number, frequencies and applicability as genetic markers. Anim. Genet., 24: 373-376.

Machado, M.A., C.S. Nascimento and M.L. Martinez, 2005. Association of BoLA-DRB3.2 locus with bovine milk production in Gir breed. Arq. Bras. Med. Vet. Zootec., 57: 380-389.

Madeja, Z., T. Adamowicz, A. Chmurzynska, T. Jankowski, J. Melonek, M. Switonski and T. Strabel, 2004. Short communication: Effect of leptin gene polymorphisms on breeding value for milk production traits. J. Dairy Sci., 87: 3925-3927.

Miretti, M.M., J.A. Ferro, M.A. Lara and E.P. Contel, 2001. Restriction Fragment Length Polymorphism (RFLP) in exon 2 of the BoLA-DRB3 gene in South American cattle. Biochem. Genet., 39: 311-324.

Mosafer, J. and M.R. Nassiry, 2005. Identification of bovine lymphocyte antigen DRB3.2 alleles in Iranian Golpayegani cattle by DNA test. Asian Aust. J. Anim. Sci., 18: 1691-1697.

Moussavi, A.R.H., M. Ahouei, M. Nassiry and A. Javadmanesh, 2006. Association of leptin polymorphisms with production, reproduction and plasma glucose level in Iranian Holstein cows. Asian Aust. J. Anim. Sci., In Press.

Nassiry, M.R., M. Heydarpour, A. Mohammadi and S. Khorsand Pariszad, 2003. Identification of bovine kappa-casein genotypes in two Iranian Holestein farms by PCR-RFLP, Brazil.

Nassiry, M.R., F. Eftekhar shahroodi and J. Mosafer, 2005. Analysis and frequency of bovine lymphocyte antigen (BoLA-DRB3) Alleles in Iranian Holstein cattle. Rus. J. Genet., 41: 817-822.

Nei, M. and W.H. Li, 1979. Mathematical model for studying genetic variation in terms of the restriction endonucleases. Proc. Nat. Acad. Sci., USA., 76: 5269-5273.

Palmer, B.R., N. Roberts, J.G.H. Hickford and R. Bickerstaffe, 1998. Rapid Communication: PCR-RFLP for *MspI* and *NcoI* in the Ovine Calpastatin Gene. J. Anim. Sci., 76: 1499-1500.

Pomp, D., T. Zou, A.C. Clutter and W. Barendse, 1997. Rapid communication mapping of leptin to bovine chromosome 4 by linkage analysis of a PCR-based polymorphism. J. Anim. Sci., 75: 1427.

Prinzenberg, E.M., S. Hiendleder, T. Ikonen and G. Erhardt, 1997. Molecular genetic characterization of new bovine κ-casein alleles CSN3F and CSN3G and genotyping by PCR-RFLP. Anim. Genet., 27: 347-349.

Schenkel, F.S., S.P. Miller, Z. Jiang, I.B. Mandel, H. Li and J.W. Wiltin, 2006. Association of a single nucleotide polymorphism in the calpastatin gene with carcass and meat quality traits of beef cattle. J. Anim. Sci., 84: 291-299.

Stone, R.T., S.M. Kappes and C.W. Beattie, 1996. The bovine homolog of the obese gene maps to chromosome-4. Mamm. Genome., 7: 399-400.

Takeshima, S., Y. Nakai, M. Ohta and Y. Aida, 2002. Characterization of DRB3 alleles in the MHC of Japanese Shorthorn cattle by polymerase chain reaction-sequence-based typing. J. Dairy Sci., 85: 1630-1632.

Tavakkolian, J., 2000. Indigenous Livestock Genetic Resources of Iran. Animal Science Research Center Publication.

Udina, I.G., E.E. Karamysheva and G.E. Sulimova, 1998. Comparative analysis of Ayrshire and Black Pied cattle breeds by histocompatibility markers. Russ. J. Genet., 34: 1668-1674.

Van Eijk, M.J.T., A. Stewart-Hayaes and A. Lewin, 1992. Extensive polymorphism of the BoLA-DRB3 gene distinguished by PCR-RFLP. Anim. Genet., 23: 483-496.