Association of *PIT1* gene and milk protein percentage in Holstein cattle

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Abstract The pituitary-specific transcription factor (*PIT-1*) gene is a candidate gene for growth, carcass and also for milk yield traits. In dairy farm animals, the main goal of the selection is the improvement of milk yield and composition. The genes of milk proteins and hormones are excellent candidate genes for linkage analysis with quantitative trait loci (QTL) because of their biological significance on the quantitative traits of interest. Thus, the objective of this study was to analyze the association between polymorphism of the *PIT1* gene and milk protein percentage in Holstein cattle sampled from a dairy farm included 1000 animals in Khorasan Razavi province, east of Iran. A total of 100 cattle were randomly sampled in the study. Genomic DNA was extracted from the whole blood. One pair primers was used for amplification of *PIT1* gene and PCR products were electrophoresed on 1% agarose gel. Then PCR products were digested with *Hinf*I restriction enzyme. The genotypic data were analyzed using PopGene software. Allelic frequencies of A and B were 0.25 and 0.75, respectively. Frequencies of AA, AB and BB genotypes were 0.06, 0.40 and 0.54, respectively. The number of observed alleles, number of effective alleles, expected heterozygosity, observed heterozygosity, mean of heterozygosity, expected hemozygosity, observed hemozygosity, Nei's index and Shanon's index were 2.00, 1.66, 0.37, 0.40, 0.38, 0.62, 0.59, 0.37 and 0.56, respectively. Results of Chi-square test showed that the population is in Hardy-Weinberg equilibrium. The results of the association study between milk protein percentage and the observed genotypes indicated that the effect of genotype on protein percentage was significant ($P<0.01$) and AB genotype had the most effect on milk protein percentage suggesting that this polymorphism can be used as a molecular marker for this trait.

Keywords: polymorphism, *PIT1* gene, PCR, Holstein cattle, milk protein

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Introduction

Iran has a cattle population of 7.9 million of which 45.9, 43.6 and 10.51% are of the indigenous, crossbred and registered (mainly Holstein) cattle. The contribution of livestock to the national economy is 4% of total GDP. FAO (1993) announced that, Iranian dairy production has undergone significant and considerable structural changes during the last two decades due to creating larger herds. According to the FAO (1993) report, artificial insemination (AI) coverage in several countries including Iran increased remarkably over the 1980s decade. In general, four groups of sires in terms of their origin are available to dairy producers through AI in Iran. These are American, Canadian, European and Iranian sires, which regardless of their origin can be further categorized to two groups: summarized or sampling sires. Summarized sires are those that have been progeny tested; thus, an estimate of their daughter's producing ability is available. Sampling sires are those that have been selected to transmit high production qualities or type-related traits based on their pedigree. However, they also need to be progeny tested to determine more accurately which sires will pass their high production and type-related traits to their daughters. Many Iranian dairy producers are reluctant to use foreign sampling sires because their daughters' performance is considered somewhat unpredictable. However, Iranian sampling sires are very reasonable and hence, some farmers prefer to use them on the repeat breeder cows as well as on the moderate to low foreign cows (Heravi Moussavi and Danesh Mesgaran**,** 2009).

Using genes concerning with economic characteristics of farm animals for marker assisted selection (MAS) can aid on the selection of animals with the most desirable breeding values. Choosing the best genotypes based on the phenotypic values of the animals for quantitative characters is difficult (Askari et al., 2011). In other words, phenotypic values do not always reflect the genotypic values especially, additive genotypic values

of the animals. The improvement of any trait in population primarily depends on its economic gain (Zamani et al., 2013). Milk yield and components are quantitative traits controlled by many genes, most of them with small effect. Dairy cattle breeders have primarily concentrated on the high milk yield per cow until now. However, the milk components should not be ignored in selection programs because component percentages tend to have negative genetic correlation with milk yield (Othman et al., 2011). Although the change in the percentage of milk components, especially the fat and protein level using nutrition may be achieved to a desirable level, this approach ignores the animal genetic effect, and also is not permanent (Soyeurt et al., 2006). In short, the planning of a breeding program intended for maintaining the desired levels of milk components as well as increasing milk yield is important. However, focus on the traits and their economic weights in selection would be linked to dairy markets, production systems, feed supply and cost, and the presence of data and its usability with the industry of countries (Shook, 2006).

Bovine PIT-1, a 291 amino acid protein with DNA-binding POU domain (De-Mattos et al., 2004), is a pituitary-specific transcription factor that is responsible for pituitary development and hormone secreting via gene expression in mammals (Othman et al., 2011). *PIT-1* gene was sequenced by Bodner et al. (1988). *Pit-1* (POU1F1) is a member of the POU-domain family of genes that play important regulatory roles in developmental processes (Dybus et al., 2004). Pit-1, an approximate 31-33-kilodalton protein (291 amino acid), was first associated with a critical role in the transcriptional regulation of growth hormone (*GH*) and prolactin (*PRL*) genes (Dybus et al., 2003). Molecular basis of this polymorphism was the silent mutation (G→A) located within exon 6 of the *Pit-1* gene (Dierkes et al., 1998). *Pit-1* gene is considered as a candidate marker for milk production due to its role in regulation of expression of *bGH* and the prolactin genes which are essential for mammary gland development and milk yield (Dybus et al., 2004). *Pit-1* gene has been sublocalized to the centromeric region (1q21-22) of *Bos taurus* chromosome 1 (Moody et al., 1995). *Pit-1* gene consists of six exons and five introns and the Pit-1 protein consists of 3 domains; POU-homeo, POU-specific and the N-terminal region which plays a role in transactivation (Haugen et al., 1993; Kopp and Jameson 1998). Mutation in the *Pit-1* gene has been reported to be responsible for the dwarf phenotypes in mice (Camper et al., 1990; Li et al., 1990). In mammals, some of the mutations in *Pit-1* gene subvert growth, prolactin and TSH hormones, and even cause abnormalities of pituitary development called hypoplasia (Renaville et al., 1997a).

Pit-1 is essential for development of somatotrope, lactotrope, and thyrotrope cells in the anterior pituitary and it transactives expression of the genes encoding GH, PRL, and TSH-b hormones. Mutations in the human *PIT-1* are responsible for a combined pituitary hormone deficiency (CPHD) with deficiency of GH, PRL or TSH hormones while the production of ACTH, LH and FSH hormones is preserved and it leads to late puberty and hypothyroidism (Bona et al.,, 2004). *Pit-1* gene is considered to be a candidate gene for the regulation of growth and development in cattle and other mammals because PRL and GH are effective in proliferation of somatotrophic cells as well as they are necessary for mammary gland development and milk yield (Zhang et al., 2009). In cattle, *Pit1* was found to be related to milk yield, protein yield, protein percentage and some conformation traits in Italian Holstein-Friesian bulls (Renaville et al., 1997a), body weight in double-muscled Belgian Blue cattle (Renaville et al., 1997b), some feeding criteria and carcass dimensions in the fattening performance of Holstein-Friesian bulls (Oprzadek et al., 2003), fat milk production in Gyr bulls (De Mattos et al., 2004), milk yield in Holstein-Friesian (Vargas et al., 2004), growth traits in Nanyang cattle (Xue et al., 2006), growth traits of Canchim animals (Carrijo et al., 2008), and also birth weight and height at withers of Geman Yellow x Qinchuan beef cattle (Zhang et al., 2009). There is no report on the study of *Pit-1* gene in Holstein cattle in Khorasan Razavi province of Iran by now. Thus, the aim of this study was to determine the allelic frequencies at the bovine *PIT1-Hinf*I locus and to investigate the relationship of the polymorphisms and milk protein percentage of Holstein cattle in Khorasan Razavi province of Iran.

Materials and Methods

A total of 100 cattle from a commercial herd containing 1000 animals, in Khorasan Razavi province of Iran were included in the study. Whole blood samples (approximately 5 mL per animals) were collected from the Jugular vein of each dairy cattle into tubes with EDTA and stored at −20°C until the DNA extraction. DNA extraction was carried out by Diatom DNA Prep Kit (Cinagen, Iran) as follows (Shojaei et al., 2011): Briefly, to an aliquot of 100 µl blood (after thawing), 400 µl of lysis buffer (Guanidin Thiocyanate, 20 mM; EDTA, 20 mM; Tris-HCl, 10 mM; Triton X100, 40 g/l; DTT, 10 g/l) was added, the mixture was vortexed and incubated at 65°C for 5 min. The cells were resuspended in 20 µl of nuclease solution (Silica gel: 4g, Guanidine solution: 100 ml) and Centrifuged for 10 sec at 12,000 ×g. The pellet was

resuspended in 200 µl of lysis buffer again. The suspended white blood cell suspension was then added to 400 µl of saline buffer (NaCl, 1M; Tris-HCL, 10 mM; KCl, 1M and EDTA, 20 mM), 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 (Ion Exchange Resin): 10%, Orange G color: 0.02%, Triton X100: 0.01%) and was incubated in 65°C for 3-5 min. Then protein was precipitated by centrifugation (3 min at 1000 ×g) and the upper layer containing the DNA was transferred to another tube. The relative purity of DNA was determined using gel electrophoresis on 1% agarose gel.

The sequences of the forward and reverse primers for the amplification of the *Pit-1*gene were:
5'-AAA CCA TCA TCT CCC TTC TT-3' and
5'-AAT GTA CAA TGT GCC TTC TGA G-3',
respectively.

The polymerase chain reaction for the *Pit-1* gene was performed in a 25 µl reaction mixture, containing 1.5 mM MgCl2, 200 µM of each dNTPs, 0.3 µM of each primers, 1X PCR buffer, 1U *Taq* polymerase (Cinagen, Iran) and 100 ng of genomic DNA template. The reaction mixture was placed in a DNA thermal cycler. Thermal cycling conditions included: an initial denaturation step at 95°C for 4 min followed by 35 cycles of 94°C for 45 sec, 59.7°C for 45 sec, 72°C for 1 min and a final extension at 72°C for 10 min.

PCR products were digested with 7 U of *HinfI* restriction enzyme at 37 °C overnight in incubator. Restriction fragments from the above PCR reactions were electrophoresed on 2% agarose gels and stained with ethidium bromide. The allele and genotype frequencies were estimated by counting method. The heterozygosity (as gene variation indicates) was calculated using the PopGene software version 1.31 (Yeh et al., 1999), according to Nei procedure (1978). The Chi-square test was used to determine whether the distribution of the genotype frequencies was in the Hardy-Weinberg equilibrium. The population genetic parameters were estimated using PopGene Version 1.31 (Yeh et al., 1999), as described by Nei (1973).

The next stage involved an analysis of association between the *Pit-1* genotypes and milk protein percentage. The following linear model was used for the statistical analysis:

$$y_{ij} = \mu + G_i + S_j + \varepsilon_{ij} \quad (1)$$

where: y_{ij} observed milk protein percentage in *ij*-th animal; µ: mean of milk protein percentage for population; G_i: fixed effects of the different genotypes of *Pit-1* (AA, AB, BB); S_j: fixed effects of sires; ε_{ij}: random error. The comparison between the different genotypes means for milk protein percentage was conducted using the Tukey method (SAS Institute 2002 Ver. 9).

Results and Discussion

The extracted DNA had good quality with no protein and phenol contamination (Figure 1). The expected size of PCR product was 451bp and DNA was successfully amplified (Figure 2). The amplified DNA fragments (451bp) were digested with *Hinf*I enzyme and electrophoretically separated to detect the genetic polymorphisms of PIT-1 gene. The point mutation (A→G) in exon VI, affecting a *Hinf*I restriction site, was used to differentiate between two alleles, A and B. The restrict-

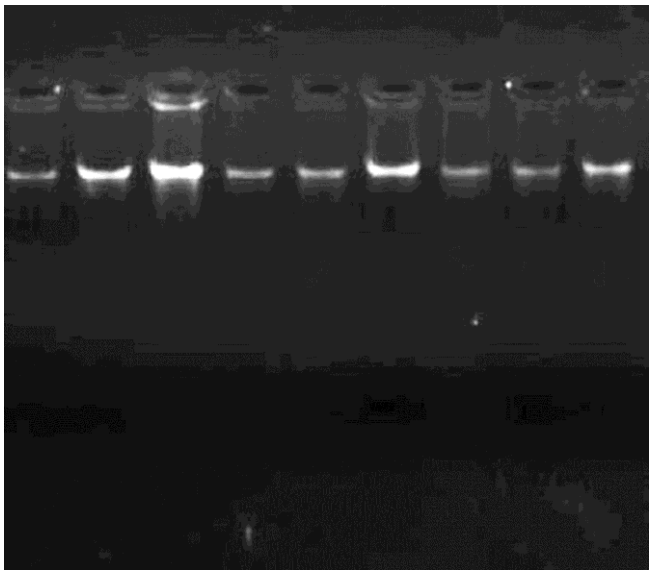

Fig. 1. Some samples of the extracted DNA from studied animals on 1% agarose gel.

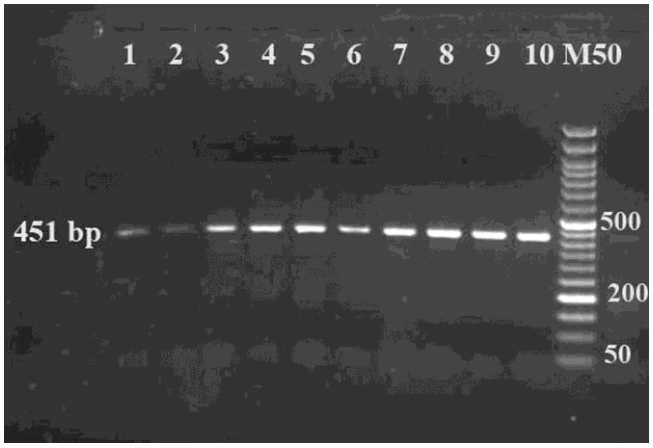

Fig. 2. Ethidium bromide-stained agarose gel of amplified PCR products representing amplification of *PIT-1* gene in Holstein cattle in Khorasan Razavi province of Iran. Lane M50: 50-bp ladder marker. Lanes 1–10: 451bp PCR products amplified from DNA of studied cattle.

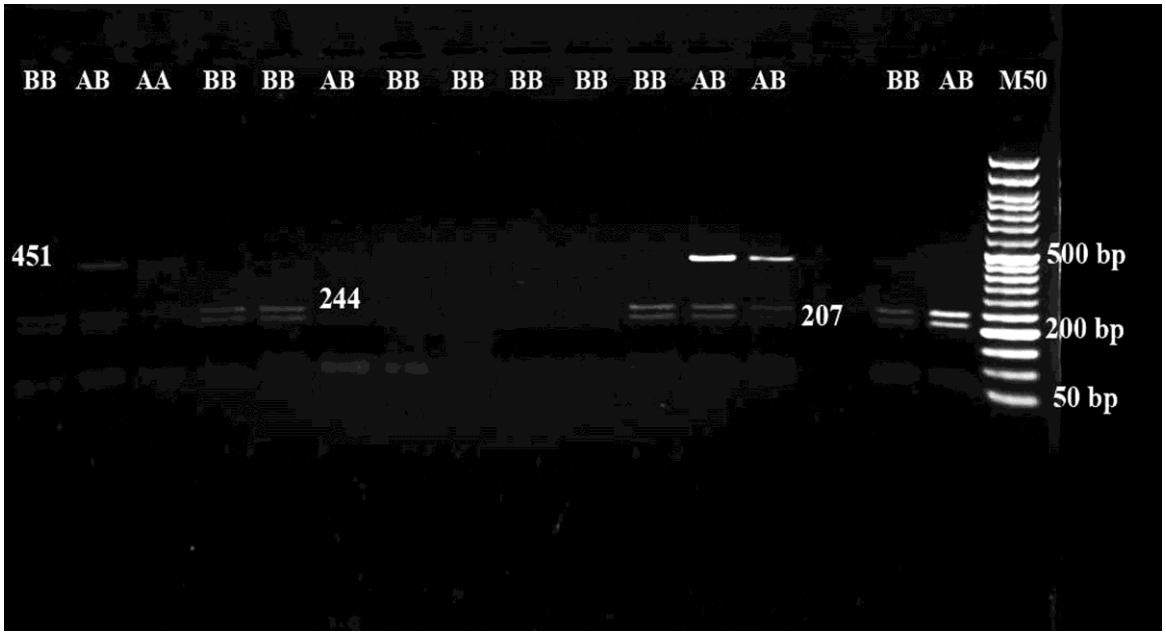

Fig. 3. PCR products digested with *Hinf*I on 2% agarose gel electrophoresis stained with ethidium bromide. AA: undigested PCR product, AB: digested PCR product (451, 244 and 207 bp) and BB: digested PCR product (244 and 207 bp).

ion fragments obtained for the PIT-1 gene polymorphism were: 244 and 207bp for BB genotype; 451, 244 and 207bp for AB genotype and 451bp (undigested fragment) for AA genotype (Figure 3).

The genotypic and allelic frequencies of Pit-1 polymorphisms (*Hinf*I 451bp) in Holstein cattle in Khorasan Razavi province of Iran has been given in Table 1. Based on these results, from 100 studied Holstein cattle 6, 40 and 54 heads were determined as AA, AB and BB genotypes, respectively. The A and B allele frequencies were estimated as 0.26 and 0.74, respectively. The population was found in the Hardy-Weinberg equilibrium based on *Hinf*I polymorphism in the present study (P>0.05).

The number of observed alleles, number of effective alleles, expected heterozygosity, observed heterozygosity, mean of heterozygosity, expected hemozygosity, observed hemozygosity, Nei's index and Shanon's index were 2.0, 1.6, 0.37, 0.40, 0.37, 0.62, 0.59, 0.37 and 0.56, respectively. Results of different studies on genotype and allele frequencies of Pit-1 (*Hinf*I) polymorphism are shown in Table 2. In most of the breeds studied in terms of Pit-1 polymorphisms (*Hinf*I 451 bp), the A allele seems to have a lower frequency values than B allele (Table 2), a situation similar to our study. In the present study, the frequency of AA genotype was lower than frequency of BB genotype confirming the results of other researchers (Table 2).

Table 1. The genotypic and allelic frequencies of *Pit-1 Hinf* I polymorphism in Holstein cattle in Khorasan Razavi province of Iran

Genotype	Number of genotypes	Genotypic frequency	Allele	Allelic frequency
AA	6	0.06	A	0.26
AB	40	0.40		
BB	54	0.54	B	0.74
Total	100	1		

The value of expected heterozygosity was calculated to be 0.37 in Holstein cattle in Khorasan Razavi province of Iran. When compared with literature, this value was lower than the findings of 0.50 in Nanyang cattle (Xue et al., 2006), 0.43 in East Anatolian Red breed cattle (Özdemir 2012), 0.47 in Brown Swiss cattle (Aytekin and Boztepe, 2013), 0.48 in East Anatolian Red cattle (Özdemir, 2012), 0.38 in Holstein cows (Edriss et al., 2008), 0.44 in Angus x Qinchuan cattle (Zhang et al., 2009), 0.39 in Charolais×Nelore cattle (Carrijo et al., 2008), 0.47 in Manzadrani cattle (Zakizadeh et al., 2007), 0.40 in Sarabi cattle (Zakizadeh et al., 2007), 0.45 in Golpayegani cattle (Zakizadeh et al., 2007), 0.47 in Sarabi cattle (Javanmard et al., 2005), 0.38 in Golpayegani cattle (Javanmard et al., 2005), 0.47 in Dashtiyari cattle (Javanmard et al., 2005), 0.47 in Golpayegani x Brown Swiss F1 cattle (Javanmard et al., 2005), 041 in Holstein-Friesian cattle (Vargas et al., 2004), 0.44 in Angus beef cattle (Zhao et al., 2004), 0.48 in Belgian Blue cattle (Renaville et al., 1997b) and 0.43 in Angus cattle (Curi et al., 2006).

This value was more than the estimates of 0.32 in Holstein cattle (Özdemir, 2012), 0.30 in Najdi cattle (Be-

Table 2. Results of different studies on genotypic and allelic frequencies of *Pit-1 Hinf*I polymorphism

Breed	Genotypic requency			Allelic frequency		Expected heterozygosity	Length of PCR fragment	Reference
	AA	AB	BB	A	B			
Brown Swiss cattle	0.12	0.51	0.37	0.37	0.63	0.47	451 bp	Aytekin and Boztepe, 2013
East Anatolian Red	0.14	0.54	0.32	0.41	0.59	0.48	260 bp	Özdemir, 2012
Holstein	0.04	0.31	0.65	0.20	0.80	0.32		
Najdi	0.36	0.30	0.67	0.18	0.82	0.30	451 bp	Beigi Nassiri *et al*., 2010
Holstein-Friesian	0.02	0.45	0.53	0.24	0.76	0.37	611 bp	Misrianti *et al*., 2010
Jordan native cattle	0	0.18	0.82	0.09	0.91	0.16	422 bp	Jawasreh *et al*., 2009
Holstein-Friesian	0.05	0.25	0.70	0.17	0.83	0.29		
Qinchuan	0.03	0.40	0.57	0.23	0.77	0.36	451 bp	Zhang *et al*., 2009
Limousin x Qinchuan	0.04	0.28	0.68	0.18	0.82	0.30		
Angus x Qinchuan	0.11	0.44	0.45	0.33	0.67	0.44		
Germany Yellow x Qinchuan	0.07	0.21	0.72	0.18	0.82	0.29		
Holstein cows (4 herds)	0.03	0.45	0.52	0.26	0.74	0.38	451 bp	Edriss *et al*., 2008
16 distinct Indian native cattle (*Bos indicus*)	0	0.12	0.88	0.06	0.94	0.12	1350 bp	Mukesh *et al*., 2008
5/8 Charolais ve 3/8 of Zebu	-	-	-	0.13	0.87	0.23	1301 bp	Carrijo *et al*., 2008
21/32 Charolais ve 11/32 Nelore	-	-	-	0.27	0.73	0.39		
Simmental	0.12	0.20	0.68	0.22	0.78	0.34	1350 bp	Viorica *et al*., 2007
Manzadrani	0.17	0.41	0.42	0.37	0.63	0.47	451 bp	Zakizadeh *et al*., 2007
Sarabi	0.08	0.38	0.54	0.27	0.73	0.40		
Golpayegani	0.11	0.45	0.44	0.34	0.66	0.45		
Holstein	0.06	0.30	0.64	0.21	0.79	0.33		
Nellore	0.79	0.21	0	0.90	0.10	0.19	1301 bp	Curi *et al*., 2006
Canchim	0.80	0.17	0.03	0.88	0.12	0.21		
1/2 Simmental	0.73	0.27	0	0.87	0.13	0.23		
1/2 Angus	0.30	0.69	0.01	0.64	0.36	0.43		
Qinchuan	-	-	-	0.23	0.77	0.36	451 bp	Yan *et al*., 2006
China Holstein-Friesian	-	-	-	0.13	0.87	0.23		
Nanyang	0.21	0.51	0.28	0.47	0.53	0.50	451 bp	Xue *et al*., 2006
Sarabi	0.45	0.34	0.21	0.62	0.38	0.47	600 bp	Javanmard *et al*., 2005
Golpayegani	0.61	0.26	0.13	0.74	0.26	0.38		
Sistani	0.84	0.16	0	0.92	0.08	0.14		
Taleshi	0.61	0.32	0.07	0.77	0.23	0.35		
Manzadrani	0.69	0.27	0.04	0.83	0.17	0.29		
Dashtiyari	0.62	0	0.38	0.62	0.38	0.47		
Golpayegani x Brown Swiss F1	0	0.77	0.23	0.39	0.61	0.47		
Holstein-Friesian	0.10	0.35	0.55	0.28	0.72	041	451 bp	Vargas *et al*., 2004
Poland Black-and-White cows	0.05	0.38	0.57	0.24	0.76	0.37	451 bp	Dybus *et al*., 2004
Gry bulls	0.90	0.10	0	0.95	0.05	0.10	~1.355 bp	De Mattos *et al*., 2004
Angus beef cattle	0.11	0.44	0.45	0.33	0.67	0.44	451 bp	Zhao *et al*., 2004
Black-and- White bulls	0.06	0.37	0.57	0.25	0.75	0.37	451 bp	Oprzadek *et al*.. 2003
Holstein	0.03	0.26	0.71	0.16	0.84	0.26	451 bp	Barreras-Serrano, 2003
Belgian Blue	0.20	0.44	0.36	0.42	0.58	0.48	451 bp	Renaville *et al*., 1997
Italian Holstein-Friesian bulls	0.02	0.32	0.66	0.18	0.82	0.31		

igi Nassiri et al., 2010), 0.16 in Jordan native cattle (Jawasreh et al., 2009), 0.29 in Holstein-Friesian cattle (Jawasreh et al., 2009), 0.36 in Qinchuan cattle (Zhang et al., 2009), 0.30 in Limousin×Qinchuan (Zhang et al., 2009), 0.29 in Germany Yellow×Qinchuan cattle (Zhang et al., 2009), 0.12 in distinct Indian native cattle (*Bos indicus*) (Mukesh et al., 2008), 0.23 in Charolais × Zebu cattle (Carrijo et al., 2008), 0.34 in Simmental cattle (Viorica et al., 2007), 0.33 in Holstein (Zakizadeh et al., 2007), 0.19 in Nellore cattle (Curi et al., 2006), 0.21 in Canchi cattle (Curi et al., 2006), 0.23 in Simmental cattle (Curi et al., 2006), 0.36 in Qinchuan cattle (Zhang *et al*., 2009), 0.23 in China Holstein-Friesian cattle (Yan et al., 2006), 0.14 in Sistani cattle (Javanmard et al.,

2005), 0.35 in Taleshi cattle (Javanmard et al., 2005),0.29 in Manzadrani cattle (Javanmard et al., 2005), 0.10 in Gry bulls (De Mattos et al., 2004), 0.26 in Holstein cattle (Barreras-Serrano, 2003), 0.31 in Italian Holstein-Friesian bulls (Renaville et al., 1997). However, similar results have been reported in other studies (e.g., 0.37 in Black and White bulls (Oprzadek et al., 2003; 0.37 in Holstein-Friesian cattle (Misrianti et al., 2010); 0.37 in Poland Black-and-White cows (Dybus et al., 2004))

High heterozygosity value in a population is due to the parental choice of increasing the frequency of heterozygotes in terms of the relevant gene. Especially, the bulls used in artificial insemination according to genes in relation to the economic traits such as milk yield and components are not pre-tested yet. This situation may alter the genetic makeup of the population by chance, and also can lead to deflection of the balance. Arora and Bhatia (2004) denoted that the high mean heterozygosity values could be attributed to low level of inbreeding, low selection pressure and large number of alleles present in a population.

The association of Pit-1 gene polymorphism with milk protein percentage in the studied population was significant (P<0.01). Comparison between the three genotypes (AA, AB and BB) based on least squares means showed that milk protein percentage of AB genotype was significantly more than those of the AA and BB genotypes (P<0.01) while milk protein percentage of AA genotype is significantly more than that of the BB genotype (P<0.01) (Table 3).

The AB genotype had higher milk protein percentage (P<0.01) when compared with the AA and BB genotypes while the AA genotype had higher milk protein percentage than the BB genotype. These results demonstrate that the allele A is dominant and the allele B is recessive. In addition, because of the superiority of heterozygous genotype over the homozygous genotype, it can be concluded that there is an over dominance gene action for *Pit-1* locus.

Our results for the association of the *PIT-1* gene and milk protein confirmed the findings from other researchers. Renaville et al. (1997a) stated that the A allele showed significant superiority over the B allele for milk yield), protein yield and also less fat percentage in Italian Holstein-Friesian bulls. Edriss et al. (2008) showed that protein percentage in AB genotype was significantly higher than the BB genotype (2.83 % vs. 2.68 %) of Holstein cows in Isfahanprovince of Iran . Parmentier et al. (1999) demonstrated significant superiority of the *Hinf*I B allele for milk (+222.4) and protein (+9.17) yields, but an inferiority for fat yield (-2.29%). The results can be interpreted as a single positive action of the A allele on protein yield and, to a lesser extent, on milk yield and fat content. This interpretation declared the milk production performance for *PIT-1* which is characterized by highest fat content other than milk yield or milk protein content. Based on our results and the literature investigating the relationship between *PIT1-HinfI* polymorphism and milk production traits, it is mentionable that A allele and AA genotype should be exploited for selection of dairy traits except for the Bos indicus cattle due to different genomic background (De Mattos et al., 2004).

Distribution differences of allele frequencies between different populations may indicate genetic differences in the base populations (Carrijo et al., 2008). In contrast to this, the results from the association analyses in different populations with the same distribution of allele frequencies may be varied. It is suggested that the linkage between milk protein and *PIT-1* should be more comprehensively studied by increasing the number of animals, molecular and quantitative data, especially taking into consideration the genotype × environment interactions and other genes affecting milk yield and components in the association trials. Besides, it would be more informative that other mutations within the *Pit-1* gene be evaluated.

Table 3. Least squares means and the standard errors of the three genotypes (AA, AB and BB) on milk protein percentage in Holstein cattle in Khorasan Razavi province of Iran

genotype	Least Squares Mean[1]	Standard Error
AA	3.26[b]	0.15
AB	3.62[a]	0.05
BB	3.22[c]	0.05

[1]Means with different superscripts differ (P<0.01).

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همبستگی ژن *PIT1* و درصد پروتئین شیر در گاو هلشتاین

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چکیده ژن فاکتور نسخه برداری ویژه هیپوفیز (*PIT-1*) یک ژن کاندیدا برای صفات رشد، لاشه و نیز تولید شیر می‌باشد. در حیوانات مزرعه‌ای، هدف اصلی انتخاب توسعه تولید و ترکیب شیر است. ژن‌های پروتئین‌ها و هورمون‌های شیر ژن‌های کاندیدای عالی، به دلیل اهمیت بیولوژیکی‌شان روی صفات کمی مورد علاقه، برای آنالیز پیوستگی با جایگاه‌های صفات کمی (QTL) هستند. بنابراین، هدف این پژوهش آنالیز همبستگی بین چندشکلی ژن *PIT1* و درصد پروتئین شیر در گاو هلشتاین نمونه برداری شده از یک گاوداری ۱۰۰۰ رأسی در استان خراسان رضوی، واقع در شرق ایران بود. از تعداد ۱۰۰ رأس گاو به تصادف در این پژوهش خونگیری شد. استخراج DNA ژنومی از خون کامل صورت گرفت. از یک جفت آغازگر برای تکثیر ژن *PIT1* استفاده و محصولات PCR روی ژل آگارز ۱ درصد الکتروفورز شدند. سپس محصولات PCR با آنزیم برشی *Hinf*I هضم شدند. داده‌های نوتیپی با استفاده از نرم‌افزار PopGene آنالیز شدند. فراوانی‌های آللی A و B به ترتیب ۰/۲۵ و ۰/۷۵ بودند. فراوانی‌های AA، AB و BB به ترتیب ۰/۰۶، ۰/۴۰ و ۰/۵۴ به دست آمد. تعداد آلل‌های مشاهده شده، تعداد آلل‌های موثر، هتروزیگوسیتی مورد انتظار، هتروزیگوسیتی مشاهده شده، میانگین هتروزیگوسیتی، هموزیگوسیتی مورد انتظار، هموزیگوسیتی مشاهده شده، شاخص نئی و شاخص شانون به ترتیب ۲، ۱/۶۶، ۰/۳۷، ۰/۴۰، ۰/۳۸، ۰/۶۲، ۰/۵۹، ۰/۳۷ و ۰/۵۶ بود. نتایج آزمون مربع کای نشان داد که جمعیت در تعادل هاردی-وینبرگ است. نتایج مطالعه همبستگی بین درصد پروتئین شیر و ژنوتیپ‌های مشاهده شده نشان داد که اثر ژنوتیپ روی درصد پروتئین معنی دار است ($P<0.01$) و ژنوتیپ AB بیشترین اثر را روی درصد پروتئین شیر داشت، لذا پیشنهاد ممکن این است که می‌توان از این چندشکلی به عنوان نشانگر ملکولی برای صفت درصد پروتئین شیر استفاده کرد.