ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 7 (2): 209-212, 2008
ISSN 1682-296X

Pit-1 Gene Polymorphism of Holstein Cows in Isfahan Province

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Abstract: A total of 262 Holstein cows of four different herd managements in Isfahan province were used to obtained polymorphism of *Pit-1* gene for a possible genetic marker information. Average frequency of the A allele among herds was 0.256 while the frequency of B allele was 0.744. Results showed there was no significant relationship between herd management and genotypic frequency, for different herds. However, BB genotype was the most frequent in the 1st, 2nd and 4th herds (0.500-0.600), while in the 3rd herd AB genotype was the most frequent one (0.564). AA genotype was the least frequent frequency (0.018-0.050) among all the genotypes. Statistical analysis showed that there was no Hardy-Weinberg equilibrium for average genotypic frequencies of different herds. Overall, the lack of equilibrium seems to be due to preference of AB genotype in the studied populations which could be as a result of selection that normally would apply in commercial herds. It should be noted that dairy producer in Iran, prefer dairy cattle for fast growing male calves and preference of fat milk daughters.

Key words: *Pit-1*, polymorphism, Holstein cattle, equilibrium, gene frequency

INTRODUCTION

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 gene expression in mammals (Cohen *et al*., 1997). *Pit-1* has been shown to control transcription of the growth hormone, prolactin (PRL) (Nelson *et al*., 1988; Mangalam *et al*., 1989), the thyroid-stimulation hormone, β-subunit (TSH- β), the growth hormone releasing hormone receptor genes and the *Pit-1* gene itself (Zhao *et al*., 2004). According to Herr *et al*. (1988) and Rosenfeld (1991) *Pit-1*, an approximately 33-kilodalton pituitary-specific protein, contains two domains, termed POU-specific and POU-homeo, which are both necessary for high-affinity DNA binding to promoters of the growth hormone and PRL genes. *Pit-1* activates the growth hormone and PRL gene expression, in part through an N-terminal transactivation domain rich in hydroxylated amino acid residues (Theill *et al*., 1989). Mutations in the human *Pit-1* are responsible for a Combined Pituitary Hormone Deficiency (CPHD) with deficiencies of growth hormone, PRL and TSH. There is more variability in the degree of hypothyroidism or delay puberty (Bona *et al*., 2004). Bovine *Pit-1* cDNA has been sequenced by Bodner *et al*. (1988). *Pit-1* was sublocalized to the centromeric region of bovine chromosome 1, located midway between TGLA57 and RM95 (Woollard *et al*., 2000). In the bovine *Pit-1* gene, the restriction fragment length polymorphism (for the *Hin*fI restriction enzyme) was identified (Moody *et al*., 1995). Molecular basis of this polymorphism was the silent mutation (G→A) located within the exon 6 of the *Pit-1* gene (Dierkes *et al*., 1998). There are a few reports on allelic and genotypic frequencies of *Pit-1* genes for some of the breeds and also relationships between these frequencies and some of the production traits (Renaville *et al*., 1997b; Zwierzchowski *et al*., 2002; Dybus *et al*., 2004; Zhao *et al*., 2004; Beauchemin *et al*., 2006).

The purpose of this study was to estimate allelic and genotypic frequencies of the *Pit-1* gene in Holstein cows of Isfahan province in Iran and test genotypic frequency for Hardy-Weinberg equilibrium.

MATERIALS AND METHODS

A total of 262 Holstein cows from four different herd managements in Isfahan province were randomly selected. Genomic DNA was extracted from the whole blood as described by Miller *et al*. (1988) with salting out method in biotechnology laboratory of Isfahan University of Technology from January-March 2007. The polymerase chain reaction was used to amplify the 451 bp DNA fragments from genomic DNA (Fig. 1). The PCR reaction contained 25-50 ng of genomic DNA, 12 pmol of each primer, 2 μL 10x PCR buffer, 1.5 mM $MgCl_2$, 200 μM dNTP and 1 unit *Taq*-polymerase in a total volume of 20 μL. The PCR primers (5-AAACCATCATCTCCCTTCTT-3) and (5-AATGTACAATGTGCCTTCTGAG-3) as described

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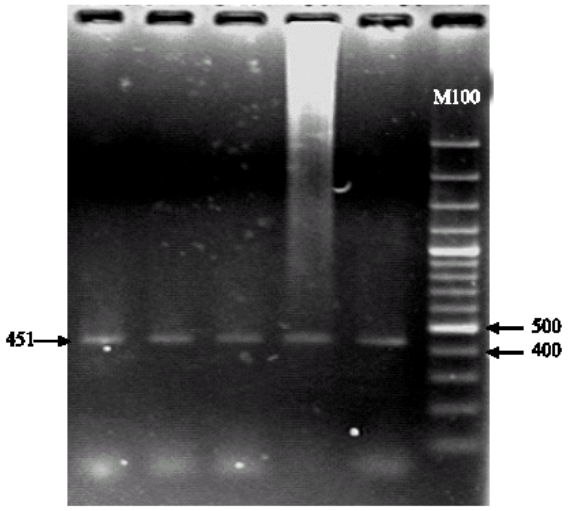

Fig. 1: PCR product of *Pit-1* locus for five different individuals. M100 column is the 100 bp-ladder plus to show the size of the PCR product (451 bp)

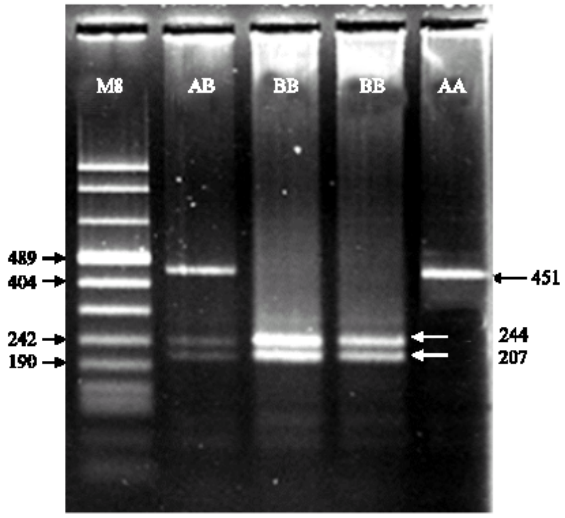

Fig. 2: *Hin*fI PCR/RFLP at the *Pit-1* locus. At right are predicted sizes of uncut (451) and cut DNA fragments (244, 207). Indicated at left are the sizes of the marker VIII

by Woollard *et al*. (1994) were designed from intron V and exon 6. PCR program was 95°C for 5 min, followed by 36 cycles of 95°C for 30 sec, 56°C for 1 min and 72°C for 2 min. The final step was at 72°C for 5 min. The PCR products were digested with the *Hin*fI restriction enzyme for the RLFP of the *Pit-1* gene, electrophoresed on 2% agarose gels and the genotypes were determined from ethidium bromide stained agarose gel under UV light.

RESULTS AND DISCUSSION

The following DNA restriction fragments were obtained for the *Pit-1* gene polymorphism: 244 and 207 bp for the BB genotype, 451, 244 and 207 for the AB and 451 bp (no digestion) for the AA (Fig. 2).

Chi-square analysis showed that there were no significant differences for herd management and genotypic frequencies (Table 1). Due to low number of observation for some genotype (AA genotype) Fisher's Exact Test was performed, there was neither significant difference between observed nor predicted ratio (Table 1).

However, BB genotype was the most frequent in the 1st, 2nd and 4th herds (0.500-0.600), but in the 3rd herd AB genotype was the most frequent genotype (0.564). The frequency of the AB genotype in the 1st, 2nd and 4th herds was followed by BB genotypic frequency (0.350-0.450). The frequency of the BB genotype in the 3rd herd was less than the AB genotypic frequency (0.418). Among all the herds, the least frequent genotype was the AA genotype (0.018-0.050). Furthermore, allelic frequency

Table 1: Genotypes and alleles frequencies of the *Pit-1* gene in different herds and the related statistical test

		Genotypes			Alleles	
Herd	N	AA	AB	BB	A	B
1st	40	0.050 (n = 2)	0.450 (n = 18)	0.500 (n = 20)	0.275	0.725
2nd	107	0.019 (n = 2)	0.449 (n = 48)	0.533 (n = 57)	0.243	0.757
3rd	55	0.018 (n = 1)	0.564 (n = 31)	0.418 (n = 23)	0.300	0.700
4th	60	0.050 (n = 3)	0.350 (n = 21)	0.600 (n = 36)	0.225	0.775

Statistics for herd ×genotype	df	Value	Prob
Chi-square	6	6.8187	0.3379
Likelihood ratio Chi-square	6	6.7799	0.3417
Mantel-Haenszel Chi-square	1	0.1976	0.6567
Fisher's exact test		3.671E-06	0.2801

analysis showed that the frequency of allele ranged from 0.225 to 0.300. As a results, the frequency of B allele ranged from 0.700 to 0.775 (Table 1).

Allelic frequencies were used to test Hardy-Weinberg equilibrium for genotypic frequencies of *Pit-1* gene (Table 2). Chi-square test result has shown that there were a significant difference between genotypic frequencies measured for average of the populations and those were expected by Hardy-Weinberg equilibrium ($p<0.01$). Comparing observed and expected genotypic frequencies showed that the AA and BB genotypes which were expected to be 0.066 and 0.553, respectively, were 0.031 and 0.519, respectively. Both frequencies were lower than expected while the AB frequency which was

Table 2: Average of the allelic and genotypes frequencies of the *Pit-1* gene in different herds and the related statistical test for Hardy-Weinberg equilibrium

		Genotypes			Alleles	
Herd	N	AA	AB	BB	A	B
Ave./Total	262	0.031 (n = 8)	0.450 (n = 118)	0.519 (n = 136)	0.256	0.744
Expected Hardy Weinberg Equilibrium		0.066	0.381	0.553		
Chi-square					8.7776	
DF					2.00	
Pr > Chi-square					0.01	
Sample size					262.00	

n = Observed number

expected to be 0.381 according to Hardy-Weinberg equilibrium was 0.450 which was lower than observed measured (Table 2).

The genetic equilibrium in the studied herd managements was not disturbed. The same result was reported by Dybus *et al.* (2004) who were working on different populations of Polish Black and White cattle. The calculated frequency of A allele in all previous studies was lower than the present study (Polish Black and White cattle, 0.25 and 0.243, Brahman steers, 0.059 and Italian Holstein-Friesian Bulls, 0.18).

There are several reports on genotypic frequency of the AA genotype in different breeds of cattle. The frequency of the AA genotype for Angus beef cattle was reported, 0.11 (Zhao *et al.*, 2004); for Polish Black and White cattle, 0.09 and 0.052 (Zwierzchowski *et al.*, 2002; Dybus *et al.*, 2004) and for Brahman steers, 0.06 (Beauchemin *et al.*, 2006); which all of them were higher than the frequency in the present study. However, in contrast the frequency of AA gene for Italian Holstein-Friesian bulls was 0.022 (Renaville *et al.*, 1997b) which were lower than the present study. Genetic disequilibrium which was found in the present study showed that the selection procedures are in the favors of AB genotype, which was higher than expected frequency in the average of different herd managements.

Previously, it was stated that the *Pit-1* gene can control the amount of growth hormone and prolactin production which are essential parts of mammary gland development and the milk yield production. *Pit-1* gene has a potential to explain genetic variations in dairy traits (Zwierzchowski *et al.*, 2002). Renaville *et al.* (1997b) in a study on polymorphism of *Pit-1* gene on Italian Holstein-Friesian bulls revealed that A allele had a positive effect on milk yield traits, body depth, angularity and rear leg set. In another report, Renaville *et al.* (1997a) found a good relationship between B allele and higher body weight at 7 months of age in double muscled Belgian Blue bulls. Also another report confirmed that there was a desirable relationship between the A allele and the daily milk yield and milk composition in Polish Black and White cows (Zwierzchowski *et al.*, 2002). Parmentier *et al.* (1999) demonstrated a significant superiority of the A allele for milk and protein yield, but an inferior effect of this allele for fat yield.

Recently, De Mattos *et al.* (2004) found that the heterozygous *Hin*fI (AB) sires were superior in relation to the *Hin*fI (BB) sires for milk and fat production in dairy Gyr bulls ($p<0.05$). In recent years in Iran, there is notable emphasis on selection of sire which has ability of producing daughter with good ability for fat milk production. Also there is good intensity of selection for those sires which are able to produced fast growing calves. However, it can be concluded that these attempts might be effective to preference of AB genotype in the populations under selection which is in agreement with the conclusions drawn by some researchers (Renaville *et al.*, 1997b; De Mattos *et al.*, 2004).

ACKNOWLEDGMENT

To Isfahan University of Technology for supporting Vahid Edriss's M.Sc. Thesis financially.

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