The Journal of Animal & Plant Sciences, 23(5): 2013, Page: 1281-1289
ISSN: 1018-7081

ASSOCIATIONS OF PIT-1 GENE POLYMORPHISM WITH MILK YIELD AND COMPOSITION TRAITS IN BROWN SWISS CATTLE

İ. Aytekin and S. Boztepe

Faculty of Agriculture, Department of Animal Science, University of Selcuk, Konya, TURKEY
Corresponding Author E-mail: aytekin@selcuk.edu.tr

ABSTRACT

The present study investigated the genetic variability of Pit-1 polymorphisms (*Hinf*I_451 bp) in a total of 301 Brown Swiss cattle, and also evaluated the possible association between the Pit-1 genotypes and dairy traits such as 305 days milk yield (MY_{305}), test day milk yield (TDMY), fat (%), protein (%), lactose (%), density (kg/m^3), solids-non-fat (%), total solids (%), freezing point (^oC), and also pH and conductivity (uS/cm) of milk. Allele frequencies were found as A: 0.374 and B: 0.626 in *Hinf*I (451 bp) polymorphism in Pit–1 gene. No significant difference was detected between the observed and the expected genotype frequencies via Hardy-Weingberg equilibrium (P>0.05). The effect of Pit-1 polymorphisms (*Hinf*I_451 bp) was not observed on milk yield and composition in Brown Swiss populations (P>0.05). The results demostrated that the Pit-1 polymorphisms (*Hinf*I_451 bp) could not be exploited as a candidate gene for selection of dairy traits in Brown Swiss cattle.

Key words: Brown Swiss, Pit-1 (*Hinf*I) polymorphism, RFLP, Milk yield and components.

INTRODUCTION

Recently, some genes concerning with economic characteristics of farm animals have been studied for marker assist selection (MAS). Use of these can aid on the selection of animals with highest breeding values. To determine the best genotypes carrying alleles by taking into account the phenotypic values of animals in quantitative characters are difficult. In other words, phenotypic values do not always reflect the genotypic values of the animals.

The improvement of any trait in population primarily depends on its economic gain. Milk yield and components are a quantitative trait controlled by many genes, each one of them with small effect. Dairy cattle breeders have primarily concentrated in 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 associations with yield traits, however, selection is limited (Looper *et al*. 2001).

Although the percentage of milk components, especially the fat and protein level with nutrition may be desirable level, this approach ignores the animal genetic effect, and also 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 in selection 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).

Recently, some genes concerning with economic characteristics of farm animals have been studied for marker assist selection (MAS). Pit-1 gene, POU1F1, POU domain class 1 transcription factor 1 (Herr *et al*. 1988), activates growth hormone (GH), prolactin (PRL) and thyrotropin (TSHβ) genes expression (Haugen *et al*. 1993; Renaville *et al*. 1997a; Kopp and Jameson 1998; Miyai *et al*. 2005). Pit-1 gene has 129 amino acid protein and this 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. It is a 31-33-kDa protein and especially synthesized in the anterior pituitary, and also is limited expression level in thyrotropes, sommatotropes and lactotropes (Haugen *et al*. 1993; Kopp and Jameson 1998). The Pit-1 protein consists of three domains; POU-homeo, POU-specific and the N-terminal region which is play a role in transactivation (Haugen *et al*. 1993; Kopp and Jameson 1998; Weatherly 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 causes abnormalities of pituitary development called hypoplasia (Renaville *et al*. 1997a). Bona *et al*. (2004) stated that Pit-1 is an 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. Mutations in the human PIT-1 are responsible for a CPHD (Combined Pituitary Hormone Deficiency) with deficiency of GH, PRL or TSH, while the production of ACTH, LH and FSH are preserved and its lead to late to reach puberty and hypothyroidism. Pit-1 gene is

considered to be a candidate gene for the regulation of growth and development in cattle and other mammals (Zhang *et al*. 2009) due to PRL and GH are effective in proliferation of somatotrophic cells (Castrillo *et al*. 1991) as well as they are necessary for mammary gland development and milk yield (Oprzadek *et al*. 2003).

Woollard *et al*. (1994) firstly identified *Hinf*I polymorphism of bovine Pit-1 gene by RFLP method. Researchers reported that the PCR product (451 bp) digested by *Hinf*I restriction enzyme revealed two alleles from in intron 5 and exon 6. As a result of A→G substitution in exon 6, A allele was not digested with *Hinf*I, but B allele shows fragments both 244 and 207 bp in length. In addition, other SNPs in the different regions of the Pit-1 gene are reached in the GenBank data base.

In cattle, Pit1 was found to be related to milk yield, protein yield, fat 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, from two lineages (Carrijo *et al*. 2008), and also birth weight and height at withers of Geman Yellow x Qinchuan beef cattle (Zhang *et al*. 2009) .

There are no reports on the study of Pit-1 gene in Brown Swiss cattle by now. The aim of this study was to determine the genotype and allele frequencies of Pit1 gene (*Hinf*I_451 bp polymorphism) and to analyze the association of this polymorphism with milk yield and compositions in Brown Swiss cattle.

MATERIALS AND METHODS

Material and Feeding: A total of 301 Brown Swiss cows from the Konuklar Farm of the General Directorate of Agricultural Enterprises, Konya Province of Turkey were used for this study. Dairy cows were separated into different feeding groups according to daily milk yield during lactation and fed *ad libitum* with a mixture of concentrated feed and forage (Table 1).

Table 1. Calculated values of nutrient offered to milking and dry cows based on dry matter intake (Yavuz 2001; Dale and Batal 2005).

		Lactation			Dry period
		I. Group	II. Group	III. Group	
Dry matter intake	(kg)	21.56	17.48	14.65	9.73
Crude protein	(%)	15.04	14.34	14.00	14.29
Metabolic energy	(ME, kcal/kg)	2767	2713	2680	2631
Crude cellulose	(%)	19.58	21.47	22.43	23.11
Neutral detergent cellulose	(NDS, %)	39.59	42.36	43.44	42.63
Acid detergent cellulose	(ADS, %)	19.23	22.14	23.35	23.06
Calcium / Phosphorus	(Ca/P)	1.84	1.88	1.84	1.78
Forage ratio	(%)	50.46	59.27	63.55	63.41

Milking and Milk samples: Cows were housed in a free-stall barn and milked twice daily in a 2 x 12 side-closed milking parlour (WestfaliaSurge, Dairy Plan C21, Version 5.2). Milk yields of each cow were obtained from herd management program during lactation. Samples of milk were taken from each cow at the milking and stored at 4–6 °C in a cool box, and also immediately analysed two times by an ultrasonic milk analyzer (LACTOSCAN MMC 30 sec Milk Analyzer, Milkotronic Ltd, Bulgaria).

For our analyses, between 2004 and 2010, a total of 377 lactation records for MY_{305} and 3529 monthly test day records for TDMY of 126 Brown Swiss were analyzed, and also 1051 monthly test day records for each milk components were analyzed in 2010. Total milk yield up to 305 lactation days (MY_{305}) was calculated using a Holland method, and others were evaluated from test day records.

***DNA extraction and PCR Assay*:** A total of 301 blood samples were collected from the Jugular vein of each dairy cattle into 4 mL tubes with EDTA and stored at −20 ^{o}C needed for DNA extraction. Genomic DNA from blood was extracted according to salting out procedure with slight modifications (Miller *et al*. 1988). After extraction, the DNA concentration of all samples was measured with the Nanodrop spectrophotometer (ND1000; NanoDrop Technologies, USA).

A 451 bp region of intron 5-exon 6 of the Pit-1 gene was amplified using a pair of primers with the following nucleotide sequences: 5’ -AAACCATCATCTCCCTTCTT-3’ and 5’-AATGTACAATGTGCCTTCTGAG-3’ (Woollard *et al*. 1994). The polymerase chain reaction mixture (10 µL) contained the following components and conditions; genomic DNA, 1.5 mmol L^{-1} $MgCl_2$ (supplied with the

enzyme), 0.2 mmol L^{-1} dNTPs, 0.3 µmol L^{-1} of each primer and 0.5 U of Taq DNA polymerase (Fermentas Life Sciences, Vilnius, Lithuania). The mixture was subjected to PCR on a thermal cycler (Techne TC-512) using the following program: initial denaturation for 10 min at 95 °C, followed by 35 cycles of 30 sec at 95 °C; 1 min at 57.1 °C; and 2 min at 72 °C followed by 10 min at 72 °C for final extension. A 20 µL aliquot of the 451 bp PCR products was digested with 6 U of *Hinf*I restriction enzyme at 37 °C overnight in incubator, the reaction being stopped by adding 6x Loading Dye (Fermentas Life Sciences, Vilnius, Lithuania).

The digested PCR products were separated in 2% agarose gel (Prona Agarose; Basica Le, Burgos, Spain), stained with ethidium bromide, viewed under UV light and scored in a gel documentation system.

Statistical analysis: The Chi-square test whether the distribution of the genotype frequencies was in the Hardy-Weinberg equilibrium was carried out by PopGene Version 1.32 (Yeh *et al*. 1997), and also heterozigosity value was determined (Nei 1973). The next stage involved an analysis of associations between the Pit-1 genotypes and dairy traits such as 305 days milk yield (MY_{305}), test day milk yield (TDMY), fat (%), protein (%), lactose (%), density (kg/m^3), solids-non-fat (%), total solids (%), freezing point (^oC),and also pH and conductivity (uS/cm) of milk.

Two models were used in this study. PROC GLM (General Linear Model) in the computer program SAS (SAS Institute 2002 Ver. 9) was used to determine the associations between Pit-1 genotypes and the MY_{305}. The following linear model was applied:

$$Y_{ijklm} = \mu + \alpha_i + \beta_j + S_k + G_l + \varepsilon_{ijklm}$$

Where: Y_{ijkml}: observed MY_{305} in *ijkl*-th animal; µ: mean of MY_{305} for population; α_i: effect of years (*i* = 2004, 2005, 2010); β_j: effect of calving month (*j*=January, February, December); S_k: effect of parity (*k* = 1, 2, 6); G_l: effect of genotypes (AA, AB, BB); ε_{ijkl}: random error

The effect of genotypes on both TDMY (kg) and milk components such as fat (%), protein (%), lactose (%), density (kg/m^3), solids-non-fat (%), total solids (%), freezing point (^oC), and also pH and conductivity (uS/cm) was analyzed by repeated measurement of mixed model (SAS Proc Mixed) used from SAS program (SAS Institute 2002 Ver. 9). The following repeated measurement of mixed model was applied:

$$Y_{ijklmno} = \mu + \alpha_i + \beta_j + S_k + G_l + \gamma_{ijklm} + p_{ijklmn} + bx_{ijklm} + \varepsilon_{ijklmno}$$

Where: $Y_{ijklmno}$: observed trait value in *ijkl*-th animal; µ: mean of trait value for population; α_i: effect of years (*i* = 2004, 2005, 2010); β_j: effect of calving month (*j*= January, February, December); S_k: effect of parity (*k* = 1, 2, 6); G_l: effect of genotypes (AA, AB, BB); γ_{ijklm} :effect of controls in *ijkl*-th animal (*m* = 1, 2, 7-10); p_{ijklmn} : subject effect; bx_{ijklm} : partial regression coefficient of days in milk (DIM) for TDMY; TDMY and conductivity for fat (%), protein (%), lactose (%), density (kg/m^3), total solids (%) and freezing point (^oC); TDMY, fat (%) and conductivity for solids-non-fat (%), TDMY for pH and conductivity (uS/cm); $\varepsilon_{ijklmno}$: random error

The effect of years (α_i) was eliminated from the models in milk components analyses, because dairy traits were obtained only from a lactation. REML method was used for unbiased estimates of variance and covariance parameters. The best repeated measures analysis using mixed models such as homogeneous variance-covariance models ((Compound Symmetry (CS), First-Order Autoregressive_AR(1) and Toeplitz (TOEP)) and heterogeneous variance-covariance models ((Unstructured (UN), Heterogeneous Compound Symmetry (CSH), Huynh-Feldt (HF) and First-Order Ante-dependence (ANTE(1))) were performed according to Akaike's Information Criterion (AIC).

After statistically analyses, the differences between any two least squares means of the traits were compared with Least Significant Difference (LSD) for MY_{305} by using MSTAT-C (1989) and for TDMY and other dairy traits by using PDMIX800 (Saxton 1998).

RESULTS and DISCUSSION

Distribution of genotype and allele frequencies: PCR products after digestion with restriction enzyme are shown in Figure 1. As seen in Figure 1, allele A was not digested with *Hinf*I, but allele B was cut at one restriction site, generating two fragments of 244 and 207 bp in length. As for the AB genotype, it can be seen as heterozygous.

Table 2 shows the genotype and allele frequencies and the expected heterozygosity of Pit-1 polymorphisms (*Hinf*I_451 bp) in Brown Swiss. As seen in Table 2, 35, 155 and 111 heads of 301 Brown Swiss cattle were determined as AA, AB and BB genotypes, respectively.

Allele A and B frequencies in Brown Swiss cattle were found as 0.374 and 0.626, respectively. The observed genotype frequencies were 0.12, 0.51 and 0.37 for AA, AB and BB, respectively. The population was found in the Hardy-Weingberg equilibrium with respect to *Hinf*I polymorphism in the present study (P>0.05). Also, expected heterozygosity value was found as 0.468 in the population.

There are several reports on genotype and allele frequencies of Pit-1 (*Hinf*I) polymorphism in different breeds of cattle (Table 3). When compared with literature in terms of allele frequencies, A allele frequency (0.374) in our study was higher than 0.188 in Italian Holstein-Friesian bulls (Renaville *et al*. 1997a), 0.155 in Holstein (Hori-Oshima and Barreras-Serrano 2003), 0.247 in Black-and-White bulls (Oprzadek *et al*. 2003), 0.331 in

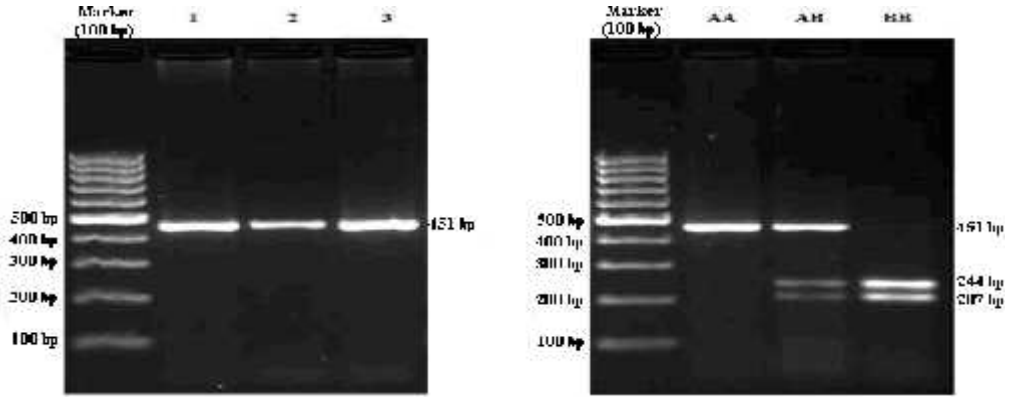

Fig 1. PCR products digested with *Hinf*I on 2% Prona agarose gel (Nu microphor) electrophoresis stained with ethidium bromide. 1, 2 and 3: PCR products, AA: undigested PCR product, AB: digested PCR product (451, 244 and 207 bp) and BB: digested PCR product (244 and 207 bp)

Table 2. The genotype and allele frequencies and the expected heterozygosity of Pit-1 *Hinf*I polymorphism in Brown Swiss.

Pit-1	N	Genotypes			Genotype frequencies			Allel frequencies		$(\chi^2)^1$
		AA	AB	BB	AA	AB	BB	A	B	
Observed	301	35	155	111	0.12	0.51	0.37	0.374	0.626	2.914^{NS}
Expected	301	41.9301	141.1398	117.9301	0.14	0.47	0.39			H_e^2
$^1\chi^2$; test of Hardy-Weingberg equlibrium, ^{2}Heterozigosity, NS; not significant (P > 0.05)										0.468

Table 3: Statement of the literature on the Pit-1 polymorphisms.

References	Breed	N	Pit-1					H_e
			Genotype frequencies			Allel frequencies		
			AA	AB	BB	A	B	
Renaville *et al*. (1997) 451 bp	Italian Holstein-Friesian bulls	89	0.022	0.315	0.553	0.188	0.812	0.305*
Renaville *et al*. (1997) 451 bp	Belgian Blue	350	0.200	0.445	0.355	0.53 0.423*	0.47 0.577*	0.488*
Hori-Oshima and Barreras-Serrano (2003) 451 bp	Holstein	196	0.026	0.257	0.717	0.155	0.845	0.262*
Oprzadek *et al*.. (2003) 451 bp	Black-and- White bulls	144	0.063*	0.368*	0.569*	0.247	0.753	0.372*
Zhao *et al*. (2004) 451 bp	Angus beef cattle	416	0.111	0.440	0.450	0.331*	0.669*	0.443*
De Mattos *et al*. (2004) ~1.355 bp	Gry bulls	40	0.900	0.100	0.000	0.95	0.05	0.095*
Dybus *et al*. (2004) 451 bp	Poland Black-and-White cows	900	0.052	0.382	0.566	0.243	0.757	0.368*
Vargas *et al*. (2004) 451 bp	Holstein-Friesian	46	0.10	0.35	0.55	0.283*	0.717*	0.405*
Javanmard *et al*. (2005) 600 bp	Sarabi	82	0.451	0.341	0.207	0.622	0.378	0.470
	Golpayegani	57	0.614	0.263	0.123	0.746+	0.254	0.379
	Sistani	38	0.842	0.158	0.000	0.921	0.079+	0.145
	Taleshi	70	0.614	0.314	0.071	0.771	0.229	0.353
	Manzadrani	26	0.692	0.269	0.038	0.827	0.173	0.286
	Dashtiyari	8	0.625	0.000	0.375	0.625	0.375	0.469
	Golpayegani x Brown Swiss F_1	13	0.000	0.769	0.231	0.385	0.615	0.473
Xue *et al*. (2006) 451 bp	Nanyang	100	0.210	0.510	0.280	0.465	0.535	0.497
Yan *et al*. (2006)	Qinchuan	218	-	-	-	0.232	0.768	0.356

451 bp	China Holstein-Friesian					0.132	0.868	0.229
	Nellore	79	0.795	0.205	0.000	0.897	0.103	0.185*
Curi *et al*. (2006)	Canchim	30	0.800	0.167	0.033	0.883	0.117	0.207*
1301 bp	1/2 Simmental	30	0.733	0.267	0.000	0.867	0.133	0.231*
	1/2 Angus	245	0.295	0.693	0.012	0.641	0.389	0.438*
	Manzadrani	96	0.167*	0.406*	0.427*	0.370	0.630	0.466*
Zakizadeh *et al*. (2007)	Sarabi	84	0.083*	0.381*	0.536*	0.274	0.726	0.398*
451 bp	Golpayegani	110	0.109*	0.455*	0.436*	0.336	0.664	0.446*
	Holstein	111	0.059*	0.297*	0.644*	0.208	0.792	0.329*
Viorica *et al*. (2007) 1350 bp	Simmental	76	0.118	0.197	0.685	0.217	0.783	0.340*
Carrijo *et al*. (2008)	5/8 Charolais ve 3/8 of Zebu	232	-	-	-	0.13	0.87	0.226*
1301 bp	21/32 Charolais ve 11/32 Nelore	277	-	-	-	0.27	0.73	0.394*
Mukesh *et al*. (2008) 1350 bp	16 distinct Indian native cattle (*Bos indicus*)	723	0.002	0.119	0.881	0.063	0.937	0.118*
Edriss *et al*. (2008) 451 bp	Holstein cows (four herds)	262	0.031	0.450	0.519	0.256	0.744	0.381*
	Qinchuan	67	0.030	0.403	0.537	0.232	0.768	0.356*
Zhang *et al*. (2009)	Limousin x Qinchuan	47	0.043	0.277	0.681	0.181	0.819	0.296*
451 bp	Angus x Qinchuan	36	0.111	0.444	0.444	0.333	0.667	0.444*
	Germany Yellow x Qinchuan	42	0.071	0.214	0.714	0.178	0.822	0.293*
Jawasreh *et al*. (2009)	Jordan native cattle	36	0.000	0.176	0.8235	0.088	0.912	0.160
422 bp	Holstein-Friesian	45	0.046	0.255	0.697	0.174	0.826	0.288
Misrianti *et al*. (2010) 611 bp	Holstein-Friesian	45	0.022	0.444	0.533	0.244+	0756+	0.369*
Beigi Nassiri *et al*. (2010) 451 bp	Najdi	84	0.0357	0.2976	0.6666	0.1845	0.8155	0.301*
Özdemir (2012)	East Anatolian Red	71	0.14	0.54	0.32	0.41	0.59	0.483*
(260 bp)	Holstein	181	0.04	0.31	0.65	0.20	0.80	0.319*

N: observed number; H_e: expected heterozigosity, *values calculated from allele frequencies and +corrected values

Angus beef cattle (Zhao *et al*. 2004), 0.243 in Black-and-White cows (Dybus *et al*. 2004), 0.283 in Holstein-Friesian (Vargas *et al*. 2004), 0.232 and 0.132 in Qinchuan cattle and Chinese Holstein cattle (Yan *et al*. 2006), 0.370, 0.274, 0.336 and 0.208 in Manzadrani, Sarabi, Golpayegani and Holstein breeds (Zakizadeh *et al*. 2007), 0.256 in Holstein Cows (Edriss *et al*. 2008), 0.232, 0.181, 0.333 and 0.178 in Qinchuan, Limousin x Qinchuan, Angus x Qinchuan and Germany Yellow x Qinchuan cattle (Zhang *et al*. 2009), 0.185 in Najdi cattle (Beigi Nassiri *et al*. 2010) and 0.20 in Holstein breed (Özdemir 2012). However, the A allele frequency was lower than 0.53 in double-muscled Belgian Blue cattle (Renaville *et al*. 1997b), 0.465 in Nanyang cattle (Xue *et al*. 2006) and 0.40 in East Anatolian Red breed (Özdemir 2012). As can be seen in references in terms of Pit-1 polymorphisms (*Hinf*I_451 bp), A allele seems to have a lower frequency values than B allele. This situation is similar to the current study.

The frequencies of AA genotype have the lowest frequency values and BB genotypes were generally determined to have a higher frequency values than AB genotypes except for Renaville *et al*. (1997b), Xue *et al*. (2006) and Edriss *et al*. (2008) studies. At the same time, the frequency of AA genotype in the present study has the same tendency with the literature, the presence of a high frequency of AB genotype was consistent with Renaville *et al*. (1997b), Xue *et al*. (2006) and Edriss *et al*. (2008) studies.

The value of expected heterozygosity was calculated to be 0.468 in Brown Swiss cattle. When compared with literature, this value was lower than findings to be as 0.488 in Belgian Blue (Renaville *et al*. 1997b), 0.497 in Nanyang cattle (Xue *et al*. 2006) and 0.483 in East Anatolian Red breed (Özdemir 2012). But, similar results such as 0.433, 0.466, 0.420 and 0.444 were obtained from Angus beef cattle (Zhao *et al*. 2004), 0.466 in Manzadrani and Golpayegani breeds (Zakizadeh *et al*. 2007), 0.420 in the third herd of Holstein cows (Edriss *et al*. 2008) and 0.444 in Angus x Qinchuan crossbred (Zhang *et al*. 2009), respectively. Also, expected heterozygosity value in this study was higher than other values reported in terms of 451 bp in the literature.

The numbers of allele and their frequencies determine the value of heterozygosity in a population. Arora and Bhatia (2004) stated that the level of variation depicted by number of alleles at each locus serves as a measure of genetic variability having direct impact on differentiation of breeds within a species. Also, its may cause some variations in phenotypes. High heterozygosity value in a population is based on 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 with

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.

Association analyses: The association of Pit-1 gene polymorphism with MY_{305}, TDMY and milk components in the population was analyzed (Table 4).

According to MY_{305} and TDMY, cows carrying AA genotype (6390 kg ± 21.43 kg) had produce more milk compare to AB (6240 kg ± 20.72 kg) and BB (6260 kg ± 20.76 kg) genotypes. With respect to milk components, generally, AA and AB genotypes showed a similar tendency and they have lower values than the BB genotype. But, take account of P values, the genotypes at Pit-1 polymorphisms (*Hinf*I_451 bp) did not show a significant association with the MY_{305}, TDMY and milk components (P>0.05). As for the differences of least squares means according to genotypes, the BB genotype was associated with an increase in test day protein percentage of 0.03% (P=0.09) and in test day total solids percentage of 0.008% (P=0.07) vs. the AB genotype. At the same time, in pH of milk, BB and AB genotypes have a higher least squares means than AA genotype such as 0.03 (P=0.02) and 0.02 (P=0.08), respectively. For all that, the results demonstrated that the Pit-1 polymorphisms (*Hinf*I_451 bp) could not be exploited as a candidate gene for selection of dairy traits in this population.

In dairy cattle, there are a few reports on relationships between Pit-1 genes (*Hinf*I polymorphism) and milk yield and components (Renaville *et al*. 1997a; Hori-Oshima and Barreras-Serrano, 2003; Vargas *et al*. 2004, De Mattos *et al*. 2004; Parmentier *et al*. 1999; Dybus *et al*. 2004 and Zakizadeh *et al*. 2007). But, studies have accelerated both the cattle and other farm animals in recent years.

Renaville *et al*. (1997a) stated that the A allele showed significant superiority over the B allele for milk yield (P<0.10), protein yield (P<0.05), some conformation traits such as body depth (P<0.10), angularity (P<0.10), rear leg set (P<0.10), and also less fat percentage (P<0.10) in Italian Holstein-Friesian bulls. Hori-Oshima and Barreras-Serrano (2003) reported AA genotype of Pit-1 with substitution of A for K allele at DGAT1 locus had significant effect on the total milk yield in Holstein cattle. The substitution effect was additive significantly on milk yield in animals with AA genotype for Pit-1 gene (296.28 kg) and a result of interaction between DGAT1 and Pit-1 genes showed that AA genotype of Pit-1 (296.28 kg) had higher average than AB (140.2 kg) and BB (145.9 kg) in terms of 305-day milk yield. Also, researchers reported that increase of animals with AA genotype for Pit-1 is most effective not only for the effect of A allele of the gene itself, but also to obtain maximum positive effects of other genes. Considered a total of 443 lactations of 46 Holstein-Friesian cows, Vargas *et al*. (2004) found that AA genotype were favorable for milk yield and not favorable for the reproductive traits taking into account the breeding values of animals. De Mattos *et al.* (2004) reported *Hinf*I variants is associated with only the fat yield of milk yield traits and bulls carrying AB genotype (16.6 kg) were superior than AA genotype (6.5 kg) for fat yield (P<0.05). They stated that this superiority resulted from the influence of allele B on these genotypes. Parmentier *et al.* (1999) demonstrated significant superiority of the HinfI B allele for milk (+222.4) and protein (+9.17) yields, but an inferiority for fat yield (-2.29%).

But, Dybus *et al*. (2004) found no associations between PIT1-*Hinf*I polymorphism and milk production traits of 900 Black-and-White cattle in five herds (P>0.05). Similarly, Zakizadeh *et al*. (2007) observed no significant association between PIT1-*Hinf*I polymorphism and milk production in Golpayegani breed and Holstein cattle of Iran (P>0.05).

We did not find associations between the Pit-1 polymorphism and production traits, in contrast with the results published by Renaville *et al*. (1997a), Hori-Oshima and Barreras-Serrano (2003), Vargas *et al*. (2004), De Mattos *et al*. (2004) and Parmentier *et al*. (1999). But, there are similar results by published by Dybus *et al*. (2004) and Zakizadeh *et al*. (2007).

Bearing in mind the literature investigating the relationship between PIT1-*Hinf*I polymorphism and milk production traits, it can be said that A allele and AA genotype should be exploited for selection of dairy traits except for Gry bulls (*Bos indicus*) 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, association analysis to be made with phenotypic values in different populations with the same distribution of allele frequencies may be varied. Increasing the number of animal and data, especially taking into consideration the genotype x environment interactions and other genes affecting milk yield and components should be made more comprehensive studies. Besides, it would be more informative that different alleles in other genes connection with the Pit-1 gene could be evaluation.

Table 4: The association of Pit-1 gene polymorphism with MY_{305}, TDMY and milk components.

Traits	*Hinf*I (451 bp) AA (n=15)	AB (n=62)	BB (n=49)	P*	Model	Factors
MY_{305} (kg)	6390 ± 219	6240±126	6260±128	0.81	GLM	Year * Month** Parity**
TDMY (kg)	21.43 ± 0.78	20.72±0.51	20.76±0.50	0.64	Mixed UN	Year * Month* Parity** DIM^{NS} Control**
Fat (%)	4.51 ± 0.09	4.48±0.05	4.55±0.05	0.58	Mixed UN	$Month^{NS}$ $Parity^{NS}$ Control* TDMY** Conductivity**
Protein (%)	3.30 ± 0.02	3.29±0.01	3.32±0.01	0.22	Mixed HF	Month** $Parity^{NS}$ Control** $TDMY^{NS}$ Conductivity**
Lactose (%)	4.79 ± 0.03	4.80±0.02	4.83±0.02	0.46	Mixed HF	Month** $Parity^{NS}$ Control** $TDMY^{NS}$ Conductivity*
Density (kg/m^3)	1030.39 ± 0.22	1030.32±0.11	1030.47±0.13	0.66	Mixed UN	Month* $Parity^{NS}$ Control* TDMY** Conductivity**
Solids-non-fat (%)	8.92 ± 0.06	8.92±0.03	8.97±0.04	0.28	Mixed ANTE (1)	Month** $Parity^{NS}$ Control** $TDMY^{NS}$ Conductivity** Fat(%)*
Total solids (%)	0.788 ± 0.005	0.786±0.003	0.794±0.003	0.19	Mixed CS	Month ** $Parity^{NS}$ Control ** $TDMY^{*}$ Conductivity**
Freezing Point (^oC)	-0.573 ± 0.004	-0.573±0.002	-0.576±0.002	0.40	Mixed CS	Month* $Parity^{NS}$ Control** TDMY* Conductivity**
pH	6.57 ± 0.01	6.59±0.01	6.60±0.01	0.06	Mixed ANTE (1)	$Month^{NS}$ Parity* Control** $TDMY^{NS}$
Conductivity (µS/cm)	4.01 ± 0.04	3.99±0.02	3.98±0.02	0.71	Mixed CSH	$Month^{**}$ Parity** $Control^{NS}$ TDMY*

*: Statistical significance level ($^*P<0.05$; $^{**}P<0.01$) ; NS: Non significance

Acknowledgements: This research was supported as a part of doctoral thesis by a grant from The Scientific Research Project Office of Selcuk University, Turkey (Project No: 09101016). We are thankful to Konuklar State Farm in General Directorate of Agricultural Enterprises.

REFERENCES

Arora, R. and S. Bhatia (2004). Genetic structure of Muzzafarnagri sheep based on microsatellite analysis. Small Rum Res. 54: 227-230.

Beigi Nassiri, M.T, Z. Biranvand, T. Hartatik, J. Fayazil and S. Tavakoli (2010). The Study of *PIT1* Gene Polymorphism in the Najdi Cattle Using PCR-RFLP Method. J. Anim. Vet. Adv. 9(15): 2001-2003.

Bona, G., R. Paracchini, M. Giordano and P. Momigliano-Richiardi (2004). Genetic defects in GH synthesis and secretion. Eur. J. Endoc. 151: 3-9.

Camper, S.A., T.L. Saunders, R.W. Katz and R.H. Reeves (1990). The Pit-1 transcription factor gene is a candidate for the murine Snell dwarf mutation. Genomics. 8: 586-590.

Carrijo, S.M, M.M. De Alencar, F.L.B. Toral and L.C. De Almedia Regitano (2008). Association of Pit1 Genotypes with growth traits in Canchim cattle. Scientia Agricola. 65(2): 116-121.

Castrillo, J.L, L.E. Theill and M. Karin (1991). Function of the homeodomain protein GHF1 in pituitary cell proliferation. Science. 253: 197-199.

Curi, R.A, D.A Palmieri, L. Suguisawa, H.N. de Oliviera, A.C Silveira and C.R. Lopes (2006). Growth and carcass traits associated with GH1/*Alu*I and POU1F1/*Hinf*I gene polymorphisms in Zebu and crossbred beef cattle. Genet. Mol. Biol. 29(1): 56-61.

Dale, N. and A. Batal (2005). Feedstuffs Reference Issue and Buyers Guide. 76: 16-22.

De Mattos, K.K, S.N. Del Lama, M.L. Martinez and A.F. Freitas (2004). Association of bGH and Pit-1 gene variants with milk production traits in dairy Gyr bulls. Pesqui. Agropecu. Bras. 39(2): 147-150.

Dybus, A., I. Szatkowska, E. Czerniawska-Piątkowska, W. Grzesiak, J. Wójcik, E. Rzewucka and S. Zych (2004). *PIT1-Hinf*I gene polymorphism and its associations with milk production traits in polish Black-and-White cattle. Arch. Tierz. Dummerstorf 47**(**6): 557–563.

Edriss, V., M.A. Edriss, H.R. Rahmani and B.E. Sayed-Tabatabaei (2008). Pit-1 gene polymorphism of Holstein cows in Isfahan Province. Biotechnology. 7(2): 209-212.

Haugen, B.R., W.M. Wood, D.F. Gordon and E.C. Ridgeway (1993). A Thyrotrope-specific Variant of Pit-1 Transactivates the Thyrotropin β Promoter. J. Biol. Chem. 268(28): 20818-20824.

Herr, W., R.A. Sturm, R.G. Clerc, L.M. Corcoran, D. Baltimore, P.A. Sharp, H.A. Ingraham, M.G. Rosenfeld, M. Finney, G. Ruvkun and H.R. Horvitz (1988). The POU domain: a large conserved region in the mammalian pit-1, oct-1, oct-2, and Caenorhabditis elegans unc-86 gene products. Genes Dev. 2: 1513-1516.

Hori-Oshima, S. and A. Barreras-Serrano (2003). Relationships between DGAT1 and PIT-1 Genes Polymorphism and Milk Yield in Holstein Cattle, Proceedings, Western Section. American Society of Animal Science. Vol 54.

Javanmard, A., N. Asadzadeh, M.H. Banabazi and J. Tavakolian (2005). The allele and genotype frequencies of bovine pituitary specific transcription factor and leptin genes in Iranian cattle and buffalo populations using PCR-RFLP. Iranian J. Biotechnology. 3, 2.

Jawasreh, K.I.Z., F. Awawdeh, I. Rawashdeh, F. Hejazeen and M. Al-Talib (2009). The Allele and Genotype Frequencies of Bovine Pituitary Specific Transcription Factor and Leptin Genes in Jordanian Cattle Population by Using PCR-RFLP. Australian J. Basic and Applied Sciences. 3(3): 1601-1606.

Kopp, P. and J.L. Jameson (1998). Thyroid Disorders, In: Jameson JL and Collins FS. eds. Principles of Molecular Medicine. Humana Press. Totowa NJ. 459-473.

Li, S., E.B. 3rd Crenshaw, E.J. Rawson, D.M. Simmons, L.W. Swanson and M.G. Rosenfeld (1990). Dwarf locus mutants lacking three pituitary cell types result from mutations in the POU-domain gene *pit-1*. Nature. 357: 528-533.

Looper, L.M., S.R. Stokes, D.N. Waldner and E.R. Jordan. (2001). Managing Milk Composition: Normal Sources of Varation, Guide D-103. http://aces.nmsu.edu/pubs/_d/d-103.html

Miller, S.A., D.D. Dykes and H.F. Polesky (1988). A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16(3): 1215.

Misriantia, R., C. Sumantria and A. Farajallahb (2010). Polymorphism Identification of Pit1 Gene in Indonesian Buffaloes (*Bubalus bubalis*) and Holstein-Friesian Cows. Media Peternakan. 33(3): 131-136.

Miyai, S., S. Yoshimura, Y. Iwasaki, S. Takekoshi, R.V. Lloyd and R.Y. Osamura (2005). Induction of GH, PRL, and TSH beta mRNA by transfection of Pit-1 in a human pituitary adenoma-derived cell line. Cell Tissue Res. 322: 269-277.

Moody, D.E., D. Pomp and W. Berendse (1995). Restriction fragment length polymorphism in amplification products of bovine PIT1 gene and assignment of PIT1 to bovine chromosome 1. Anim. Genet. 26: 45-47.

MSTAT. (1989). MSTAT User's guide: Statistics (version 5). Michigan State University, Michigan, USA.

Mukesh, M., M. Sodhi, R.C. Sobti, B. Prakash, R. Kaushik, R.A.K. Aggarwal and B.P. Mishra (2008). Analysis of bovine pituitary specific transcription factor-Hinf I gene polymorphism in Indian zebuine cattle. Livest. Sci. 113: 81-86.

Nei, M., (1973). Analysis of gene diversity in subdivided populations. Proceedings of The National Academy of Sciences of The United States of America. 70: 3321-3323.

Oprzadek, J., K. Flisikowski, L. Zwierzchowski and E. Dymnicki (2003). Polymorphisms at loci of Leptin (LEP), Pit1 and STAT5A and their association with growth, feed conversion and carcass quality in Black-and- White bulls. Anim. Sci. Pap. Rep. 21(3): 135-145.

Özdemir, M (2012). Determination of Pit-1/Hinf1 Polymorphism in Holstein and Native Ear cattle raised as genetic resource in Turkey. The J. Anim. and Plant Sciences. 22(1): 25-28.

Parmentier, I., D. Portetelle, N. Gengler, A. Pradi, C. Bertozzi, L. Vleurick, R. Gilson and R. Renaville (1999). Candidate gene markers associated with somatotropic axis and milk selection. Domes. Anim. Endocrin. 17: 139-148.

Renaville, R., N. Gengler, E. Vrech, A. Prandi, S. Massart, C. Corradini, C. Bertozzi, F. Mortiaux, A. Burny and D. Portetelle (1997a). Pit-1 Gene Polymorphism, Milk Yield, and Conformation Traits for Italian Holstein-Friesian Bulls. J. Dairy Sci. 80: 3431-3438.

Renaville, R., N. Gengler, I. Parmentier, F. Mortiaux, S. Massart, C. Bertozzi, A. Burny and D. Portetelle (1997b). Pit-1 gene *Hinf*I RFLP and growth traits in doublemuscled Belgian Blue cattle. J. Anim. Sci. 75 (Suppl. 1): 146.

SAS Institute. (2002). SAS/STAT 9 User's Guide – Procedures, SAS Inst. Inc., Cary, NC.

Saxton, A.M. (1998). A macro for converting mean separation output to letter groupings in Proc Mixed. In Proc. 23rd SAS Users Group Intl., SAS Institute, Cary, NC, 1243-1246.

Shook G.E., (2006). Major Advances in Determining Appropriate Selection Goals. J. Dairy Sci. 89: 1349-1361.

Soyeurt, H., P. Dardenne, A. Gillon, C. Croquet, S. Vanderic, P. Mayeres, C. Bertozzi and N. Gengler (2006). Variation in Fatty Acid Contents of Milk and Milk Fat within and across Breeds. J. Dairy Sci. 89: 4858-4865.

Vargas, L.D., V.E. Gana and I.F. Escudero (2004). Pit-1 gene polymorphism in dairy cows from Central Chile. Archivos de Zootecnia. 53(202): 217-220.

Viorica, C., A. Vlaic and I. Gaboreanu (2007). H*inf*I Polymorphism of K-Casein and Pit1 Genes in Romanian Simmental Cattle. Lucrări ştiinţifice Zootehnie şi Biotehnologii. 40(1): 59-61.

Weatherly, K.L. (1998). Regulation of Pituitary Genes by the Transcription Factor, Pit-1, in the Domestic Turkey, Virginia Polytechnic Institute and State University Master of Science in Animal and Poultry Sciences, Blacksburg, Virginia.

Woollard, J., C.B. Schmitz, A.E. Freeman and C.K. Tuggle (1994). *Hinf*I polymorphism at the bovine Pit-1 locus. J. Anim. Sci. 72: 3267.

Xue, K., H. Chen, , S. Wang, , X. Cai, B. Liu, C.F. Zhang, C.Z. Lei, X.Z. Wang, Y.M. Wang and H. Niu (2006). Effect of genetic variations of the POU1F1 gene on growth traits of nanyang cattle. Acta Genetica Sinica. 33(10): 901-907.

Yan, L.J., B. Liu, X.T. Fang, H. Chen, R.F. Zhang, B. Bao and H.J. Zhang (2006). Analysis of POU1F1 gene polymorphisms in Qinchuan cattle and Chinese Holstein cattle. Yi Chuan. 28(11): 1371-1375.

Yavuz, H.M. (2001). Basic Principles of Livestock Nutrition and Some Scientific Approaches to the Compound Feed Production. Farmavet Pharmaceutical Industry and Trading Joint Stock Company Publication, Istanbul.

Yeh, F.C., Y. Rongcai, T. Boyle, Z. Ye and J.M. Xiyan. (1997). POPGENE, The User-friendly Shareware for Population Genetic Analysis. Molecular Biology and Biotechnology Centre. University of Alberta, Edmonton, Alberta, Canada.

Zakizadeh, S., M. Reissmann, G. Rahimi, N.A. Javaremi, P. Reinecke, S.R. Mirae-Ashtiani and M.M. Shahrbabak (2007). Polymorphism of Bovine POU1F1 Gene: allele frequencies and effects on milk production in three Iranian native breeds and Holstein cattle of Iran. Pakistan J. Biol. Sci. 10(15): 2575-2578.

Zhang, C., B. Liu, H. Chen, X. Lan, C. Lei, Z. Zhang and R. Zhang (2009). Associations of a *Hinf*I PCR-RFLP of POU1F1 gene with growth traits in Qinchuan cattle. Anim. Biotechnol. 20(2): 71-74.

Zhao, Q., M.E. Davis and H.C. Hines (2004). Associations of polymorphisms in the Pit-1 gene with growth and carcass traits in Angus beef cattle. J. Anim. Sci. 82: 2229-2233.