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Pit-1 Gene Polymorphisms in Anatolian Black, Holstein Friesian, Brown Swiss and Simmental Cattle Reared in Turkey

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ABSTRACT

The aim of this study is to determine the genetic variation of the Pit 1 gene and comparison polymorphisms between four cattle breeds (Anatolian Black, Holstein Friesian, Brown Swiss, and Simmental). A total of two hundred animals were used for this purpose in four different cattle breeds, each with 50 heads. Genetic variations between breeds were identified via RFLP method by PCR. The allele frequency A and B for Anatolian Black, Holstein Friesian, Brown Swiss, and Simmental was 0.260, 0.740; 0.320, 0.680; 0.100, 0.900; 0.230, 0.770 respectively. While the genotype frequency AA, AB and BB for Anatolian Black, Holstein Friesian, Brown Swiss, and Simmental was 0.10, 0.32 and 0.62; 0.10, 0.44 and 0.46; 0.00, 0.20 and 0.80; 0.10, 0.26 and 0.64 respectively. According to the chi-square test, all breeds were found to be in Hardy–Weinberg equilibrium (P>0.05). As a result, it can be said that with a more comprehensive study that will include economic traits in these breeds, revealing association analyses would be more informative in the future.

Keywords: Pit-1, Genetic variations, Cattle breeds, Allele frequency, Genotype frequency, Hardy–Weinberg equilibrium

1. Introduction

Improvement of livestock has focused on the selective breeding of individuals with superior phenotypes. Most economic traits generally controlled by a large number of genes in animals which are largely influenced from the genotype and environment (Williams 2005). It seems to be difficult to determine the best genotypes carrying alleles by taking into account the phenotypic values of animals in quantitative characters (Aytekin & Boztepe 2013). More recently, new technologies and methods such as QTL and candidate gene approach have emerged to determining the characteristics of these genes. One of the candidate genes that affect the production of milk, protein quality, protein content, body fat percentages, immunity, growth and development traits in cattle is Pit-1 gene.

Pit-1 gene, known as POU1F1, is a pituitary-specific transcription factor responsible for pituitary development and hormone expression in mammals (Cohen et al. 1996). It is an essential part of the body development process. It activates growth hormone gene, prolactin activator, Thyroid-stimulating hormone and receptor of hormone which is releasing growth hormone (Pytlewski et al. 2018). The bovine Pit-1 gene is located in chromosome 1 (BTA1) and consists 6 exons and 5 introns. Pit-1 gene coding for a protein consisting of 129 amino acids (33 kDa) with DNA-binding POU domain (Moody et al. 1995; Thuy et al. 2018). The pit-1 gene is one of the strong candidate genes which associated with body weight, average daily gains, milk production and reproduction traits in cattle (Chauhan et al. 2015; Moravcíková et al. 2013; Thuy et al. 2018). Woollard et al. (1994) firstly identified *Hinf*I polymorphism of bovine Pit-1 gene by RFLP method. Molecular basis of this polymorphism was the silent mutation (G→A) located within the exon 6 of the Pit-1 gene (Moravcíková et al. 2013).

The aim of this study is to determine the genetic variation of the Pit 1 gene and to compare polymorphism between Anatolian Black, Holstein Friesian, Brown Swiss, and Simmental cattle breeds.

2. Material and Methods

Whole blood samples were collected from Konya city for Holstein Friesian and Brown Swiss, Kütahya for Simmental cattle and Ankara city for Anatolian Black cattle. Ethical approval was given by the Faculty of Veterinary Medicine ethical committee (No:2021/125). The genetic analyses were performed in Animal Science Biotechnology Laboratory, Faculty of Agriculture, Selcuk University. A total of 200 heads of animals, 50 heads of each breed were used in the study. EDTA-containing tubes were used to prevent blood clotting during sample collection. Blood samples were stored at 20 °C. Blood samples were taken from the tail vein of cattle. Genomic DNA was extracted from whole blood using the Quick Gene DNA whole blood kit S (DB-S) (KURABO, Japan). The primer sequences and PCR conditions are given in Table 1. The PCR was achieved in a reaction volume of 10 μL containing 1 μL DNA, 5 μL of 2X Dream *Taq* Green PCR Master Mix (Thermo Scientific, USA), 0.30μL for each primer (10 μ mol) (Macrogen, Turkey) and 3.4 μL distilled water. PCR products were digested with fast digest enzyme (Thermo Scientific) which containing 5 μL PCR product, 8.5 μL distilled water, 1 μL 10X buffer and 0.5 μL restriction enzyme (total of 15 μL). Digestion products were separated on 3% agarose gel at 85 V for 50 min, in 0.5X TBE buffer stained by ethidium bromide with used 100bp plus DNA marker (Vivantis, Malaysia). The results were checked under ultraviolet lights.

The Chi-square test whether the distribution of the genotype frequencies was in the Hardy-Weinberg equilibrium was carried out by using PopGene32 ver. 1.32 (Yeh et al. 1997).

Table 1- The primer sequence, fragment sizes, PCR condition and restriction enzyme for Pit-1 gene

Gene	*Primer sequence*	*Base pair*	*Reference*	*PCR conditions*	*Restriction Enzyme*
Pit-1	5′- AAACCATCATCTCCCTTCTT-3′ 5′- AATGTACAATGTGCCTTCTGAG-3′	451	Woollard et al. (1994)	95 °C 10m, 95 °C 30s, 57.1 °C 1m, 72 °C 2 m, 35 cycles 72 °C 10m	*Hinf*I

3. Results

A 451 bp region of intron 5-exon 6 of the Pit-1 gene was amplified. The polymorphism was observed after products were digested with *Hinf*I enzyme. Digestion of the PCR fragment of Pit-1 with *Hinf*I resulted in fragment lengths of 451 bp for AA; 451, 244, 207 bp for AB and 244, 207 for BB (Figure 1). The allele and genotype frequency for four cattle breeds are given in Table 2.

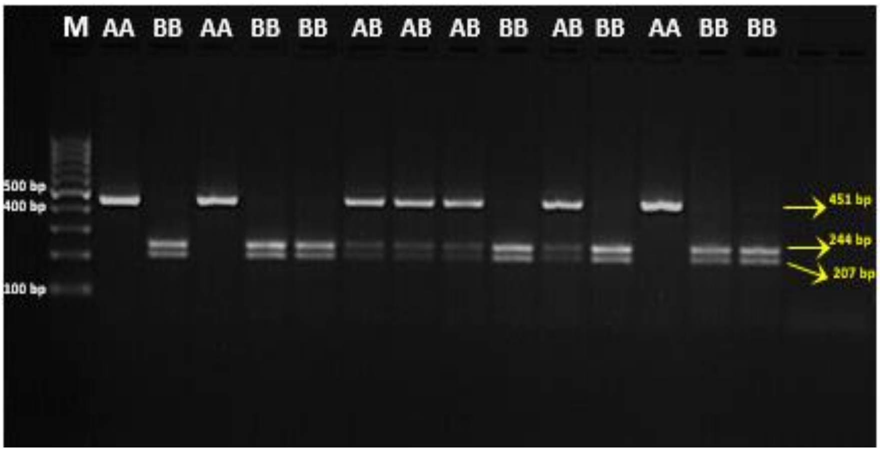

Figure 1- Agarose gel electrophoresis of digested products of Pit-1 gene with *Hinf*I restriction enzyme in cattle; M: 100bp Plus DNA Ladder (Vivantis Technologies), AA: 451 bp, BB: 244 and 207 bp and AB: 451, 244 and 207 bp

Table 2- The genotype and allele frequencies of Pit-1/HinfI polymorphism in four cattle breeds

Breeds	*N*	*Genotype frequencies*			*Allele frequencies*		
		AA	*AB*	*BB*	*A*	*B*	*χ2*
AB	50	0.100	0.320	0.620	0.260	0.740	1.800 (P>0.05)
HF	50	0.100	0.440	0.460	0.320	0.680	0.006 (P>0.05)
BS	50	0.000	0.200	0.800	0.100	0.900	0.617 (P>0.05)
SIM	50	0.100	0.260	0.640	0.230	0.770	3.536 (P>0.05)

AB: Anatolian Black; HF: Holstein Friesian; BS: Brown Swiss; SIM: Simmental; P>0.05: in Hardy-Weinberg equilibrium

It is found that AA genotype frequency in Anatolian Black, Holstein-Friesian and Simmental is similar. In contrast AA genotype frequency of Brown Swiss was showed a difference from other cattle breeds. The highest A allele frequency was found in Holstein-Friesian breed as 0.320, while the highest B allele frequency was found as 0.900 in Brown Swiss breed. According to allele frequency, the most variation (heterozygosity) is genetically in HF (0.44), but the least BS (0.18).

4. Discussion

Pit-1 is an important candidate gene that associated with milk yield and growth traits. Pit-1 includes the two most frequent alleles A and B. It assumed that the gene that carrying the A allele is associated with milk yield and that carrying the B allele with growth traits and fat percentage. In this study, the A allele frequency in Anatolian Black, Holstein Friesian, Brown Swiss and Simmental determined as 0.260, 0.320, 0.100 and 0.230 respectively. On other hand the B allele frequency determined as 0.740, 0.680, 0.900 and 0.770 respectively. The results revealed that the B allele frequency is higher than A frequency in four cattle breeds. These results are in agreement with most of the demonstrated previous studies as detailed below. B allele frequency in various cattle breeds was as follows; 0.81 in Italian Holstein bulls (Renaville et al. 1997), 0.84 in Holstein (Hori-Oshima & Barreras-Serrano 2003), 0.75 Poland Black cattle (Dybus et al. 2004), 0.76 in Qinchuan and 0.868 in China Holstein (Yan et al. 2006), 0.72 in Sarabi (Zakizadeh et al. 2007), 0.78 in Simmental (Coşier et al. 2007), 0.87 in Charolais (Carrijo et al. 2008), 0.81 in Limousin and 0.82 in Angus (Zhang et al. 2009), 0.91 in Jordan native cattle (Jawasreh et al. 2009), 0.90 in Romanian Black cattle (Carsai et al. 2012), 0.98 in Bali (Jakaria and Noor 2015), 0.99 in Grati-Ongole Grade (Hartati et al. 2018). A allele frequency in various cattle breeds was as follows: in Italian Holstein-Friesian Bulls 0.188 (Renaville et al. 1997), in Holstein cows 0.170 (Heidari et al. 2012), in East Anatolian Red 0.41 (Özdemir 2012), in Romanian Black and White and Romanian Grey Steppe 0.100 and 0.250 (Carsai et al. 2012), in Slovak Spotted cattle 0.29 (Moravcíková et al. 2013), in Bali Cattle 0.018 (Jakaria and Noor 2015), in Sahiwal cattle 0.194 (Chauhan et al. 2015), in Holstein 0.253 (Yasemin et al. 2017), in Holstein 0.32 (Bayram et al. 2017), in Holstein Frisian dairy cows bred in Vietnam 0.216 (Thuy et al. 2018).

Briefly, it can be seen in the Table 3, the A allele frequency was generally found to be less than the B allele in previous studies in different breeds for Pit-1 gene. A similar tendency has also been expressed by Aytekin and Boztepe (2013) as a result of the relationship between Pit1-*Hinf*I polymorphism and milk production traits, it can be suggested that A allele and AA genotype are exploited for selection of dairy traits. The present investigation may provide additional base data for future genetic assessments of these breeds.

Table 3- Statements of the literature on the Pit-1 polymorphisms

References	*bp*	*Breeds*	*N*	*Genotype frequencies*			*Allel frequencies*	
				AA	*AB*	*BB*	*A*	*B*
Renaville et al. (1997)	451	Italian Holstein-Friesian bulls	89	0.022	0.315	0.553	0.188	0.812
	451	Belgian Blue	350	0.200	0.445	0.355	0.53 0.423*	0.47 0.577*
Hori-Oshima and Barreras-Serrano (2003)	451	Holstein	196	0.026	0.257	0.717	0.155	0.845
Oprządek and Flisikowski (2003)	451	Black-and- White bulls	144	0.063*	0.368*	0.569*	0.247	0.753
Zhao et al. (2004)	451	Angus beef cattle	416	0.111	0.440	0.450	0.331*	0.669*
Mattos et al. (2004)	1.355	Gry bulls	40	0.900	0.100	0.000	0.95	0.05
Dybus et al. (2004)	451	Poland Black-and-White cows	900	0.052	0.382	0.566	0.243	0.757
Vargas et al. (2004)	451	Holstein-Friesian	46	0.10	0.35	0.55	0.283*	0.717*
Javanmard et al. (2005)	600	Sarabi	82	0.451	0.341	0.207	0.622	0.378
		Golpayegani	57	0.614	0.263	0.123	0.746^{+}	0.254
		Sistani	38	0.842	0.158	0.000	0.921	0.079^{+}
		Taleshi	70	0.614	0.314	0.071	0.771	0.229
		Manzadrani	26	0.692	0.269	0.038	0.827	0.173
		Dashtiyari	8	0.625	0.000	0.375	0.625	0.375
		Golpayegani x Brown Swiss F_1	13	0.000	0.769	0.231	0.385	0.615
Kai et al. (2006)	451	Nanyang	100	0.210	0.510	0.280	0.465	0.535
Zakizadeh et al. (2007)	451	Manzadrani	96	0.167*	0.406*	0.427*	0.370	0.630
		Sarabi	84	0.083*	0.381*	0.536*	0.274	0.726
		Golpayegani	110	0.109*	0.455*	0.436*	0.336	0.664
		Holstein	111	0.059*	0.297*	0.644*	0.208	0.792
Coşier et al. (2007)	1350	Simmental	76	0.118	0.197	0.685	0.217	0.783
Carrijo et al. (2008)	1301	Charolais	232	-	-	-	0.13	0.87
		Nelore	277	-	-	-	0.27	0.73

Table 3 (Continued) - Statements of the literature on the Pit-1 polymorphisms

References	*bp*	*Breeds*	*N*	*Genotype frequencies*			*Allel frequencies*	
				AA	*AB*	*BB*	*A*	*B*
Mukesh et al. (2008)	1350	Indian native cattle (*Bos indicus*)	723	0.002	0.119	0.881	0.063	0.937
Edriss et al. (2008)	451	Holstein cows (four herds)	262	0.031	0.450	0.519	0.256	0.744
Zhang et al. (2009)	451	Qinchuan	67	0.030	0.403	0.537	0.232	0.768
		Limousin x Qinchuan	47	0.043	0.277	0.681	0.181	0.819
		Angus x Qinchuan	36	0.111	0.444	0.444	0.333	0.667
		Germany Yellow x Qinchuan	42	0.071	0.214	0.714	0.178	0.822
Jawasreh et al. (2009)	422	Jordan native cattle	36	0.000	0.176	0.8235	0.088	0.912
		Holstein-Friesian	45	0.046	0.255	0.697	0.174	0.826
Misrianti et al. (2010)	611	Holstein-Friesian	45	0.022	0.444	0.533	0.244^{+}	0756^{+}
Biranvand et al. (2010)	451	Najdi	84	0.0357	0.2976	0.6666	0.1845	0.8155
Özdemir (2012)	260	Eastern Anatolian Red	71	0.14	0.54	0.32	0.41	0.59
		Holstein	181	0.04	0.31	0.65	0.20	0.80
Carsai et al. (2012)	451	Romanian Black and White cattle/ high milk production individuals	60	0	0.182	0.818	0.091	0.909
		Romanian Black and White cattle/ low milk production individuals	60	0	0.200	0.800	0.100	0.900
		Romanian Grey Steppe	60	0	0.500	0.500	0.250	0.750
Heidari et al. (2012)	1355	Holstein	100	-	-	-	0.170	0.830
Aytekin and Boztepe (2013)	451	Brown Swiss	301	0.12	0.51	0.37	0.374	0.626
Moravcíková et al. (2013)	260	Slovak Spotted cattle	110	0.087	0.417	0.496	0.2955	0.7045
Jakaria and Noor (2015)	451	Bali	245	0.00	0.04	0.96	0.018	0.982
		Madura	68	0.00	0.07	0.93	0.037	0.963
		Pesirir	100	0.01	0.13	0.86	0.075	0.925
		Aceh	25	0.00	0.08	0.92	0.040	0.960
		Katingah	50	0.00	0.10	0.90	0.050	0.950
Chauhan et al. (2015)	600	Sahiwal cattle	77	0.0389	0.3116	0.6493	0.1948	0.8051
Ahmadi et al. (2015)	611	Holstein	57	0.35	0.36	0.59	0.22	0.78
Ebrahimi Hoseinzadeh et al. (2015)	451	Holstein	100	0.06	0.40	0.54	0.26	0.74
Trakovická et al. (2015)	260	Slovak Simmental	288	0.052	0.347	0.600	0.226	0.774
Yasemin et al. (2017)	447	Holstein	146	0.548	0.3973	0.5479	0.2534	0.7466
Bayram et al. (2017)	600	Holstein	350	0.176	0.286	0.536	0.32	0.68
Hartati et al. (2018)	1301	Grati-Ongole Grade	107	0.000	0.009	0.991	0.005	0.995
Thuy et al. (2018)	451	Holstein	125	0.080	0.272	0.648	0.216	0.784
Gökcan (2019)	451	Holstein	52	0.019	0.231	0.750	0.135	0.865

N: observed number; *: Values calculated from allele frequencies and $^{+}$corrected values

5. Conclusions

The present investigation can be used as an indication for improvement of economically traits in the dairy cattle and for determination of the status of these four breeds reared in Turkey in addition studies are needed to perform an association researches with economically traits.

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