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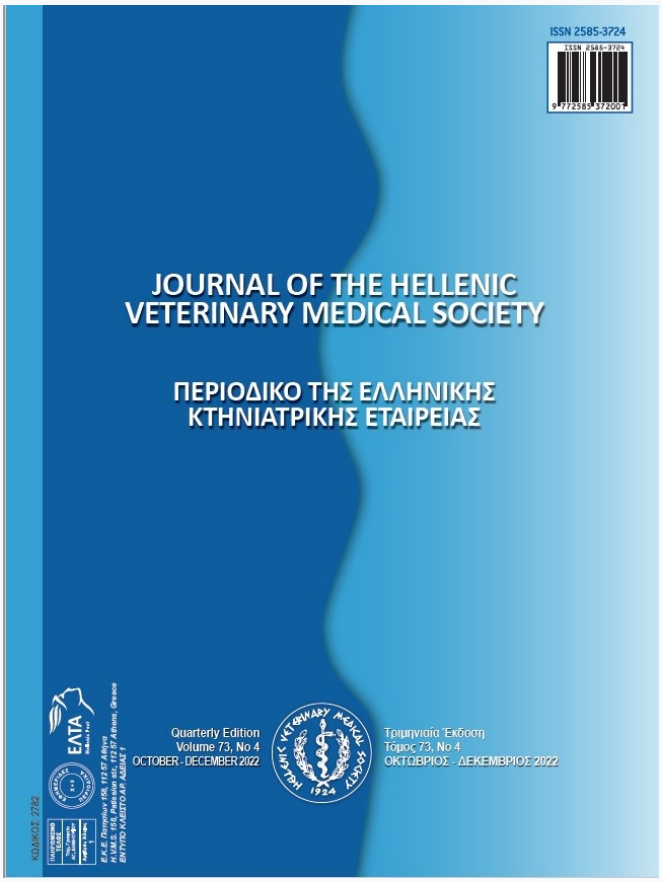

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Research article
Ερευνητικό άρθρο

The relationships between *Pit-1* gene polymorphism and some performance traits in simmental and brown swiss breeds

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ABSTRACT: This study's objective was to investigate the genotypic structures of the *Pit-1* gene from genomic DNA samples taken from the Simmental and Brown Swissbreeds and determine the distribution of genotype and allele frequencies of bovine breeds and reveal the relationships between the detected genotypes and some performance traits. *Pit-1*/*Hinf*I gene polymorphisms were determined using the PCR-RFLP method from genomic DNA samples obtained from Brown Swiss and Simmental cattle used in the study. According to the Hardy-Weinberg genetic test, the distribution of genotype frequencies of the studied population was found to be in equilibrium ($p>0.05$) in both breeds. Genotype frequencies of AA, AB, and BB alleles of the *Pit-1* gene were determined as 5.73%, 36.42%, and 57.85%, respectively, forthe Simmental breed, while the genotype frequencies for the Brown Swiss breed were determined as 9.43%, 42.86%, and 48.01%, respectively. Concerning the Simmental breed, the actual milk yield was determined as 5953 ± 364.2, 5212 ± 993.4, 5507 ± 889.5 kg, the 305-day milk yield as 5953 ± 276.8, 5642 ± 782.7, 5427 ± 246.7 kg, the lactation period as 307 ± 47, 293 ± 29, 303 ± 22 days, the daily milk yield as 19.53 ± 6, 18.49 ± 8, 17.81 ± 2 kg for AA, AB, and BB genotypes, respectively. Concerning the Brown Swiss breed, the actual milk yield was determined as 3606 ± 253, 3558 ± 530, 3999 ± 099 kg, the 305-day milk yield as 4920 ± 217, 4462 ± 900, 4635 ± 870 kg, the lactation period as 222 ± 36, 240 ± 04, 258 ± 37 days, the daily milk yield as 17.62 ± 2, 15.49 ± 2, 16.04 ± 3 kg for AA, AB, and BB genotypes, respectively.In the statistical analysis, the relationships between the performance traits studied and *Pit*-1 genotypes in Simmental and Brown Swiss cattle were not proven to be statistically significant.

Keywords: *Pit*-1, Polymorphism, Simmental, Brown Swiss, Performance traits, PCR-RFLP

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INTRODUCTION

Agriculture and animal husbandry play a vital role in meeting the nutritional needs of people. The main purpose of animal husbandry is profitable breeding with higher meat and milk yields per animal. In today's world, there are dairy cattle breeds with high capacity in terms of milk yield that are raised widely. In line with the breeding activities carried out in different countries according to ecological environmental conditions, important cattle breeds, such as Brown Swiss, Jersey, Guernsey, Ayrshire, Holstein, and Simmental breeds, come to the fore (Özek, 2015). The Brown Swiss and Simmental breedsare among the most common cattle breeds in Turkey. They are considered robust breeds with high capability to adapt to different conditions and with combined yield traits (Özhan et al., 2012).

While nutrition, genetic factors, breed, and epigenetic factors play a role in mammary gland productivity, the synthesis and secretion of milk are also associated with the lactation process (Qiang et al., 2014; Do and Ibeagha-Awemu, 2017). The lactation process varies depending on the effects of care, nutrition, and molecular mechanisms. These differences at the molecular level appear due to genetic and epigenetic changes. Since it is known that epigenetic effects play a significant role in the development of the mammary gland, studies have shown that the *Pit*-1 gene is involved in this process (Dinçel, 2018).

The inhibition of *Pit*-1 synthesis leads to a significant reduction in the proliferation of PRL, GH-producing cell lines, resulting in a considerable decrease in PRL and GH expression (Beigi et al., 2010; Selvaggi and Dario, 2011; Heidari et al., 2012). It has been suggested that the expression of the *Pit*-1 gene is superior to the growth hormone and prolactin genes and the expression of hormone-specific activators (Scully and Michael, 2000). The *Pit*-1 gene in cattle, which has a 129 amino acid protein, is a member of the DNA-binding POU family of the homeodomain transcription factor and is sublocalized to the centromeric region of bovine chromosome 1 located between the TGLA57 and RM95 loci (Moddy et al., 1995; Moddy et al., 1996). With QTL detection, 1q21-q22 chromosome region has also been claimed to make a positive contribution to animal production (Woollard et al., 2000).

The *Pit*-1 gene plays an important role in regulating the growth and development of living organisms (Sobrier, 2016). The *Pit*-1 gene, responsible for the growth hormone gene in the organisms of mammals, has been reported to be polymorphic using the *Hinfl* enzyme in cattle (Moddy et al., 1996). While it is stated that the use of polymorphic markers in breeding programs can make selection more accurate and efficient (Özdemir, 2012), it has been shown that PRL and GH are affected by the inhibition of *Pit*-1, causing decreased milk production, and the *Pit*-1 gene has a significant potential as a genetic marker to evaluate the traits of milk production (Trakovicka et al., 2014).

Previous studies have reported that the B allele of the *Hinfi* polymorphism in exon 6 of the *Pit*-1 gene is observed at higher frequencies, the related polymorphism is strongly associated with the protein percentage, and the AB genotype has higher milk yield and milk protein percentage than the AA and BB genotypes in cattle (Zwierzchowski et al., 2002; Cosier et al., 2007; Yang et al., 2010; Heidari et al., 2012; Hoseinzadeh et al., 2015). Some other studies have revealed an association of the *Pit*-1 AA genotype and the A allele with high milk yield and protein content (Groza et al., 2005; Viorica, 2006; Lin jia et al., 2009; Hussain, 2016; Bayram et al., 2017). They have also shown that polymorphisms in intron 5 of *Pit*-1 are significantly associated with body weight, average daily profit, and 6, 12, 18, and 24-month chest circumferences, and the A allele can be advantageous in terms of growth traits in Chinese cattle (Tang, 2012). The BB genotype in the Slovak Simmental cow population was reported to have a significant effect on milk performance (Trakovicka et al., 2015). Some studies investigating the relationships between *Pit*-1/*Hinf*I polymorphism and milk production traits in cattle have detected no relationship (Dybus et al., 2004; Aytekin and Boztepe, 2013; Arnim et al., 2017; Özdemir et al., 2018; Zabeel et al., 2018; Pozovnikova et al., 2020).

The aim of this studywas to examine by the PCR-RFLP method the genotypic structures of the *Pit*-1 gene from genomic DNA samples taken from Brown Swiss and Simmental cattle raised in two private enterprises in Erzurum, Turkey, determine the distribution of genotype and allele frequencies of cattle breeds in terms of the relevant gene, and investigate the relationships between genotypic structures and some performance traits.

MATERIALS AND METHODS

The research material consists of blood samples taken from 70 Brown Swiss cattle and 71 Simmental

cows raised in private enterprises in Erzurum and genomic DNA obtained from each sample.

The primary sequence of the *Pit*-1 gene(F: 5′-ACT CGC TAT TAC ACA ATA GGA GCC T-3′, R: 5′-TCC TGC CAA CTC ACC TCC C-3′), designed by Ozdemir (2012), was used to amplify a 260 bp fragment in cattle. In our study, amplification reactions were performed in a final volume of 25 µl, as seen in Table 1. PCR amplification was performed at different times under the same conditions (Table 1).

Visualization of PCR products

To determine whether amplification took place after the PCR process, the PCR product was run on a 1.2% agarose gel at 80 Watt for 25 min. The gel was prepared by adding 0.60 g of agarose, 50 ml TBE 1X, and 8 µl of ethidium bromide. It was kept in the microwave for 2 min and then poured into the cuvette to be loaded with agarose gel. Then, the combs were placed in the cuvette, and it was waited for 20-25 min for freezing and polymerizing. When the combswere frozen, 8 µl of the prepared mixture was loaded into the wells of each comb. During this addition, bromophenol was used for dyeing and placed in the combs. After visualization under UV light, the amplified PCR products were stored at -20 °C.

PCR-RFLP

Approximately 10 µl of each amplified sample was taken and placed in 0.2 ml sterile Eppendorf tubes. Six to eight units of *HinfI,* a restriction enzyme for the relevant region (5'-3' recognition region: GA^TC), 6-8 µl of RE buffer (buffer R and buffer Tango), and 6 µl of distilled water were added, and 5 µl of mineral oil was poured. Incubation was carried out at 37 °C°C for 10-12 hours. Afterward, 3 µl of bromophenol dye, a loading buffer, was added to each of the restricted samples. All products were moved around on the parafilm with the help of a micropipette to remove the mineral oil. Of the restricted PCR products, 8 µl was run on a 3% agarose gel prepared earlier and placed in the combs after its treatment with bromophenol. It was then visualized under UV light after 150 min of electrophoresis at 45 Volts. Genotyping processes were completed with the help of standard markers according to the band sizes of the products.

Statistical analyses

Pit-1 allele frequencies were determined by gene counting. The Chi-square (X^2) test was carried out to check whether the populations were in Hardy-Weinberg equilibrium or not. The relationships between some yield traits and genotypic structures of Brown Swiss and Simmental cattle raised in private enterprises were investigated.The study examined performance traits, such as actual milk yield, 305-day milk yield, daily milk yield,and lactation period. In the analysis of the obtained data, SPSS statistical software was used on the basis of the general linear model (Harvey, 1990). In the yield traits addressed for the Brown Swiss and Simmental breeds, the focus was set on external intermittent environmental factors such as genotype, lactation number,and calving season.

The following statistical model was used according to the yield traits in the study.

Y_{ijkl}: $\mu+a_i+b_j+c_k+e_{ijkl}$

$Y_{ijkl:}$ The value of any cow's performance traits (actual milk yield, 305-day milk yield, lactation period, and daily milk yield)

µ: population mean

a_i: i^{th} genotype effect (i:1-3; AA:1, AB:2, BB:3)

Table 1. PCR components of the *Pit*-1 gene and the PCR program applied

PCR component		**PCR program**		
Material	Volume	Temperature	Cycle	Stage
dNTP	1 µl	5 min at 95°C	1 cycle	Start
Taq	0.5-1.0 U			
MgCl2	25 µM	50 sec at 60°C	35 cycles	Denaturing
PCR buffer	5 µL(10x)	50 sec at 61°C	35 cycles	Annealing
Primer F	1 µM	45 sec at 60°C	35 cycles	Extension
Primer R	1 µM			
Genomic DNA	100ng	5 min at 72°C	1 cycle	Final extension
distilled water	10 µl			

b_j: effect of the j^{th} lactation order (j:1-3; 1st lactation:1, 2nd lactation:2, 3rd lactation:3)

c_k: effect of the k^{th} calving season (I:1-2;1: winter-spring, 2:summer-autumn)

e_{ijkl}: Marginal error

In the model used, all the factors, except for the error,were considered constant, and the error was accepted to berandom.

RESULTS AND DISCUSSION

PCR was conducted on each DNA sample isolated from the bloods of Simmental and Brown Swiss cattle, and they were run on a 1.2% agarose gel to obtain DNA bands. Figure 1 shows the image of PCR products on the agarose gel under UV light.

The DNA samples obtained from Simmental and Brown Swiss cattle were amplified in the PCR device and restricted with the *Hinf*I restriction endonuclease enzyme. The polymorphic regions of the *Pit*-1 gene were identified under UV light as a result of the electrophoresis of the 3% agarose gel prepared. Theoretically, bands are produced at the lengths of AA; 260 bp, BB; 190 bp and 70 bp, AB: 260 bp, 190 bp and 70 bp. Figure 2 shows the image of an exemplary agarose gel belonging to the PCR-RFLP result under UV light.

Genetic Structure of Breeds in Terms of Pit-1 Gene Polymorphism

In our study, AA, AB, and BB phenotypes were identified, depending on the *HinfI* restriction site polymorphism of the *Pit*-1 gene. The identified phenotypes and gene frequencies calculated in% distributionsare given in Table 2, and the H-W genetic equilibrium is given in Table 3.

The BB phenotype of the *Pit*-1 gene constituted the phenotype group observed at a high rate in the

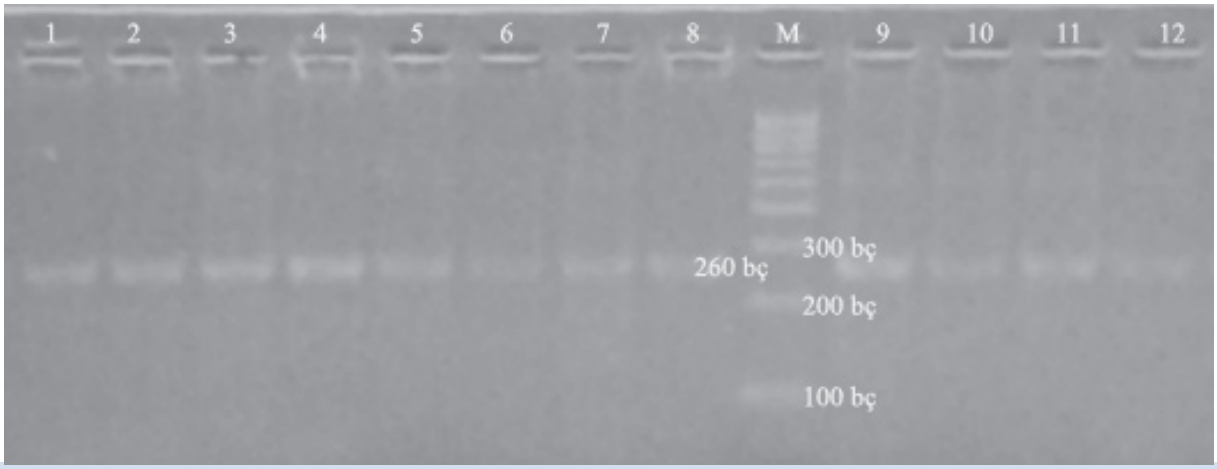

Figure 1. Agarose gel image of PCR products under UV light (M:1000-100 bp, *Pit*-1:260 bp)

Figure 2. PCR-RFLP gel image of the *Pit*-1 gene (AA;260bp, BB;190 bp and 70 bp, AB:260 bp, 190 bp and 70 bp, M: Marker)

Brown Swiss breed by 51.4% and in the Simmental breed by 62.0% (Table 2). The order of the phenotypes was BB>AB>AA and did not change according to the breeds. The gene frequency of the B allele of the *Pit*-1 gene was determined as 69% in the Brown Swiss breed and 76% in the Simmental breed.

Concerning the previous research conducted on the *Pit*-1 gene in different cattle breeds, the BB>AB>AA test analysis results reported in the studies carried out by Rennaville et al. (1997) on Italian Holstein-Friesian cattle breeds, by Dybus et al. (2004) on Polish black and white cows, by Viorica et al. (2007) on the Simmental breed, by Mukesh et al. (2008) on Indian native cattle breeds, by Zhang et al. (2009) on Qinchuan, Limousin x Qinchuan, Angus x Qinchuan, Germany yellow x Qinchuan breeds, by BeigiNassiri et al. (2010) on the Najdi cattle breed, and by Özdemir (2012) on the Holstein cattle breed were consistent with the results we obtained in the current study. The gene frequencies of the phenotype distributions of the *Pit*-1/*Hinf*I polymorphism were also consistent with our results.

The distributions of genotype frequencies in the Brown Swiss and Simmental breeds were in the Hardy-Weinberg genetic equilibrium (P>0.05) (Table 3).

Concerning the *Pit*-1 gene, the results of the Hardy-Weinberg genetic equilibrium test were found to be consistent with this studyin the studies conducted by Renaville et al. (1997) on Italian Friesian cattle, by Dybus et al. (2004) on Black and White Holstein cattle breeds, by Özdemir (2012) on East Anatolian Red and Holstein cattle breeds, by Hussain (2016) on Iraqi crossbred cattle, and by Aytekin and Boztepe (2013) on the Brown Swiss cattle breed.

Relationship of thePit-1 Gene Phenotypes with Some Performance Traits

The relationships of *Pit*-1 genotypes with some performance traits, such as actual milk yield, 305-day milk yield, lactation period, and daily milk yield,were investigated. The least squares means and standard errors of the *Pit*-1 gene genotypes in terms of some yield traits are presented in Table 4.

Table 2. Genotype Distributions of the *Pit*-1/*Hinf*I polymorphism and Allele Gene Frequencies in Breeds

Phenotype	Brown Swiss		Simmental	
	n	%	n	%
AA	9	12.9	7	9.9
AB	25	35.7	20	28.1
BB	36	51.4	44	62.0
Allele Gene Frequency (%)	**A**	**B**	**A**	**B**
	31	69	24	76

Table 3. *Pit*-1 genotype frequencies of Brown Swiss and Simmental cattle and the results of the Hardy-Weinberg genetic equilibrium test

Breeds	N	Observed	Expected	X^2 test	P
		AA/AB/BB	AA/AB/BB		
Brown Swiss	70	9/25/36	6.60/29.79/33.60	0.1783	NS
Simmental	71	7/20/44	4.07/25.86/41.07	0.05620	NS

NS: Non significant

Table 4. The least squares means and standard errors of *Pit*-1 Gene genotypes in terms of some yield traits

Breed	Genotype	N	Actual Milk Yield(kg)		305-Day Milk Yield(kg)		Daily Milk Yield(kg)		Lactation Period (day)	
			x̄±Sx̄		x̄±Sx̄		x̄±Sx̄		x̄±Sx̄	
Simmental	**AA**	15	5488	364.2	5953	276.8	19.53	6.11	307	47
	AB	23	5212	993.4	5642	782.7	18.49	8.31	293	29
	BB	52	5507	889.5	5427	246.7	17.81	2.70	303	22
Total		90	5464	192.2	5639	450.7	18.65	5.00	304	73
Brown Swiss	**AA**	18	3606	253	4920	217	17.62	2.91	222	36
	AB	62	3583	530	4462	900	15.49	2.27	240	04
	BB	70	3999	99	4635	870	16.04	3.00	258	37
Total		150	160	627	4672	996	16.38	6.25	240	26

The genotypes of the *Pit*-1 gene were determined as AA, AB, and BB, and the percentage frequencies were calculated as 5.73%, 36.42%, and 57.85% for the Simmental breed and 12.9%, 35.7%, and 51.4% for the Brown Swiss breed, respectively. The frequency of the A allele was 0.24, and the frequency of the B allele was 0.76 in the Simmental breed. The frequency of the A allele was 0.31, and the frequency of the B allele was 0.69 in the Brown Swiss breed. According to the Hardy-Weinberg genetic equilibrium test, it was observed that the populations examined for both breeds were in equilibrium (P>0.05).

In both breeds, the effect of *Pit*-1 genotypes on the examined performance traits was not significant (P>0.05). In the analyses, the AA genotype of the *Pit*-1 gene was observed to have a higher mean than the AB and BB genotypes in 305-day milk yield. Likewise, the AA genotype was superior to the AB and BB genotypes in daily milk yield. However, the effect of the difference in these genotype means was statistically insignificant (P>0.05)

Upon reviewing the previous similar studies on this subject, Rennaville et al. (1997) found the effect of the A allele on milk yield to be superior to the B allele in their study on Italian Friesian cattle. In their study on Polish Black and White cattle, Zwierzchowski et al. (2002) reported that the A allele had higher milk yield than the B allele. Contrary to these studies, Dybus et al. (2004) stated that they could not detect any relationship between the *Pit*-1 genotype and milk yield traits in Black and White cattle. Similar to our study, Aytekin and Boztepe (2013) reported no relationship between *Pit*-1 genotypes and 305-day milk yield in the Brown Swiss cattle breed, whereas Dybus et al. (2004) found this relationship to be significant in Polish Black and White cows. Among the studies investigating the relationship between milk yield and the *Pit*-1 gene in cattle, this relationship was not found significant in the study of Aytekin and Boztepe, (2013) on the Brown Swiss cattle breed, in the study of Hussain (2016) on cattle breeds in the Iraq region, and finally in the study of Arnim et al. (2017) on Pesisir cattle breeds.Zwierzchowski et al. (2002) and Dybus et al. (2004) reported a significant effect of lactation period and *Pit*-1/*Hinf*I polymorphism in their studies on black and white cows.

CONCLUSION

Genotypic structures of the *Pit*-1 gene polymorphism of each cattle were identified using the PCR-RFLP method on genomic DNA samples obtained from the Simmental and Brown Swiss breeds. Both populations were observed to be in genetic equilibrium in terms of the investigated *Pit*-1 gene polymorphic region. It was revealed that the effects of the detected *Pit*-1 genotypic structures on all yield traits (actual milk yield, daily milk yield, 305-day milk yield, and lactation period) were not statistically significant. This necessitates conducting intensive studies on different breeds and populations.

CONFLICT OF INTEREST

None declared by the authors.

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