Kocatepe Veterinary Journal

Kocatepe Vet J. (2024) 17(4):386-392

DOI: 10.30607/kvj. 1485628

RESEARCH ARTICLE

Pit1/Hinfi Polymorphism in Holstein Cattle in Afyonkarahisar Province

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ABSTRACT

Pit1 (Pituitary Specific Transcription Factor-1) gene has been shown to be resided on the centromeric region of chromosome 1 in cattle. This gene, also known as POUF1F has responsible for development of pituitary gland and hormone expression in mammals. Pit1 deficiency causes a dwarfism phenotype in mice, dogs and fish. Previous studies have demonstrated to associated with polymorphisms of the Pit1 gene and growth and reproductive performance and milk, meat yield traits. In the cattle, a HinfI polymorphism on Pit1 gene (c.1178G>A) have been reported that there is an association between milk and reproduction yield parameters. In this study, we aimed existence and distribution of Pit1/HinfI polymorphism in 81 head Holstein cattle in Afyonkarahisar province. In our study, we defined that there were 5 AA, 30 AG and 46 GG genotypes in our survey population. We also detected to frequency of A allele as 0.25 and the G allele as 0.75. We calculated to genetic index values such as PIC (0.3027418) and Heterozygosity (0.3742044) using RStudio package. Chi-square value was found 0.049 and was exhibited to survey population in Hardy-Weinberg equilibrium. The Pit1/HinfI polymorphism is a potential option for use in marker-assisted selection studies in light of these findings for Holstein cattle in Afyonkarahisar province.

Keywords: HinfI, Holstein sığır, PCR-RFLP, Pit1

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Afyonkarahisar'da Yetiştirilen Holstein Sığırlarda Pit1/Hinfi Polimorfizmi

ÖZ

Pit1 (Hipofize Özgü Transkripsiyon Faktörü-1) Sığırlarda 1.kromozomun sentromerik bölgesinde bulunan yaklaşık 129 aminoasitten oluşan bir protein kodlayan bir gendir. Memelilerde hipofiz bezinin gelişiminden ve hormon ekspresyonundan sorumlu olan Pit1 geni, POUF1F olarak da bilinmektedir. Pit1 eksikliğinde farelerde, köpeklerde, balıklarda cücelik fenotipi gözlenmektedir. Yapılan çalışmalar, Pit1 genindeki polimorfizmlerin sığırlarda, koyunlarda, domuzlarda, tavuklarda büyüme, üreme performansı, et ve süt verimi özellikleriyle ilişkili olduğunu göstermiştir. Sığırlarda Pit1/HinfI (c.1178G>A) polimorfizminin süt verimi ve döl verimi parametreleriyle ilişkili olduğu ifade edilmektedir. Bu çalışmada, Afyonkarahisar ilinde yetiştirilen 81 baş Holstein sığır Pit1/HinfI polimorfizminin varlığı ve dağılımının belirlenmesi amaçlanmıştır. PCR-RFLP tekniği kullanılarak hayvanların bu polimorfizm açısından genotiplendirilmiştir. Çalışma sonucunda 81 hayvandan 5 tanesinin AA, 30 tanesinin AG ve 46 tanesinin GG genotipinde olduğu bulunmuştur. G allelinin frekansı 0,75, A allelinin frekansı ise 0,25 olarak hesaplanmıştır. Genetik indeks değerlerinden PIC değeri 0,3027418 ve Heterozigotluk değeri 0,3742044 olarak hesaplanmıştır. χ^2 değeri 0,049 bulunmuş olup, popülasyonun Hardy-Weinberg dengesinde olduğu ortaya konulmuştur. Bu bulgular ışığında Pit1/HinfI polimorfizminin Afyonkarahisar ilinde yetiştirilen Holstein sığırlar için markör destekli seleksiyon çalışmalarında kullanılabilecek potansiyel bir aday olduğu düşünülmektedir.

Anahtar kelimeler: HinfI, Holstein sığır, PCR-RFLP, Pit1

To cite this article: Gezer Bilecen P. Demirtaş E. Erdoğan M. Uğuz C. Pit1/Hinfi Polymorphism in Holstein Cattle in Afyonkarahisar Province. Kocatepe Vet J. (2024) 17(4):386-392

Submission: 27.05.2024 **Accepted**: 25.11.2024 **Published Online**: 04.12.2024

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INTRODUCTION

Pituitary specific transcription factor -1 (Pit1) gene has been shown to be resided on the centromeric region of chromosome 1 in cattle. This gene encodes a protein of 129 amino acids and approximately 33 kDa in weight. (Moody et al. 1995; Selvaggi et al. 2011; Thuy et al. 2018). Pit1 gene is liable for the expression of hormones and the development of pituitary gland in mammals (Doosti et al. 2011). It has been shown that the expression of prolactin hormone in lactotroph cells (Supowit et al. 1992) and growth hormone in somatotroph cells (Tuggle and Trenkle, 1996) in anterior pituitary is controlled by Pit1 gene. The Pit1 protein, also called POUF1F, contains a POU domain for high affinity binding to target DNA (Ingraham vd., 1990, Hendriks-Stegeman vd., 2000). The Pit1/POUF1F protein binds to the promoter areas of the genes that encode growth hormone and prolactin hormone via its POU domain, resulting in the stimulation of transcription (Mangalam vd., 1989, Trokavicka vd., 2015).

Mutations on Pit1 gene may cause the inadequate expression of GH, PRL and TSH hormones in anterior pituitary (Cohen et al. 1996; Renaville et al. 1997). Dwarfism in mouse (Li et al. 1990, Flurkey et al. 2002), fish (Nica et al. 2004) and dogs (Lantinga-van Leeuwen et al. 1999) showed that Pit1 deficiency is responsible for growth hormone regulation.

Pit1 gene polymorphisms have been reported to show an association between growth and reproduction traits in cattle (Pytlewski et al. 2018), buffaloes (Zghair and Hassoni, 2021), sheep (Bai et al. 2016), swine (Piórkowska et al. 2015) and chickens (Nie et al. 2008). Pit1 gene polymorphisms are correlated to the first calving age, weaning, and the daily gain weight of calves in Limousine cattle (Pytlewski et al. 2022). These polymorphisms are reported to affect the milk yield and the components of milk, such as the amounts of lipids and proteins (De Mattos et al. 2004; Edriss et al. 2009, Zhou et al. 2016; Thuy et al. 2018).

Initially, Wollard et al. (1994) showed using by HinfI restriction enzyme that there was a transition mutation from G to A in 451 bp length region (c.1178G>A) on Pit1 gene. This polymorphism has been shown to be associated with reproductive parameters such as the first calving age, insemination numbers, calf and the cow weight during calving in Holstein cattle (Pytlewski et al. 2018). Pit1/HinfI polymoprhism was also associated with milk yield and milk composition in Holstein and Sahiwal cattle (Renaville vd., 1997; Hosseinzadeh et al. 2015a; Chauhan et al. 2015; Anggraeni et al. 2020). However, it has been reported that there is no relation between polymorphism and dairy traits in Simmental and Brown Swiss breeds (Aytekin and Boztepe, 2013; Sönmez and Ünal, 2023).

Previous studies have shown that Pit 1 gene polymorphisms are a potential marker for selection studies. In this study, we purposed to detect the existence and distribution of Pit1/HinfI polymorphism in 81 Holstein cattle in Afyonkarahisar province.

MATERIAL and METHODs

In this study, blood samples collected from Holstein cattle and laid up at -80 °C in the Medical Biology and Genetics laboratory in Afyon Kocatepe University-Faculty of Veterinary Medicine were used.

In this study, blood samples previously collected from Holstein cattle and laid up at -80 °C in the Medical Biology and Genetics Laboratory of Afyon Kocatepe University, Faculty of Veterinary Medicine were used. All procedures were approved by the local ethics committee (AKÜ-HADYEK-260-20) Afyon Kocatepe University.

DNA Isolation

DNA was isolated from blood samples using by spin-column method. Ten micro liter proteinase K, 200 µl blood sample and 200 µl Extraction buffer were added into 1.5 ml microcentrifuge tubes and vortexed for 10-15 seconds. Mixture was then incubated at 56 ℃ for 15 minutes. After the completion of incubation, 210 µl Binding Buffer was added into the lysate and transferred into the spin column and centrifuged at 8000 rpm for 1 minute. Aliquates of 650 µl Wash buffer I was added into the spin-column and centrifuged again at 8000 rpm for 1 minute. Collection tube was discarded and 500 µl Wash Buffer II was added into tube emptied tube and centrifuged at 8000 rpm for 1 minute. Collection tube was discarded and 250 µl Wash Buffer II was added into spin column and centrifuged at 14000 rpm for 3 minutes. After centrifugation, column was transferred to new 1.5 ml microcentrifuge tube and 100 µl TE (10 mMTris- 1mM EDTA, pH: 8.0) buffer was added into column and incubated at room temperature for 5 minutes. When incubation was completed, tubes centrifuged at 8000 rpm for 1 minute. Isolated DNA was stored at -80 ℃.

Polymerase Chain Reaction (PCR)

Primer sequences used was represented in Table 1. Primers optimal annealing temperature was determined as 56 ℃ by using Gradient PCR process. Primer pairs for target region at intron between 5 and 6 exons were used as described by Woollard et al (1994). Primer pairs were checked for hairpin and dimerization by using Primer3 programmed.

Table1. Primer sequences for Pit1 gene amplification

Primer	Sequence	Tm (Temperature Melting)
Forward	AAACCATCATCTCCCTTCTT	
5'→3'		56℃
Reverse 5'→3'	AATGTACAATGTGCCTTCTGAG	

PCR amplification was performed by using Dream Taq Polymerase kit 5U/ µl (Thermofisher Scientific-Litvania). 10 x PCR buffer 1 µl, 0.3 mM Forward primer, 0.3 mM Reverse Primer, 0.3 mM dNTP mix, 0.0625 µl (1.25 U) Dream Taq DNA polymerase, 1.5 µl DNA (~ 15 ng) was added into 0.2 ml PCR tubes. PCR conditions was shown in Table 2. When PCR process was completed, PCR products were run in %2 agarose gel electrophoresis at 90 Volt and viewed at UV imaging system (Vilber Lourmat BIO-VISION).

Table 2. PCR conditions

Component	Volume (µl)	Incubation Temperature
Nuclease Free Water	5.5	
Fast Digest Green Buffer	1	37℃-30 minute
HinfI Restriction Enzyme	0.5	80℃-15 minute (for inactivation of enzyme)
PCR product	8	
Total	15	

RFLP-Restriction Fragment Length Polymorphism

After completion of PCR, the PCR products were cut by using the HinfI restriction endonuclease for the detection of polymorphism.

Protocol for RFLP was shown in Table 3. HinfI restriction enzyme was used to detect the genotypes from PCR product. DNA fragments obtained from RFLP was run in 3% agarose gel electrophoresis at 90 Volt for 30 minutes and then viewed at UV imaging system.

Statistical Analysis

R-Studio package was used to allele and genotype frequencies with this PIC value and Heterozygosity value. We also calculated the genetic index value such as PIC value and Heterozygosity value, using RStudio Package. The population's Hardy-Weinberg equilibrium was defined using the chi-square (χ^2) test.

Table 3. Contents of RFLP reaction

Component	Volume (µl)	Incubation Temperature
Nuclease Free Water	5.5	
Fast Digest Green Buffer	1	
HinfI Restriction Enzyme	0.5	37℃-30 minute
PCR product	8	80℃-15 minute (for inactivation of enzyme)
Total	15	

RESULTS

In this study, the target region of Pit1 gene (between intron 5-exon6) was amplified by PCR.. RFLP analysis was performed for genotyping by HinfI enzyme. In RFLP, 451 bp length DNA fragment was cut using HinfI into 244 and 207 bp length two fragments (Figure 1). In the surveyed population (81 head Holstein cattle in Afyonkarahisar), we determined that there were 5 AA, 30 AG and 46 GG genotypes. Frequency of G allele appeared as 0.75, whereas A allele was 0.25.

We also determined the PIC value as 0.3027418 and the heterozygosity value as 0.3742044 using the R-Studio package (Table 4). If PIC value ranges from 0.25 to 0.50, this polymorphism can be used for marker as mid-level informativeness (Bostein et al. 1980; Selvaggi and Dario, 2011). Our PIC value, demonstrated that the Pit1/HinfI polymorphism could be a mid-level informative marker to use in selection studies.

The survey population was in Hardy-Weinberg equilibrium in terms of HinfI polymorphism in the target region of Pit1 gene, according to our results (P>0.05). As a result of the χ^2 test calculation based on the observed and expected genotype frequencies that was 0.049 and this value was below the 0.05 significance level (TD1:0.05= 3.841) with 1 degree of freedom in the χ^2 distribution table.

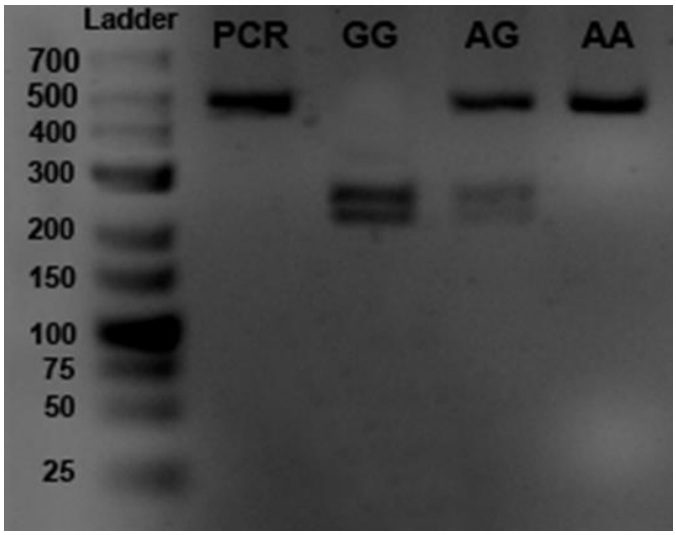

Figure 1: Results of RFLP. Only 451 bp in fragment in AA genotype, 244 and 207 bp fragments in GG genotype, all fragments in heterozygote AG.

Table 4. Genotypic and allelic frequencies and genetic index values

Frequency of Allele			Frequency of Genotype			χ^2
	Count	Proportion		Count	Proportion	
A	40	0.25	AA	5	0.06	
G	122	0.75	AG	30	0.37	
			GG	46	0.57	0.049

Genetic Indexes	
PIC value	0.3027418
Hu (Heterozygosity)	0.3742044

DISCUSSION

In 350 Holstein cattle, Bayram et al. (2017) found that the frequency of the G allele was 0.68 and the A allele was 0.32. According to Hosseinzadeh et al. (2015b), the frequency of the G allele was 0.74 and the frequency of the A allele was 0.26 in Holstein cattle in the HinfI restriction site. Misrianti et al (2010) they defined that the frequency of G allele was 0.75 and the A allele was 0.25 in Holstein cattle, exactly the same in our study. Genotype frequency was also AA 2%, AG 44%, and GG 53%. Besides, there is no detection of Pit1/HinfI polymorphism in 320 Indonesian buffalo. In the case of this polymorphism, the buffalo population was monomorphic (Ref). In 1024 Polish Holstein cattle, Pytlewski et al (2018) reported that the G allele frequency was 0.795 and the A allele frequency was 0.205. AA, AG, and GG genotypes frequencies also notified as 4.7 %, 31.5 % and 63.7 %, respectively. In 125 Holstein cattle, the frequency of the G allele was 0.784 and the frequency of the A allele was 0.216, according to Thuy et al (2018). The frequency of the GG, AG, and AA genotypes was determined to be 8%, 27.2%, and 64.8%, respectively. Dybus et al (2004) demonstrated frequency of G allele was 0.757 and A allele was 0.243 in 900 head of Holstein cattle in Poland. Frequency of AA, AG and GG genotypes also reported 5%, 38% and 56%, respectively, in the same study. Edriss et al (2009) reported to frequency of G allele was 0.744 and A allele was 0.256. Genotype frequencies were also reported as AA 3%, AG 45%, and GG 52%. All of these studies were conducted on Holstein cattle breeds. The results of all these studies were close to each other and the results of our studies were similar to the results of those studies.

In 288 Simmental cattle, Trokavicka et al. (2015) reported that the frequency of G allele was 0.774 and the A allele was 0.225. In Simmental cattle (n=67), the frequency of the G allele was 0.58 and the frequency of the A allele was 0.42 and the population under examination was in Hardy-Weinberg equilibrium according to Sönmez & Ünal (2023). Aytekin and Boztepe (2013) reported the frequency

of G allele was 0.626 in 301 Brown Swiss cattle, while the A allele was 0.374. Studies conducted in other dairy cattle breeds including Simmental and Brown Swiss showed that there were approximately similar results in Holstein breed. Ardıçlı et al. (2023) determined that the frequency of G allele was 0.7778 and the A allele was 0. 2222. Genetic index values such as the PIC value and heterozygosity were noted as 0.2859 and 0.3457, respectively.

In 2009, Zhang and colleagues examined in Chinese native breed Qinchuan and its various crossbreed (Pure Qinchuan-QQ n=67, Limusin x Qinchuan-LQ n=47, Angus x Qinchuan- AQ n=36 and Germany Yellow x Qinchuan-DQ n=42) for HinfI/Pit1 polymorphism. They noted the frequency of the G allele was 0.768, 0.819, 0.667, and 0.88, whereas the A allele was 0.232, 0.181, 0333, and 0.178, respectively, in QQ, LQ, AQ, and DQ cattle. The Pit1/HinfI polymorphism allele frequencies in Podolica cattle were reported by Selvaggi and Dario (2011) to be A = 0.3 and G = 0.7 and 14.42% AA, 31.73% AG, and 53.85% GG were the genotype frequencies. In addition, the PIC value was calculated as 0.332 which was in parallel with our results. According to Moravčiková et al (2013) frequency of G allele was 0.704 and A allele was 0.295 at 110 spotted regions in Slovak cattle and the population was Hardy-Weinberg equilibrium. The G allele frequency was detected as 0.659 and the A allele frequency was 0.341 in 296 Auliekol cattle of Kazakhstan. As Taipova et al (2020) reported that the studied population was in Hardy-Weinberg equilibrium. De Mattos et al (2004) investigated to the Pit1/HinfI polymorphism which was used for progeny testing Gyr bulls. The results of this investigation indicated that the frequency of G allele 0.95 and A allele was 0.05, whereas genotype frequencies were AG 10 % and GG 90%. AA genotype was not observed in this study. Hartati et al (2018) defined the allelic frequencies A and G alleles as 0.005 and 0.995 in 107 Indonesian native cattle breeds. Moreover, they found the genotype frequencies as AG 0.9% and 99.1% GG. Additionally, they showed that there was no AA genotype in the population and the population was in Hardy-Weinberg equilibrium. On the contrary to our findings, they determined to PIC value extremely low as 0.009. In this population, the genetic diversity concerning the targeted polymorphism is notably low. These two studies did not coincided to AA genotype and the ratio of heterozygosity was very low. Thus, frequency of A allele was extremely low. Zghairand and Hassoni (2021) determined that the frequency of G allele was 0.90 and the A allele was 0.10 in 27 buffaloes. They did not obtain any AA genotype in the population. This research showed that the A allele originating from only heterozygous individuals is present in the population. Therefore, this study reveals very important findings since it is the only study in which the A allele was detected in buffaloes. Gritsienko et al. (2020) reported that the frequency of the G allele was 0.69, 0.63 and 0.50. The A allele was also 0.31, 0.37 and 0.50 in Ukrainian native breeds including Ukrainian red (n = 32), Ukrainian black mottled (n = 32), and Ukranian red mottled (n = 28), respectively. It has been determined that G allele frequency is 0.71 and the A allele frequency is 0.29 in 69 Russian Holstein, (Pozovnikova et al (2020).

The frequency of A allele was found as 0.356 and the G allele was found 0.644 in 104 Anatolian Black (native Turkish cattle breed) by Sakar and Zülkadir (2022). Genotype frequencies also noted AA 9%, AG 51,9% and GG 38,5%. They identified to heterozygosity value (He) as 0.458. Aytekin and Bayraktar (2022) showed that the frequency of G allel was 0.74, 0.68, 0.90 and 0.77 in Anatolian Black, Holstein, Brown Swiss, and Simmental cattle, respectively. AA genotype was not detected in the population. The A allele was 0.26, 0.32, 0.1 and 0.23. This study demonstrated the minimum frequency of the A allele in Brown Swiss breed and also the maximum frequency in Holstein, as shown in other studies. Toğyar ve Özdemir (2023), investigated to the polymorphism in 70 Brown Swiss and 71 Simmetnal cattle. They determined frequency of G allele was 0.69 in Brown Swiss and 0.76 in Simmental cattle. The A allele frequency was 0.31 in Brown Swiss and 0.24 in Simmental cattle.

Khaizaran et al (2014) studied Pit1/HinfI polymorphism on 101 Palastinian Holstein, 18 crossbred and 25 native breed cattle. The frequencies of A allele were showed 0.31, 0.66 and 0.78 and the G allele were 0.68, 0.33 and 0.22, respectively in the mentioned breeds. It was observed that the frequency of the G allele was higher in the Holstein, which has been known to have high dairy characteristics in comparison to the Palestinian native and hybrid cattle breeds. Similarly, Doosti et al. (2011) investigated to the polymorphism in 224 Holstein cattle and 210 Iranian native cattle. In native cattle, they determined frequency of G allele was 0.25 and the A allele was 0.75. However, in the Holstein cattle, they defined frequency of G allele was 0.701 and the A allele was 0.298. These studies indicated that there is a high frequency of A allele in native cattle breeds than that of Holstein cattle breed.

CONCLUSION

In conclusion, Pit1 gene region is polymorphic in Holstein cattle breed in Afyonkarahisar. Wild type G allele is common while A allele is rare. Thus, Pit1/HinfI polymorphism is convenient to use for marker assisted selection study. Our study did not declare an association with any yield traits or phenotypic data. However, previous other studies were proved to Pit1/HinfI polymorphism affects on milk yield, milk composition, meat quality and reproductive traits.

Conflict of Interest: The author declares no conflict of interest.

Authors Contribution Rate: PGB: %35, ED: %35, ME: %15 CU: %15

Ethics Committee Information: All animal-related procedures were approved by the local ethic committee (approval AKUHADYEK 49533702/25) at Afyon Kocatepe University.

Financial support: The study was supported by Afyon Kocatepe University, BAP coordinating.

Explanation: This study is summarized from the first author's Master's thesis.

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