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Identification of Pituitary-Specific Transcription Factor-1 (PIT-1) Genotype in Bali Cattle

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ABSTRACT

This study aimed to identify the genotype of the gene Pituitary-specific transcription factor 1 (Pit-1) in Bali cattle (*Bos sondaicus*). A total of 20 Bali cattle (13 males and seven females) were used in this study. DNA samples were isolated from blood samples using the phenol-chloroform extraction method. This study uses the PCR-RFLP method. Amplification of specific DNA fragments (size 451 bp, intron regions are stretching from 4 to exon 6) of the Pit-1 gene Bali cattle samples was performed using a pair of specific primers (forward: 5'-AAA-CCA-TCA-TCT-CCC-TTC-TT -3 ', and reverse: 5'-AAT-GTA-CAA-TGT-GCC-TTC-TGA-G-3'). Based on the RFLP analysis/*Hin*fI, all Bali cattle research resulted in a uniform (monomorphic) band pattern. The band sizes were 244 bp and 207 bp, and the band patterns were thus identified as genotype BB, so it is known that the frequency of the A and B alleles is at 0 and 1. The frequency of the A allele was not found in Bali cattle (*Bos sondaicus*) and showed a significant difference with other cattle breeds (*Bos taurus* and *Bos indicus*).

Keywords: Bali cattle, Pit-1 gene, PCR-RFLP, restriction enzyme *Hin*fI

INTRODUCTION

Bali cattle (*Bos sondaicus*) are native Indonesian beef cattle that need to be preserved, and their productivity is increased. The development of molecular technology today can help improve productivity and maintain the purity of the nation. Through this technology, it is possible to identify polymorphisms of loci in genes and non-genes in the Bali cattle genome. The polymorphic loci that were found to be associated with livestock traits with the economic value could be used as molecular markers to assist in the selection program for improving livestock traits.

Pituitary transcription factor-1 (Pit-1), also known as growth hormone factor-1 (GHF1), is a pituitary-specific transcription factor that plays a role in activating the expression of prolactin, thyrotropin and growth hormone genes in the anterior pituitary gland (Tuggle and Trenkle, 1996).), the GHRH receptor gene (Lin et al., 1992), the Pit-1 gene itself (Rhodes et al., 1993), and is also a factor controlling the differentiation and proliferation of cells of the pituitary gland (Renville et al., 1997[a]; Zhao, 2004).

In cattle, Pit-1 is a protein composed of 291 amino acids (De Mattos et al., 2004) and is encoded by a single gene. The Pit-1 gene in cattle (*Bos taurus*) is located at the centromere region of chromosome 1 (Moody et al., 1995). Pit-1 is a member of the POU domain (Pit-Oct-Unc), which contains proteins, a group of transcriptional regulators that have a crucial role in cell differentiation and division (Mangalam et al., 1989). Therefore, the gene encoding Pit-1 was selected as a candidate gene for milk performance, growth, and carcass traits in several cattle breeds (Renaville et al., 1997b; Woollard et al., 1994; Moody et al., 1995).

Pit-1 gene polymorphism in cattle (*Bos taurus*) was first reported by Woollard et al. (1994) in exon 6 using the RFLP/*HinfI* technique, which found two alleles: A and B. Molecularly, this polymorphism arises as a result of a point mutation, namely the substitution of adenine with guanine (A207G) located at exon 6 of the Pit-1 gene (Woollard et al., 1994; Dierkes et al., 1998). The *Hin*fI polymorphism in exon 6 of the Pit-1 gene (Pit-1/E6/*Hin*fI locus) was later found in cattle breeds belonging to the *Bos taurus* and *Bos indicus* groups (Table 1). Some researchers have even found a relationship between these polymorphic loci with growth, body weight, carcass, and lactation performance traits and stated that the Pit-1/E6/*Hin*fI locus could be used as a molecular marker for these traits in the studied cattle (Renaville et al., 1997a; Woollard et al., 1994; Moody et al., 1995).

This study aimed to identify the HinfI genotype in exon 6 of the Pit-1 gene (Locus Pit-1/E6/*Hin*fI) in Bali cattle (*Bos sondaicus*). This identification is important for at least two purposes: (1) providing basic information in the framework of finding molecular markers for genetic improvement of growth characteristics of Bali cattle if the evaluation results reveal that the locus is polymorphic; however (2) if the Pit-1/E6/*Hin*fI locus is found to be monomorphic, the

genotype of that locus can be used as an additional indicator in addition to the existing indicators to assess the purity of the Bali cattle population in a particular area as an effort to conserve Bali cattle.

MATERIALS AND METHODS

The material used in this study was a DNA sample of Bali cattle isolated from blood samples of 20 Bali cattle (unrelated) using the phenol-chloroform extraction method (Sambrook et al., 1989). The 20 Bali cattle in the study were adult cattle consisting of 13 males and seven females, which are Balinese cattle owned by smallholder farmers in the Manokwari Regency, West Papua, Indonesia.

In this study, identification of the Pit-1 genotypes in Bali cattle used the PCR-RFLP method. The steps were amplifying specific DNA fragments, digestion of specific DNA fragments using the *Hin*fI restriction enzyme, electrophoresis of digestion products, and data analysis to determine the allele frequency and genotype of Pit-1 Bali cattle.

Amplification of specific DNA fragments (451 bp in size, spanning from the intron 4 to exon six region of the Pit-1) gene was performed by the Polymerase Chain Reaction (PCR) technique. The primers used are: forward: 5'-AAA-CCA-TCA-TCT-CCC-TTC-TT-3' and reverse 5'-AAT-GTA-CAA-TGT-GCC-TTC-TGA-G-3 ' (Woollard et al., 1994). The specific DNA fragment sequence (451 bp) is presented in Figure 2. The specific DNA fragment amplification process was initiated by adding 19µL dH_2O, 2µL DNA solution, and 2µL each primer into the Pure TagTM Ready-To-GoTM PCR Beads tube (Amersham Biosciences). The PCR conditions were programmed according to the instructions of Woollard et al. (1994), as follows: initial denaturation of 95^oC, 5 minutes, followed by 35 cycles of amplification (denaturation 95^oC, 30 seconds, annealing 56^oC, 60 seconds, 72^oC extension, 120 seconds), and extra extension at 72^oC, 5 minutes.

The obtained amplicon was digested with the restriction enzyme HinfI (5'..g▼antc.. 3'), produced by MBI Fermentas. A total of 5µL amplicon, 1µL HinfI restriction enzyme, 1µL buffer 10x, and 3µL dH_2O were put in a 1.5 ml microtube and homogenized, then incubated in a water bath for 3 hours at 37^oC. Digestion products were electrophoresed on 1.5% agarose gel containing Gold Nucleic Acid Stain in TBE buffer. The electrophoresis begins by taking 5µL of digestion product, mixing it with 2µL of loading buffer, and then putting it into the gel well. Running gel was carried out at 100 volts for 30 minutes using a DNA marker (DirectLoadTM Wide Range DNA Marker, Sigma). The electrophoresis results were examined under ultraviolet light, then photographed with a digital camera. Genotype identification was made by comparing the banding pattern produced by each sample with DNA markers.

The data obtained were estimated by allele and genotype frequencies of the studied loci using the procedure of Nei and Kumar (2000), as follows:

$$X_i = (2n_{ii} + \Sigma n_{ij}) /(2n)$$

$$X_{ii} = n_{ii}/n$$

were,
Xi = i-th allele frequency
Xii = ii-th genotype frequency
nii = number of samples with ii genotype
nij = number of samples with ij genotype
n = number of all samples.

A locus is classified as polymorphic if the highest allele frequency found in the population does not exceed 0.99 (Nei and Kumar, 2000). On the other hand, a locus is classified as monomorphic if it does not meet the polymorphic criteria above.

RESULTS AND DISCUSSION

A specific DNA fragment of 451 bp in size (ranging from the intron region 4 to exon 6) of the Bali cattle Pit-1 gene was successfully amplified (Figure 1) using a pair of primers as recommended by Woollard et al. (1994). Based on the nucleotide sequences available in GenBank: EF090616.2 and EF090617.2, it is known that there is a single nucleotide mutation [G (Guanine) A (Adenine)] at position 207. This variation can be detected by nucleotide cleavage treatment using the restriction enzyme HinfI. Figure 2 shows the sequence of target DNA fragments in the Pit-1 gene not recognized by HinfI (allele A, GenBank: EF090617.2) and recognized by HinfI (allele B, GenBank: EF090616.2).

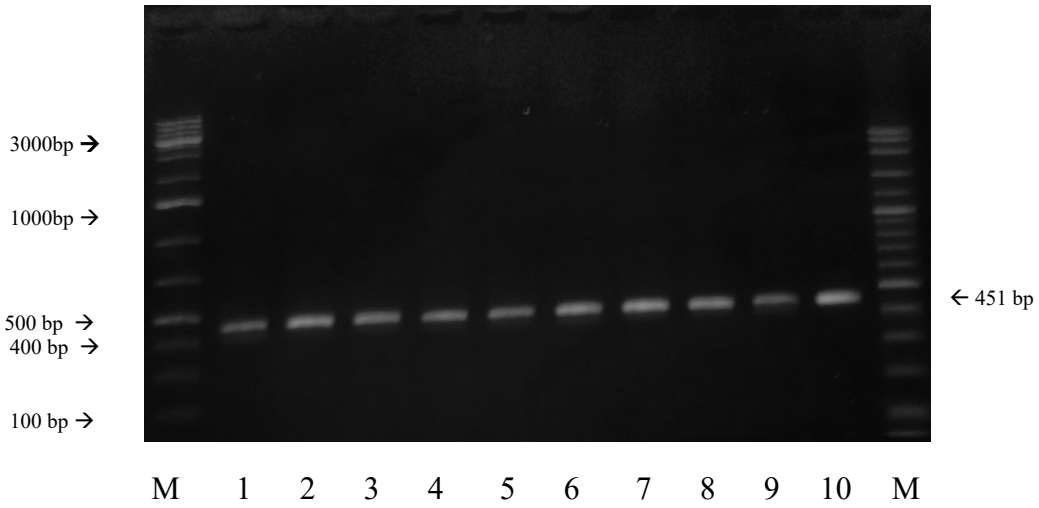

Figure 1. Visualization of the amplification of specific DNA fragments (451 bp, extending from intron 4 to exon 6) in the Pit-1 gene of Bali cattle. (M: DNA marker; No. 1 – 10: amplicons)

Alel A

Primer Forward →

```
  1 aaaccatcat ctcccttctt tcctgccaac tccccacctc ccagtattgc tgctaaagac
 61 gccctggaga gacactttgg agaacagaat aagccttcct ctcaggagat cctgcggatg
121 gctgaagaac taaacctgga gaaagaagtg gtgagggttt ggttttgtaa ccgaaggcag
181 agagaaaaac gggtgaagac aagcctaaat cagagtttat ttactatttc taaggagcat
241 ctcgaatgca gataggctct cctattgtgt aatagcgagt gtttctactt ttcattcctt
301 tctcttctcc agccaaaata gaaattagtt atttggttag cttcaaaaaa tcacatcagt
361 aatttttgca gaagtgtttc ttttctactt taaaaataaa tacaatttaa attatgttga
421 tgaattattc tcagaaggca cattgtacat t
```

← Primer Revese

Alel B

Primer Forward →

```
  1 aaaccatcat ctcccttctt tcctgccaac tccccacctc ccagtattgc tgctaaagac
 61 gccctggaga gacactttgg agaacagaat aagccttcct ctcaggagat cctgcggatg
121 gctgaagaac taaacctgga gaaagaagtg gtgagggttt ggttttgtaa ccgaaggcag
181 agagaaaaac gggtgaagac aagcctg▼aatcagagtttat ttactatttc taaggagcat
241 ctcgaatgca gataggctct cctattgtgt aatagcgagt gtttctactt ttcattcctt
301 tctcttctcc agccaaaata gaaattagtt atttggttag cttcaaaaaa tcacatcagt
361 aatttttgca gaagtgtttc ttttctactt taaaaataaa tacaatttaa attatgttga
421 tgaattattc tcagaaggca cattgtacat t
```

← Primer Revese

Figure 2. The sequence of target DNA fragments in the Pit-1 gene is not recognized by *Hin*fI (A allele, GenBank: EF090617.2) and recognized by *Hin*fI (B allele, GenBank: EF090616.2). Mutation site at position 207 (G to A). (HinfI sequence: 5'..g▼antc.. 3')

Based on the *Hin*fI cleavage pattern (sequence: 5'..g▼antc.. 3') on the specific DNA fragment (451 bp) of the Pit-1 gene in cattle, there are three possible genotypes that can be found at the Pit-1/E6/*Hin*fI locus, i.e., AA genotype indicated by one band: 451 bp, AB genotype indicated by the presence of three bands: 451bp, 244 bp, and 207 bp; and BB genotype was indicated by the presence of two bands: 244 bp and 207 bp. The three genotypes consist of two alleles which can be illustrated in Figure 3.

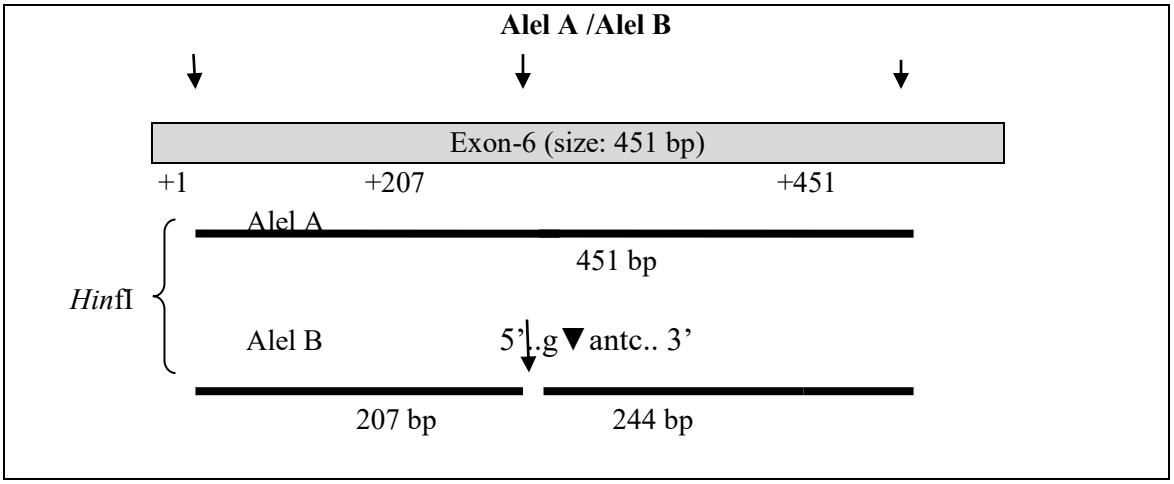

Figure 3. Grouping of genotypes based on the *Hin*fI cleavage pattern (sequence: 5'..g▼antc.. 3') on specific DNA fragments (451 bp) of the Pit-1 gene in cattle. [AA genotype (one band: 451 bp), AB genotype (three bands: 451 bp, 244 bp, and 207 bp); and BB genotype (two bands: 244 bp and 207 bp)].

In this study, cutting a specific DNA fragment of 451 bp in size using the *Hin*fI enzyme resulted in a uniform band pattern, namely a band pattern consisting of 2 fragments: band 1 (244 bp) and band 2 (207 bp). This banding pattern was identified as allele B. Figure 4 shows the results of *Hin*fI cuts in all samples of Bali cattle. Thus, the Pit-1/E6/*Hin*fI locus of all Bali cattle studied (20 heads) was BB genotype, while AA and AB genotypes were not found in this study. In this regard, the frequency of BB genotype from the Pit-1/E6/*Hin*fI locus in Bali cattle was 100%, while the AA and AB genotypes were 0% (zero percent), respectively. Based on the genotype frequencies, it is known that the frequencies of the A and B alleles of the Pit-1/E6/*Hin*fI loci in Bali cattle are 0 and 1. By finding the frequencies of the two alleles, it is known that the Pit-1/E6/*Hin*fI locus in Bali cattle is monomorphic because the highest allele frequency found in the population exceeds 0.99 (Nei and Kumar, 2000).

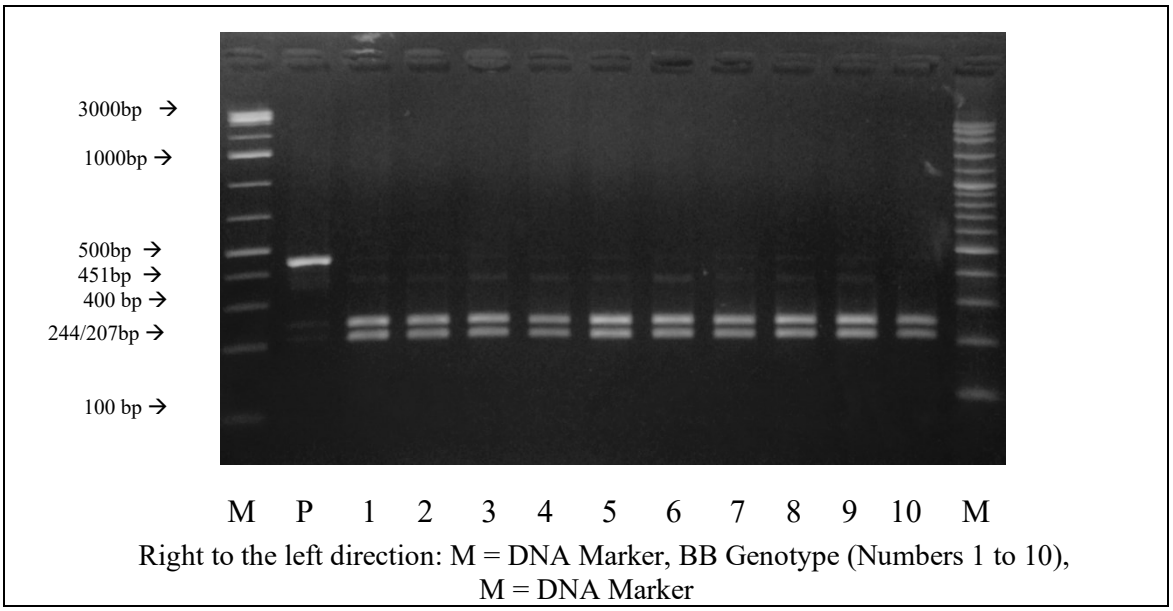

Figure 4. Visualization of Bali cattle amplicon cutting results using HinfI

In this study, it was clear that the allele A from the Pit-1/E6/*Hin*fI locus in Bali cattle (*Bos sondaicus*) was classified as a rare allele, maybe not even found. This phenomenon can be one of the distinguishing factors between Bali cattle (*Bos sondaicus*) and cattle belonging to the *Bos taurus* and *Bos indicus* groups (Table 1). The distinction between the Bali cattle (*Bos sondaicus*) and the

Bos taurus and *Bos indicus* breeds were also found at the kappa-casein (KCN) locus in exon-4 (KCN/E4/*Hin*dIII locus), where the A allele at the KCN/E4/*Hin*dIII locus commonly found in *Bos taurus* and *Bos indicus*, not in Bali cattle (*Bos sondaicus*) (Mu'in and Supriyantono, 2012). Previously, Mu'in (2008) also found growth hormone loci at exon 5 (GH/E5/*Alu*I locus) in Bali cattle; only the L allele was found, while *Bos taurus* and *Bos indicus* found L and V alleles. These three differentiators deserve consideration. as an additional indicator in addition to the existing indicators to assess the purity of the Bali cattle population in a specific area to conserve Bali cattle.

Table 1. Allele frequencies of the Pit-1/E6/*Hin*fI loci in several breeds of cattle

Breeds	N	Allele frequencies of Pit-1/E6/*Hin*fI locus		Sources
		A	B	
Gyr	40	0.950	0.050	De Mattos et al. 2004
Sistani	38	0.921	0.079	Javanmard et al. 2005.
Nellore	79	0.897	0.103	Curi et al. 2006.
Canchim	30	0.883	0.117	Curi et al. 2006.
½ Simmental	30	0.867	0.133	Curi et al. 2006.
Mazandrani	26	0.827	0.173	Javanmard et al.. 2005.
Taleshi	70	0.771	0.229	Javanmard et al. 2005.
Golpaygani	57	0.746	0.254	Javanmard et al. 2005.
Iranian native cattle	210	0.750	0.250	Doosti et al. 2011.
½ Angus	245	0.641	0.359	Curi et al. 2006.
Nanyang	100	0.465	0.535	Xue et al. 2006
Belgia Blue	350	0.423	0.577	Renaville et al. 1997.
East Anatolian Red	71	0.410	0.590	Ozdemir, 2012
Golpayegani x B. Swiss F_1	13	0.385	0.615	Javanmard et al. 2005.
Brown Swiss	301	0.374	0.626	Aytekin and Boztepe, 2013.
Mazandrani	96	0.370	0.630	Zakizadeh et al.. 2007.
Golpaygani	110	0.336	0.664	Zakizadeh et al.. 2007.
Angus	416	0.331	0.669	Zhoa et al. 2004
Holstein	224	0.300	0.700	Doosti et al. 2011.
Sarabi	84	0.274	0.726	Zakizadeh et al.. 2007.
Holstein	262	0.256	0.744	Edriss et al.. 2008.
Friesian Holstein	45	0.250	0.750	Misrianti et al. 2010.
Romanian Grey Steppe	60	0.250	0.750	Carsai et al. 2012.
Black-and-White bulls	145	0.247	0.753	Oprzadek et al. 2003.
Holstein-Friesian	45	0.244	0.756	Misrianti et al. 2010.
Polish Black-and-White	900	0.243	0.757	Dybus et al. 2004.
Romanian Simmental	76	0.217	0.783	Viorica et al. 2007.
Iranian Holstein	111	0.208	0.792	Zakizadeh et al.. 2007.
Holstein	181	0.200	0.800	Ozdemir, 2012
Italian Holstein-Friesian	89	0.188	0.812	Renaville et al. 1997.
Najdi	84	0.184	0.816	Beigi Nassiri et al..2010
Holstein - Friesian	45	0.174	0.826	Jawasreh et al. 2009.
5/8 Charolais : 3/8 Zebu	232	0.130	0.870	Carrijo et al. 2008.
Yakutian	42	0.119	0.881	Lazebnaya et al., 2010.
Romanian Black & White	60	0.090	0.910	Carsai et al.. 2012.
Jordan native cattle	36	0.088	0.912	Jawasreh et al. 2009.
Bali Cattle (*Bos sondaicus*)	20	0.000	1.000	In this study

*) Pit-1/E6/*Hin*fI locus, which is a locus located on exon 6 of the Pit-1 gene. The genotype of this locus can be detected by the restriction enzyme *Hin*fI.

Research reports that studied the relationship between Pit-1/E6/*Hin*fI locus polymorphisms with growth traits and meat production in cattle breeds were relatively few compared to cow's milk production and quality characteristics, both in *Bos taurus* and *Bos indicus*. Renaville et al. (1997[a]) found a close relationship between the B allele of the Pit-1/E6/*Hin*fI locus and body weight at seven months of age in double-muscled cattle of Belgian Blue

bulls. Renaville et al. (1997a) also found a relationship between Pit-1/E6/*Hin*fI locus polymorphisms with growth, body weight, and carcass characteristics in Italian Holstein-Friesian bulls. However, other researchers did not find a significant relationship between Pit-1/E6/*Hin*fI locus polymorphisms with growth and carcass traits in Angus cattle (Zhoa et al., 2004).

Viorica (2007) identified the Pit-1/E6/HinfI locus polymorphism in 76 Romanian Simmental cattle and found that the frequency of the A allele was lower than that of the B allele. It was also informed that cows carrying the A allele produced a high amount of milk and had a lower body depth. The fat percentage is low because it has a high milk yield, but the fat is nearly constant. Renaville et al. (1997b) showed that there was high genetic variation at the Pit-1/E6/HinfI locus in FH cattle and that the A allele (especially the AB genotype) had an effect on milk yield and its components. Parmentier et al. (1999) also found a significant advantage of the A allele for milk yields and protein yields but low self-esteem for fat yields.

Based on this study which involved only 20 samples of Bali cattle (unrelated), it is known that the Pit-1/E6/HinfI locus in Bali cattle is monomorphic, so the effect of the Pit-1/E6/HinfI locus on growth characteristics of Bali cattle cannot be studied. The same research involving a larger number of samples may need to be carried out to clarify this study's results.

CONCLUSION

In Bali cattle (*Bos sondaicus*) there was a substitution of adenine with guanine (A207G) located at exon 6 of the Pit-1 gene, so at this locus, only the BB genotype (monomorphic) was found. This phenomenon is not the same as that found in *Bos taurus* and *Bos indicus*, so the monomorphism found at this locus can be an indicator to assess the purity of the Bali cattle population for conservation purposes.

REFERENCES

Ayteki, I. and S. Boztepe. 2013. Associations of Pit-1 Gene Polymorphism with Milk and Composition Traits in Browns Swiss Cattle. The Journal of Animal & Plant Sciences 23 (5): 12811289.

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. Journal of Animal and Veterinary Advances 9 (15): 2001-2003.

Carrijo, S.M., M.M. de Alencar, F.L. B. Toral., L.C. de Almeida Regitano. 2008. Associationof Pit-1 genotypes with growth traits Canchim cattle. Sci. Agric. (Piracicaba, Braz.) 65 (2):116-121.

Carsai, T.C., V.A. Balteanu, A. Vlaic, and V. Cosier. 2012. The Polymorphism of Pituitary Factor 1 (POU1F1) in Cattle. Animal Science and Biotechologies 45(1): 142 – 146.

Curi, R.A., D.A. Palmieri, L. Suguisawa, H.N. Olievera, A.C. Silviera and C.R. Lopes. 2006. Growth and carcass traits associated with GH/AluI and POU1F1/HinfI gene polymorphism in Zebu and crossbred beef cattle. Genet. and Mol Biotech. 29: 56-61.

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

Dierkes, B., B. Kriegesmann, B.G. Baumgartner and B. Brening, 1998. Partial genomic structure of the bovine Pit-1 gene and characterization of a HinfI transition polymorphism in exon 6. Anim. Genet. 29:405.

Doosti, A., A.Arshi and B. Momeni, 2011. Moleculer study of PIT1 gene polymorphism in Holstein and Iranian native cattle. African Journal of Agricultural Research 6 (19): 4467-4470.

Dybus, A., I. Szakowska, E. Czerniawska-Piatkowska, W. Grzesiak, J. Wojcik, E. Rzewucka, and S. Zych. 2004. PIT-HinfI gene polymorphism and its associations with milk production traits in polish Black-and-White cattle. Arch. Tierx., 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.

Janvanmard, A., N. Asadazadeh, H.M. 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. Biotecnol. 3:104-108.

Jawasreh, K.I.Z., F. Awawdeh, I. Rawashdeh, F. Hejazeen and M. Al-Thalib. 2009. The allele and genotype frequencies of bovine

pituitary specific transcription factor and leptin genes in Jordanian cattle population by using PCR-RFLP. Austr. J. Basic ad Appl. Sci., 3:1601-1606.

Lazebnaya, I.V., O.E. Lazebny, and G.E. Sulimova. 2010. Study of genetic variation in Yakutian cattle (*Bos taurus* L) using the prolactin *bPRL*, growth hormone *bGH*, and transcription Factor *bPit*-1 genes. Russian Journal og Genetics, 46(3):377-380.

Lin, C.R., S.C. Lin, C.P. Chang, and M.G. Rosenfeld. 1992. Pit-1 dependent expression of the receptor for growth hormone releasing factor mediates pituitary cell growth. Nature 260: 765-768.

Mangalam, H.J., V.R. Albert, H.A. Ingraham, M. Kapiloff, L. Wilson, C. Nelson, H.Elsholtz, and M.G. Rosenfeld. 1989. A pituitary POU-domain protein, Pit-1, activities both growth hormone and prolactin promoters transcriptionally. Genes Dev. 3: 946-958.

Misrianti, R., C. Sumantri, and A. Farajallah. 2010. Polymorphism identification of Pit1 gene in Indonesia buffaloes (*Bubalus bubalis*) and Holstein-Friesian cows. Media Peternakan, 33(3): 131-136.

Moody, D.E., D. Pomp, and Werndes, 1995. Restriction fragmen length polymorphism in amplification products of bovine Pit1 gene and assignment of Pit1 to bovine chromosome 1. Anim. Gene 26:45-47.

Mu'in, M.A. dan A. Supriyantono. 2012. Identification of κ-casein polymorphism in Bali cattle. J. Agricultural Science and Technology A. 2(3A): 397 – 401.

Nei, M. and D. Kumar. 2000. Molecuar evolution and phylogenetics. Oxford University Press, New York.

Oprzadek J., K.Flisikowski, L. Zweirzchowski and E. Dymnicki. 2003. Polymorphism at loci of leptin (LEP), Pit-1 and STA-T5A and their association with growth, feed conversion and carcass quality in Black-and-White bulls. Anim. Sci. *Pap.* and Rep. 21:223-231.

Ozdemir, M. 2012. Determination of PIT-1/HinfI polymorphism in Holstein and Native Ear cattle raised as genetic resource in Turkey. The Journal of Animal & Plant Science 22(1): 25-28.

Parmentier, I.N., D. Portetelle, N. Gengler, A. Pradi, and C. Bertozzi, 1999. Candidate gene markers associated with somatotropic axis and milk selection. Domestic Anim. Endocrinol. 17:139-148.

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

Renaville, R., N. Gengler,E. Vrech, A. Prandi, S. Massart, C. Corradini, C. Bertozzi, F. Mortiaux, A. Burny and D. Portetelle, 1997[b]. Pit-1 gene polymorphism, milk yield, and conformation traits for Italian Holstein-Friesian bulls. J. Dairy Sci. 80:3431-3438.

Rhodes, S.J., R. Chen, G.E. DiMattia, K.M. Scully, K.A. Kalla, S.C. Lin, V.C. Yu, and M.G. Rosenfeld. 1993. A tissue-specific enhancer confers Pit-1-dependent morphogen inducibility and autoregulation on the Pit-1 gene. Genes Dev. 7: 913-932.

Sambrook, J., Fritcsh and T. Maniatis. 1989. Molecular Cloning Laboratory Manual. Second Edition, Vol. 3. Cold Spring Harbor Laboratory Press. USA.

Tuggle, C.K. and A. Trenkle, 1996. Control of growth hormone synthesis. Domestic Animal Endocrinology 13: 1-33.

Viorica, C., A. Vlaic, and I. Gaboreanu. 2007. HinfI Polymorphism of k-Casein and Pit1 Genes in Romanian Simmental Cattle. Zootehniesi Biotehnologi 40(1): 59 – 64.

Woollard, J., C.B. Schmitz, A.E. Freeman, and C.K. Tuggle. 1994. Rapid communication: HinfI polymorphism at the bovine PIT1 locus. Journal Animal of Science 78: 3267.

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

Zakizadeh, S., M. Reissmann, G. Rahimi, A. Nejati Javaremi, P. Reinecke, S.R. Mirae-Ashtiani, and M. Moradi Shahrbabak. 2007. Polymorphism of the Bovine POU1F1 Gene: Allele Frequencies and Effect on Milk Production in Three Iranian Native Breeds and Holstein Cattle of Iran. Pakistan Journal of Biological Science 10: 2575 – 2578.

Zhao, Q., M.E. Davis, and H.C. Hines. 2004. Associations of polymorphism in the Pit-1 gene with growth and carcass traits in Angus beef cattle. Journal of Animal Science 82 (8): 2229-2233.