The polymorphism in g.1256G>A of bovine pituitary specific transcription factor-1 (bPIT-1) gene and its association with body weight of Pasundan cattle

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ABSTRACT

Bovine Pituitary specific transcription factor 1 (bPit-1) is one of amino acid that controlling pituitary gland in mammals. The pituitary gland is important for secretion of growth hormone from growth genes. This study was carried out to detect polymorphism in the exon 6 of bPit-1 (g.1256G>A) in Pasundan cattle using PCR-RFLP method and its association with body weight. Total of 69 heads (15 males and 54 females) of Pasundan cattle from breeding station (BPPIBT-SP Ciamis, West Java) were used in this study. Research showed that two genotypes of bPit-1/Hinf1 gene were identified in this study i.e GG (0.90) and AG (0.10) with frekuensi allele sebesar 0,05 (A) dan 0,95 (G). Nilai polymorphism informative content (PIC) and jumlah allele efektif (n_e) yang diperoleh masing-masing sebesar 0,09 (low) dan 1,11. Nilai Chi-square (χ^2) pada populasi sampel sebesar 0,20 dan masih dalam keseimbangan Hardy-Weinberg (χ^2<5,99). Disimpulkan bahwa polimorfisme pada gen bPit-1/Hinf1 sapi Pasundan di pusat pembibitan termasuk rendah dan tidak berasosiasi dengan berat badan.

Kata kunci: sapi Pasundan, gen bPit-1, PCR-RFLP, berat badan

ABSTRACT

Bovine Pituitary specific transcription factor 1 (bPit-1) is one of amino acid that controlling pituitary gland in mammals. The pituitary gland is important for secretion of growth hormone from growth genes. This study was carried out to detect polymorphism in the exon 6 of bPit-1 (g.1256G>A) in Pasundan cattle using PCR-RFLP method and its association with body weight. Total of 69 heads (15 males and 54 females) of Pasundan cattle from breeding station (BPPIBT-SP Ciamis, West Java) were used in this study. Research showed that two genotypes of bPit-1/Hinf1 gene were identified in this study i.e GG (0.90) and AG (0.10) with allele frequencies of 0.05 (A) and 0.95 (G). The polymorphic informative content (PIC) and number of effective allele (n_e) values were 0.09 (low) and 1.11. The Chi-square (χ^2) value in the population studied was 0.20 and in Hardy-Weinberg equilibrium (χ^2<5.99). It was concluded that the polymorphism of bPit-1/Hinf1 in Pasundan cattle included of low category and was not associated with body weight.

Keywords: Pasundan cattle, bPit-1 gene, PCR-RFLP, body weight
INTRODUCTION

Pasundan cattle is one of native cattle in Indonesia decided by Ministry of Agriculture No: 1051/Kpts/SR.120/10/2014. Pasundan cattle was created from crossbreeding between Bos indicus and Bos javanicus since hundred years ago. This cattle was adapted well in West Java Province and kept by the farmers as beef cattle. Recently, the genetic improvement of Pasundan cattle was supported by local government through breeding station of Balai Pengembangan Perbadian dan Inseminasi Buatan Ternak - Sapi Potong (BPPIBT-SP) Ciamis, West Java. As the Pasundan breeding center, BPPIBT-SP Ciamis must be capable to increase livestock’s productivity through livestock selection. Recently, livestock selection can be conducted based on single nucleotide polymorphism (SNP) in the gene that controlling productivity and called as the candidate gene (Dekkers, 2004; Van Eenennaam et al., 2007).

There are many growth hormone family genes that were used as molecular selection in cattle i.e. insulin-like growth factor 1 (IGF-1), insulin-like growth factor binding protein 3 (IGFBP-3), growth hormone (GH), growth hormone receptor (GHR), growth hormone releasing hormone (GHRH) and pituitary specific transcription factor (Pit-1) genes. The bovine Pit-1 gene is one of the candidate gene that potential for molecular selection in cattle (Sumantri et al., 2011; Oner et al., 2017). The bPit-1 gene was located at centromeric region of chromosome 1 (1q21-22) and consists of five introns and six exons (Woollard et al., 2000). The bPit-1 gene was synthesized at anterior pituitary gland and has 291 amino acid protein (31-33 kDa) with DNA binding POU domain class 1 transcription factor 1 (POUF1) that is responsible for pituitary development and hormone secreting gene expression in mammals, activating expression of growth hormone, prolactin and thyrotropin β-subunit genes (de Mattos et al., 2004).

Previous studies reported that one SNP was in the exon 6 of bPit-1 gene at position g.1256G>A based on GenBank: Y15995 (Javanmard et al., 2005; Misrianti et al., 2010; Aytekin and Boztepe, 2013; Nahavandi et al., 2010; Chauhan et al., 2015; Bayram et al., 2017). Moreover, SNP of g.1256G>A can be detected by HinfI restriction enzyme through PCR-RFLP method (Dybus et al., 2003). Several studies reported that polymorphism of bPit-1/HinfI were associated with growth traits in Canchim (Carrijo et al., 2008) and fat percentage in dairy Gyr (de Mattos et al., 2004). Despite, many researches also reported that polymorphism of bPit-1/HinfI were not associated with milk performance traits in Slovak Simmental (Trakovicka et al., 2015), Brown Swiss (Aytekin and Boztepe, 2013), Friesian Holstein (Heidari et al., 2012), Hoseinzadeh et al., 2015; Ozdemir et al., 2016) and Polish Black and White (Dybus et al., 2004), growth and carcass traits in crossbred cattle (Curi et al., 2006), body weight and body measurements in Limousine cattle (Dybus et al., 2003) and superovulation response in Friesian Holstein (Sumantri et al., 2011).

Identification genotype of bPit-1/HinfI gene in Pasundan cattle is important as the basic information for molecular selection in the future. Despite, the information regarding to bPit-1 gene of Pasundan cattle so far is not reported. The objectives of this study were to identify the polymorphism in the exon 6 of bPit-1 gene and to investigate the influence of genotype type related to body weight in a herd of Pasundan cattle.

MATERIALS AND METHODS

Blood Samples and DNA Extraction

A total of 69 heads of Pasundan cattle (15 males and 54 females) from breeding station (BPPIBT-SP Ciamis, West Java Province) were used for blood sampling purpose. Blood samples (3-5 mL) were taken from cocygeal vein using venoject and collected in vaccutainer tubes containing anticoagulant (K2EDTA). The blood samples were used in the DNA extraction kit process using the Genomic DNA Mini kit (Geneaid Biotech Ltd., Taiwan) following the manufactures instruction. The extracted DNA was recorded and stored at -20°C for next analysis.

PCR Amplification of bPit-1 Gene

The primer sequences for PCR analysis was adoped from Nahavandi et al. (2010) i.e Pit-1F: 5'-GAGCCTACATGAGACAAGCATC-3' and Pit-1R: 5'-AAATGTACAATGTGCCTTCTGA-3'. This primer was amplified Pit-1 gene along 610 bp according to the reference sequence (Figure 1). The polymerase chain reaction (PCR) reagents were as follows: 2.7 μL of KAPA2G Robust PCR Kit (Kapa Biosystems, Cape Town, South Africa); each 0.80 μL of forward and reverse primers (200 ng/μL); 2.0 μL of DNA samples; and ddH2O up to 7.0 μL. The PCR was carried out in
mastercycler gradient machine (Eppendorf, Germany). The PCR program was set up as follows: initial denaturation at 94°C for 5 minutes; denaturation at 94°C for 30 seconds; annealing at 64°C for 30 seconds; initial extension at 72°C for 30 seconds and final extension at 72°C for 5 minutes. The PCR product was visualized using 1.0% agarose gel (Vivantis, Malaysia). The gel was stained with GelRed™ (Biotium, USA). Total 3.0 μL of 100 bp DNA ladder (Vivantis, Malaysia) was used as molecular size marker. The electrophoresis (110 V; 30 minutes) analysis was used for visualization PCR product with GBOX Documentation System (Syngene, UK).

**Genotyping of bPit-1 Gene using RFLP Technique**

Analysis of restriction fragment length polymorphism (RFLP) was applied for genotyping of Pit-1 gene in this study. The mixture was consisted of 4.20 μL of PCR product; 0.28 μL of Hinfl restriction enzyme (GA*NTC); 0.70 μL buffer and ddH₂O up to 7.0 μL. Then, the mixtures were incubated at 37°C for 1 h. Digested products were analyzed using electrophoresis (110 V; 30 minutes) analysis was used for visualization PCR product with GBOX Documentation System (Syngene, UK).

**Statistical Analysis**

Data of body weight (BW) were analyzed applying a linear mixed model as follows:

\[ Y_i = \mu + G_i + e_i \]

Where:

- \( Y_i \) : dependent variable (BW)
- \( \mu \) : overall mean
- \( G_i \) : fixed effect of the \( j \)th genotype (AA, AG, GG)
- \( e_i \) : random residual effect

The genotype data of in all samples were used to estimate allele frequencies, heterozigosity, polymorphic informative content (PIC), number of effective allele (\( n_e \)) and Chi-square (\( \chi^2 \)) values as follow:

The allele frequencies were calculated using formula from Sadeghi et al. (2008) as follows:

\[ X_i = \frac{2(N_{ii} + N_{ij})}{2N} \]

Where:

- \( X_i \) : frequency of \( i \)th allele
- \( N_{ii} \) : number of genotype \( A_iA_i \)
- \( N_{ij} \) : number of genotype \( A_iA_j \)
- \( N \) : number of observation

The heterosigosity values were calculated using formula from Nei and Kumar (2000) as follows:

\[ H_e = 1 - \sum_{i=1}^{n} X_i^2 \]

and

\[ \text{SE} = \sqrt{\text{Var}}_{H_e} \]
RESULTS AND DISCUSSION

The Pit-1 gene fragments was successfully amplified using PCR technique for all sample and resulted in a single product of 610 bp (Figure 2). The RFLP analysis showed the fragments obtained for the bPit-1/HinfI polymorphism were 367 and 243 bp for GG genotype; 610, 367 and 243 bp for the AB genotype as presented in Figure 3. The statistical analysis for bPit-1/HinfI polymorphism is presented in Table 1. Genotype AA (610 bp) was not observed in this study and similar to the other breeds cattle such as Golpayegani × Brown Swiss (Javanmard et al., 2005) and Gyr (de Mattos et al., 2004). Despite, Jakaria and Noor (2015) reported that AA genotype in the bPit-1/HinfI gene are absence in many Indonesian native cattle such as Aceh, Katingan and Bali cattle. Therefore, the frequency of AG genotype in this study was 0.10 and similar to Katingan (Jakaria and Noor, 2015). The frequency of A allele in the present study was under 0.10 and similar to native cattle in Indonesia (Madura, Pesisir, Aceh, Katingan, Bali) and Brazil (Gyr) is presented in Table 2.

The PIC value in the present study is low (PIC<0.25) and describes that the genetic diversity of bPit-1/HinfI is not effective for
molecular selection in Pasundan cattle. Low PIC value in the bPit-1/HinfI of Pasundan cattle can be affected by selection system in smallholder farmer. Moreover, limitation number of sires in the population might be caused the low value of PIC (Agung et al., 2017). The ne value of bPit-1/HinfI gene in Pasundan cattle was 1.11 and reveals that B allele as the dominant allele in this gene. The genetic diversity of bPit-1/HinfI gene in the animal studied under Hardy-Weinberg (HW) equilibrium and can be caused by random mating still occurred in the research site. The He and Ho values in the present study was similar (0.10) and reveal that the animal studied under HW equilibrium. Body weight of Pasundan cattle in GG genotypes was not significantly different from AG genotypes (Table 3). No association between bPit-1/HinfI gene polymorphism and body weight in the present study might be caused by low number of sample.

Dybus et al. (2003) reported that in polymorphism of bPit-1/HinfI gene was not associated with body weight in Limousine cattle and similar to the present study. In contrast, Renaville et al. (1997a) reported that A allele in the bPit-1/HinfI gene was found to be superior for milk traits and body measurements in Italian Frieasian Holstein. Moreover, Sumantri et al. (2011) reported that genotype AA in the bPit-1/HinfI gene of FH cows had the highest of ovulation rate rather than other genotypes. The bPit-1/HinfI gene of Pasundan cattle in this study can not be used as molecular selection for body weight. Detection of the polymorphism in the other region of bPit-1 gene i.e. 5’UTR/promotor, other exons, intron and 3’UTR is important to obtain the genetic marker for productivity traits through marker assisted selection (MAS) program in the future.
Table 2. Polymorphism of the Exon 6 of bPit-1/HinfI Gene with Different PCR Product according to the Previous Study

| Breed                        | Species   | Location | N  | PCR product (bp) | Genotype frequency | Allele frequency |
|------------------------------|-----------|----------|----|------------------|--------------------|------------------|
| Holstein-Friesian1           | Bos taurus| Indonesia| 45 | 610              | 0.02 0.44 0.53    | 0.25 0.75        |
| Brown Swiss2                  | Bos taurus| Turkey   | 301| 610              | 0.12 0.51 0.37    | 0.37 0.63        |
| Sarabi3                      | Bos taurus| Iran     | 82 | 610              | 0.45 0.34 0.21    | 0.68 0.38        |
| Golpayegani x Brown Swiss4    | Bos taurus| Iran     | 13 | 610              | 0.00 0.77 0.23    | 0.38 0.62        |
| Turkish Holstein-Friesian5    | Bos taurus| Turkey   | 352| 610              | 0.18 0.29 0.53    | 0.32 0.68        |
| Slovak Simmental6             | Bos taurus| Slovakia | 288| 260              | 0.05 0.35 0.60    | 0.23 0.77        |
| Slovak Spotted Cattle7        | Bos taurus| Slovakia | 110| 260              | 0.05 0.50 0.45    | 0.30 0.70        |
| Holstein-Friesian8            | Bos taurus| Turkey   | 181| 260              | 0.04 0.31 0.65    | 0.20 0.80        |
| East Anatolian Red9           | Bos taurus| Turkey   | 71 | 451              | 0.14 0.54 0.32    | 0.41 0.59        |
| Italian Holstein- Fr. bull10  | Bos taurus| Italia   | 89 | 451              | 0.02 0.32 0.55    | 0.19 0.81        |
| Belgian Blue11                | Bos taurus| Belgia   | 350| 451              | 0.20 0.45 0.35    | 0.42 0.58        |
| Angus12                      | Bos taurus| USA      | 416| 451              | 0.11 0.44 0.45    | 0.33 0.67        |
| Polish Black and White13      | Bos taurus| Poland   | 900| 451              | 0.05 0.38 0.57    | 0.24 0.76        |
| Iranian Holstein- Fr. cow14   | Bos taurus| Iran     | 262| 451              | 0.03 0.45 0.52    | 0.26 0.74        |
| Chilean Holstein- Fr.15       | Bos taurus| Chile    | 46 | 451              | 0.10 0.35 0.55    | 0.28 0.72        |
| Qinchuan16                   | Bos taurus| China    | 218| 451              | 0.03 0.40 0.57    | 0.23 0.77        |
| Limousine17                  | Bos taurus| Poland   | 130| 451              | 0.07 0.41 0.52    | 0.27 0.73        |
| Podolica18                   | Bos taurus| Italy    | 104| 451              | 0.14 0.32 0.54    | 0.30 0.70        |
| Holstein-Friesian19          | Bos taurus| Iran     | 100| 451              | 0.06 0.40 0.54    | 0.26 0.74        |
| Sahiwal20                    | Bos indicus| India   | 77 | 610              | 0.04 0.31 0.65    | 0.19 0.81        |
| Najdi21                      | Bos indicus| Iran     | 84 | 451              | 0.04 0.30 0.66    | 0.18 0.82        |
| Madura22                     | Bos indicus| Indonesia| 68 | 451              | 0.00 0.07 0.93    | 0.04 0.96        |
| Pessis22                     | Bos indicus| Indonesia| 100| 451              | 0.01 0.13 0.86    | 0.08 0.92        |
| Aceh22                       | Bos indicus| Indonesia| 25 | 451              | 0.00 0.08 0.92    | 0.04 0.96        |
| Katingan22                   | Bos indicus| Indonesia| 50 | 451              | 0.00 0.10 0.90    | 0.05 0.95        |
| Nellore23                    | Bos indicus| Brazil   | 79 | 1301             | 0.80 0.20 0.00    | 0.90 0.10        |
| Canchim24                    | B. ind x B. tau| Brazil | 219| 1301             | 0.77 0.19 0.04    | 0.87 0.13        |
| Gyr25                        | B. indicus| Brazil   | 40 | 1355             | 0.00 0.10 0.90    | 0.05 0.95        |
| Bali22                       | Bos javanicus| Indonesia| 245| 610              | 0.00 0.04 0.96    | 0.02 0.98        |

N = number of sample; 1Misrianti et al. (2010); 2Ayetkin and Boztepe (2013); 3Nahavandi et al. (2010); 4Javanmard et al. (2005); 5Bayram et al. (2017); 6Trakovic et al. (2015); 7Moravcikova et al. (2013); 8Ozdemir et al. (2017); 9Ozdemir (2012); 10Renaville et al. (1997a); 11Renaville et al. (1997b); 12Zhao et al. (2004); 13Dybus et al. (2014); 14Edriss et al. (2008); 15Vargas et al. (2014); 16Yan et al. (2011); 17Dybus et al. (2003); 18Selvaggi and Dario (2011); 19Hoseinzadeh et al. (2015); 20Chauhan et al. (2015); 21Beigi et al. (2010); 22Jakaria and Noor (2015); 23Curi et al. (2006); 24Carrio et al. (2008); 25de Mattos et al. (2004)
**CONCLUSION**

Single nucleotide polymorphism of g.1256G>A in the bPit-1 gene had low of genetic diversity and was not associated with body weight in Pasundan cattle. The AA genotype was not detected in the present study. In addition, the A allele in bPit-1/HinfI gene of animal studied included of rare allele with low frequency.

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