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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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ABSTRAK

Bovine Pituitary specific transcription factor 1 (bPit-1) merupakan suatu asam amino yang berfungsi untuk mengontrol perkembangan kelenjar pituitary pada sapi. Kelenjar pituitary berfungsi untuk mensekresikan hormon pertumbuhan yang dihasilkan oleh gen-gen pertumbuhan. Penelitian ini bertujuan untuk mendeteksi polimorfisme pada ekson 6 gen *bPit-1* (*g.1256G>A*) dengan metode *PCR-RFLP* dan mengetahui pengaruhnya terhadap berat badan sapi Pasundan. Sampel yang digunakan sebanyak 69 ekor (15 jantan dan 54 betina) dan berasal dari pusat pembibitan (BPPIBT-SP Ciamis, Jawa Barat). Hasil penelitian menunjukkan bahwa terdapat dua genotipe gen *bPit-1/Hinf*I pada sapi Pasundan di pusat pembibitan (BPPIBT-SP Ciamis, Jawa Barat) yaitu GG (0,90) dan AG (0,10) dengan frekuensi alel sebesar 0,05 (A) dan 0,95 (G). Nilai *polymorphism informative content* (PIC) dan jumlah alel efektif (n_e) yang diperoleh masing-masing sebesar 0,09 (rendah) 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/Hinf*I 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 controling 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/*Hinf*I 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. respectively. 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/*Hinf*I 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 Agriculure No: 1051/Kpts/SR.120/10/2014. Pasundan cattle was created from crossbreding 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 gevernment through breeding station of *Balai Pengembangan Perbibitan 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 controling 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 *Hinf*I restriction enzyme through PCR-RFLP method (Dybus *et al.,* 2003). Several studies reported that polymorphism of bPit-1/*Hinf*I 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/*Hinf*I 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 e*t al.,* 2016) and Polish Black and White (Dybus e*t al.,* 2004), growth and carcass traits in crossbred cattle (Curi e*t 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/*Hinf*I 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^{o}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 amplifed 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

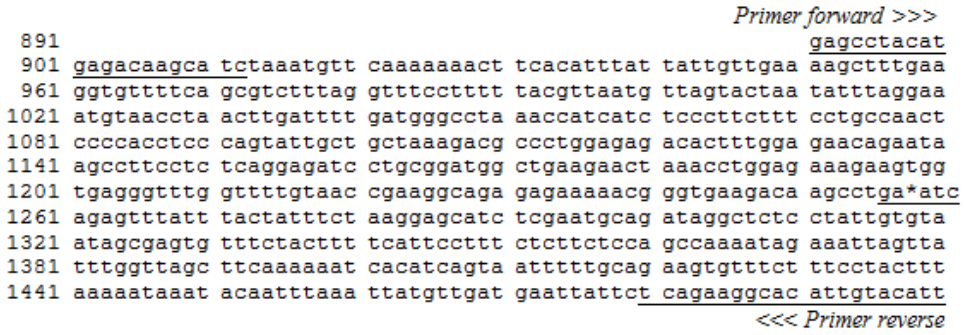

Figure 1. The primer position (underline) and *Hinf*I restriction enzyme site (ga*Ntc) in bPit-1 gene based on combination sequences from GenBank: Y15995 (891-1301) and AM490263 (1302-1500).

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 *Hinf*I restriction enzyme (GA*NTC); 0.70 µL buffer and ddH_2O up to 7.0 µL. Then, the mixtures were incubated at 37°C for 1 h. Digested products were analyzed using electrophoresis (110 V; 1 h) on 2.0% agarose gel with 3.0 µL of 100 bp DNA ladder. The digested product was stained with GelRed™ and captured with GBOX Documentation System. Samples with AA genotypes were consisted of one DNA fragment (610 bp). Samples with AG genotype consisted of three DNA fragments (610 bp, 367 bp and 243 bp). While, samples with GG genotype consisted of two DNA fragments (367 bp and 243 bp).

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)

μ : 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 (χ^2) values as follow:

The allele frequencies were calculated using formula from Sadeghi e*t 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

$$SE = \sqrt{Var_{He}}$$

$$Var_{He} = \frac{2}{2(2n - 1)} X$$

$$X = \left[2(2n - 2)\left(\sum X_i^3 - \left(\sum X_i^2\right)^2\right) + \left(\sum X_i^2 - \left(\sum X_i^2\right)^2\right)\right]$$

$$H_o = \frac{X_{ij}}{N}$$

Where:

H_e : expected heterozigosity

H_o : observed heterozigosity

X_i : frequency of i^{th} allele

X_{ij} : frequency of heterozygote genotype

N : number of observation

SE : standard error

The PIC value was calculated using formula from Hildebrand *et al.* (1992) as follows:

$$PIC = 1 - \sum_{i=1}^{n} X_i^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2X_i^2 X_j^2$$

PIC : polymorphic informative content

X_i : frequency of i^{th} allele

X_j : frequency of j^{th} allele

The n_e value was calculated using formula from Nei and Kumar (2000) as follows:

$$n_e = \frac{1}{\sum_{i=1}^{n} X_i^2}$$

Where:

n_e : number of effective allele

X_i : frequency of i^{th} allele

The χ^2 value was calculated using formula from Nei and Kumar (2000) as follows:

$$\chi^2 = \sum_{i=1}^{n} \frac{(O_i - E_i)^2}{E_i}$$

Where:

χ^2 : Chi-square value

O_i : number of observed i^{th} genotype

E_i : numberof expected i^{th} genotype

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/*Hinf*I 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/*Hinf*I 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/*Hinf*I 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/*Hinf*I is not effective for

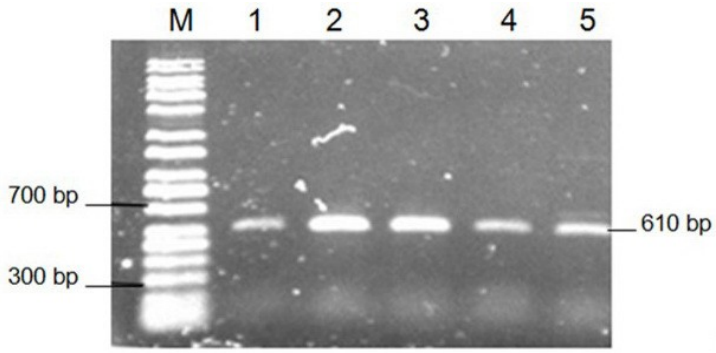

Figure 2. The amplification of bPit-1 gene showed on1% agarose gel. M: DNA ladder 100 bp; lanes 1-5: PCR products amplified from DNA of animal studied

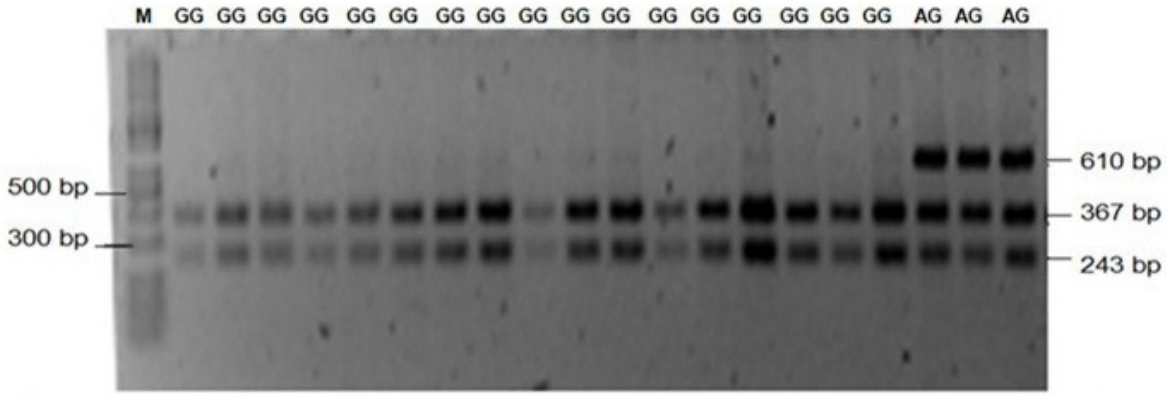

Figure 3. The result of PCR-RFLP analysis in bPit-1/*Hinf*I gene of Pasundan cattle separated on 2% agarose gel consisted of two genotypes of GG (367 bp and 243 bp) and AG (610 bp, 367 bp and 243 bp). M: DNA ladder 100 bp

Table 1. Genetic Characterization in the Exon 6 of bPit-1/*Hinf*I Gene in Pasundan Cattle at the Breeding Station

Genotype Frequency (N)			Allele Frequency		H_e (SE)	H_o	PIC	n_e	χ^2
AA	AG	GG	A	G					
0.00 (0)	0.10 (7)	0.90 (62)	0.05	0.95	0.10 (0.07)	0.10	0.09	1.11	0.20*

N= number of sample; SE= standard error; χ^2= Chi square value; H_e= expected heterozigosity; H_o= observed heterozigosity; PIC= polymorphism informative content; n_e= number of effective allele; *under Hardy-Weinberg equilibrium ($\chi^2_{2;\,0.05} = 5.99$)

molecular selection in Pasundan cattle. Low PIC value in the bPit-1/*Hinf*I 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 e*t al., 2*017). The n_e value of bPit-1/*Hinf*I 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/*Hinf*I gene in the animal studied under Hardy-Weinberg (HW) equilibrium and can be caused by random mating still occured in the research site. The H_o and H_e 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/*Hinf*I 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/*Hinf*I 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/*Hinf*I gene was found to be superior for milk traits and body measurements in Italian Frieasian Holstein. Morever, Sumantri *et al.* (2011) reported that genotype AA in the bPit-1/*Hinf*I gene of FH cows had the highest of ovulation rate rather than other genotypes.

The bPit-1/*Hinf*I 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/*Hinf*I Gene with Different PCR Product according to the Previous Study

Breed	Species	Location	N	PCR product (bp)	Genotype frequency			Allele frequency	
					AA	AG	GG	A	G
Holstein-Friesian[1]	*Bos taurus*	Indonesia	45	610	0.02	0.44	0.53	0.25	0.75
Brown Swiss[2]	*Bos taurus*	Turkey	301	610	0.12	0.51	0.37	0.37	0.63
Sarabi[3]	*Bos taurus*	Iran	82	610	0.45	0.34	0.21	0.68	0.38
Golpayegani x Brown Swiss[4]	*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 Simmental[6]	*Bos taurus*	Slovakia	288	260	0.05	0.35	0.60	0.23	0.77
Slovak Spotted Cattle[7]	*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. bull[10]	*Bos taurus*	Italia	89	451	0.02	0.32	0.55	0.19	0.81
Belgian Blue[11]	*Bos taurus*	Belgia	350	451	0.20	0.45	0.35	0.42	0.58
Angus[12]	*Bos taurus*	USA	416	451	0.11	0.44	0.45	0.33	0.67
Polish Black and White[13]	*Bos taurus*	Poland	900	451	0.05	0.38	0.57	0.24	0.76
Iranian Holstein-Fr. cow[14]	*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
Qinchuan[16]	*Bos taurus*	China	218	451	0.03	0.40	0.57	0.23	0.77
Limousine[17]	*Bos taurus*	Poland	130	451	0.07	0.41	0.52	0.27	0.73
Podolica[18]	*Bos taurus*	Italy	104	451	0.14	0.32	0.54	0.30	0.70
Holstein-Friesian[19]	*Bos taurus*	Iran	100	451	0.06	0.40	0.54	0.26	0.74
Sahiwal[20]	*Bos indicus*	India	77	610	0.04	0.31	0.65	0.19	0.81
Najdi[21]	*Bos indicus*	Iran	84	451	0.04	0.30	0.66	0.18	0.82
Madura[22]	*Bos indicus*	Indonesia	68	451	0.00	0.07	0.93	0.04	0.96
Pesisir[22]	*Bos indicus*	Indonesia	100	451	0.01	0.13	0.86	0.08	0.92
Aceh[22]	*Bos indicus*	Indonesia	25	451	0.00	0.08	0.92	0.04	0.96
Katingan[22]	*Bos indicus*	Indonesia	50	451	0.00	0.10	0.90	0.05	0.95
Nellore[23]	*Bos indicus*	Brazil	79	1301	0.80	0.20	0.00	0.90	0.10
Canchim[24]	*B. ind x B. tau*	Brazil	219	1301	0.77	0.19	0.04	0.87	0.13
Gyr[25]	*B. indicus*	Brazil	40	1355	0.00	0.10	0.90	0.05	0.95
Bali[22]	*Bos javanicus*	Indonesia	245	451	0.00	0.04	0.96	0.02	0.98

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

Table 3. Association of bPit-1/*Hinf*I Gene polymorphism with Body Measurements and Body Weight of Pasundan Cattle at the Breeding Station

Group / Genotype	Body weight (kg)
Heifer (1 PPI)	
GG (N = 21)	163.90±21.49
AG (N = 3)	184.33±22.81
Cow (2 PPI)	
GG (N = 23)	232.97±23.92
AG (N = 2)	228.50±10.61
Bull (3 PPI)	
GG (N = 8)	362.88±39.81
AG (N = 2)	407.00±32.53

PPI= pairs of incisors; N= number of observation

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/*Hinf*I gene of animal studied included of rare allele with low frequency.

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