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Short Communication: Identification of Growth Hormone gene polymorphism for beef cattle in Pesisir Selatan District, West Sumatra, Indonesia

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Abstract. *Putra DE, Sumadi, Kanazawa T, Hartatik T. 2016. Short Communication: Identification of Growth Hormone gene polymorphism for beef cattle in Pesisir Selatan District, West Sumatra, Indonesia. Biodiversitas 17: 711-715.* This study aimed to determine gene polymorphism of growth hormone of domestic cattle in Pesisir Selatan District of West Sumatra. The research was conducted at Laboratory of Animal Genetics and Breeding Faculty of Animal Science, UGM from August 2013 to January 2014. Blood samples were collected from 66 individuals consist of 15 Pesisir cattle, 15 SimPes cattle, 15 SimPO cattle, 15 Bali cattle and 6 PO cattle. DNA was extracted from each blood samples after SDS-proteinase K digestion, and used for PCR-amplification for a region of growth hormone gene (211 bp), and then the PCR products were analyzed for restriction fragment length polymorphism (RFLP) using *Alu*I restriction enzyme. The results showed that GH gene of Pesisir, PO and Bali cattle are monomorphic, which frequencies of L allele was 1.00 and V allele was 0.000 while these LL genotype was 1.000. Frequency of L and V alleles in SimPO and SimPes cattle were 0.634, 0.366 and 0.700, 0.300, respectively. SimPO and SimPes cattle were polymorphic, LL and LV of SimPO cattle was 0.733 and 0.267 as well as SimPes cattle which LL and LV was 0.600 and 0.400, respectively. The correlation between genotype and the performance (body weight and body size) was not significant. The present study indicates that polymorphism of growth hormone gene in *Alu*I site could not yet be used as a molecular marker for body weight and body size of beef cattle.

Keywords: Domestic cattle, Growth Hormone gene, polymorphism

INTRODUCTION

Pesisir Selatan District was known as a place for developing the Pesisir cattle as local cattle. The populations of Pesisir cattle in Pesisir Selatan District reach 78.322 head at year 2011 (Statistic of Pesisir Selatan 2012). There are also Bali cattle as one of cattle of Indonesia origin and the other local cattle of Indonesia and the product of crossbreeding with exotic bull such as such as PO, SimPO, Limpo and SimPes. The development of cross breed cattle as resources of beef cattle in Indonesia was very potential in this area. However the study about genetic resources is still face the limitation of the information. The development of molecular genetics has opened up opportunities to determine the level of genetic diversity at the DNA level. It can be used to identify the genetic potential of livestock. Pesisir cattle are unique in body size, very small but have higher value of carcass than those of Bali cattle. The increasing number of crossbreed cattle such as SimPO, Limpo and SimPes were being one of basic reason on molecular studies to growth hormone (GH) gene which predicted to be one of major gene plays an important role in the growth process (Etherton and Bauman 1998).

The polymorphism in exon V has been observed when digested by the *Alu*I enzyme (GH-*Alu*I), and the 2 alleles called L (*leucine* in the 127th codon) and V (*valine* in the 127th codon) are distinguished (Zhang et al. 1993). The diversity of genes in cattle can be identified by the method restriction fragment length polymorphism (RFLP). The PCR-RFLP technique is an easier way and is efficient to identify the nucleotide sequence variation in DNA gene fragments of the cattle. This technique has been successfully employed to identify the growth hormone gene polymorphism in local cattle of Indonesia such as Madura cattle and its crossbreed with Limousin (Hartatik et al. 2013; Volkandari et al. 2013). The role of GH gene in appearance in cattle gives very obvious effect so that allegedly there are differences in GH gene between Pesisir cattle and Bali cattle and others cattle. Reis et al. (2001), Dario et al. (2005), Sutarno (2010), Akis Akad et al. (2012), Moravcikova et al. (2012), Deepika and Salar (2013), Korkmazagaoglu and Akyuz (2013), Sari et al. (2013), and Akcay et al. (2015) has conducted studies on the effects of GH gene polymorphism on productivity of beef cattle. Study about GH 211 bp in buffalo also reported by Hussain et al. (2014). Akcay (2015) also investigated that GH is a candidate gene for selection program in beef cattle.

The present study aimed to identify the polymorphism of growth hormone gene in local beef cattle in Indonesia and to study the association with body weight and body size. These basic data can be used as a potential marker assisted selection in the future.

MATERIALS AND METHODS

Samples collection

Sixty six blood samples were collected from Indonesia beef cattle that consisted of Pesisir cattle (15), Simmental x Pesisir or SimPes cattle (15), Bali cattle (15), Simmental x PO or SimPO (15) and PO (6). These cattle distributed to three villages at Pesisir Selatan District, West Sumatra Province, and were managed by farmers by their traditional ways. Three milliliter of blood samples were collected from jugular vein into vacuum test tubes, which contained K_3EDTA as an anticoagulant. Blood samples were stored at -20C until use.

DNA Extraction

DNA was extracted from blood samples using an SDS-PK (sodium dodecyl sulfate-proteinase K) method described by Sambrook et al. (1989) and Sulandari and Zein (2003), with modifications. The blood sample (approx. 200 µL) was mixed with 800 µL buffer A solution in an Eppendorf tube for 1.5 mL, then centrifuged at 10,000 rpm for five minutes. After removal of the supernatant, the pellet was resuspended with 300 µL buffer A solution, then centrifuged again at 10,000 rpm for five minutes. This step was repeated until the pellet color ~~is~~ become pale. The pellet was ~~a~~dded with 270 µL of buffer B and further added with 30 µL buffer C, then the mixture were incubated at 50°C for overnight. The next day, the mixture was added with 71 µL of 5 M NaCl solution, shacked vigorously for 15 second, and then was centrifuged at 10,000 rpm for 10 minutes. The top layer (approx. 300 µL) was transferred into a new 1.5 mL Eppendorf tube, added with 600 µL of 96% Ethanol, and mixed slowly. After emergence of DNA, then the tube was centrifuged at 12.000 rpm for 10 minutes. The supernatant was carefully deposed, and the DNA pellet was washed by addition of 100 µL of 70% ethanol and subsequent centrifugation at 12.000 rpm for 5 minutes. The supernatant was discarded and the DNA pellet was air-dried until it became semi-transparent. The dried DNA was added with 100 µL of TE (Tris-EDTA) solution (pH 7.4) or aquabidest sterile (Otsuka) then left for overnight to dissolve DNA.

Polymerase Chain Reaction (PCR)

Amplification of DNA fragments of 211 bp of a specific region of growth hormone gene was performed by polymerase chain reaction technique. The following set of primers was used according to Reis et al. (2001): *GH-forward*, 5' GCT GCT CCT GAG GGC CCT TC 3'; and *GH-reverse*, 5' CAT GAC CCT CAG GTA CGT CTC CG 3'. The amplification was performed using a final volume of 30 µL containing 15 µL PCR Kit KAPA (KAPA Biosystems), 11.25 µL aquabidest, *forward* and *reverse* primer 1.5 µL (10 pmol/µL) respectively, and 0.75 µL DNA template (20-100 ng). PCR was performed in thermocycler (PEQLAP Primus 25 Advance). The amplification condition for GH gene were an initial denaturation at 97°C for 1 minute 30 seconds, denaturation at 94°C for 1 minute, annealing at 62°C for 1 minute, elongation at 72°C for 1 minute. Steps two to four were repeated for 30 cycles and the reaction ended with a final extension at 72°C for 10 minutes (Mu'in 2008). The PCR products were separated on a 0.8% agarose gel, stained with ethidium bromide, and visualized under UV light in UV Transilluminator. Photographs were taken using digital camera (Canon EOS 600D).

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP)

Growth hormone gene variants were identified by a PCR-RFLP method. *Alu*I restriction enzyme (5'-AG | CT-3') was used in this analysis. The total volume of PCR-RFLP mixture was 15 µL containing 5 µL of PCR product, 0.5 µL of *Alu*I restriction enzyme (x U/µL, Fermentas, Life Science), 1.5 µL of 10x buffer tango, and 8 µL *aquabidest* that incubated at 37°C for 3 hours in multiheater (EYELA). PCR-restriction fragments were separated by electrophoresis on 2% agarose gel in 1x TBE buffer at 50 V for 2 hours, and visualized under UV light after staining with ethidium bromide. Photographs were taken as described above.

Statistics analysis

The frequency of alleles and genotypes were estimated following the method describe by Falconer and Mackay (1989):

$$p^2+2pq+q^2=1$$

Where:
p^2 = frequency of LL
$2pq$ = frequency of LV
q^2 = frequency of VV

Chi-square (X^2) analysis was used for finding the genetic equilibrium in population.

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

Association analysis between genotype and quantitative traits (body weight and body measurement) used SPSS version 20.0 program

RESULTS AND DISCUSSION

In the present study, 66 individuals from 5 Indonesian beef cattle breeds (Pesisir, PO, Bali, SimPO (Simmental x PO), and SimPes (Simmental x Pesisir)) were genotyped for GH-*Alu*I locus using PCR-RFLP technique. A pair of primer for GH gene (Reis et al. 2001) was used for amplifying 211 bp DNA fragment (see, Figure 1, Lane 2). Single Nucleotide Polymorphism (SNP) in exon 5 (at codon 127) of the bovine GH gene was located in the PCR product. The SNP has been found in the all of beef cattle populations and caused changed amino acids Leucine to Valine (GTC to GTG). It is a point mutation (a transversion mutation) in position 2141 of GH gene (GenBank accession Number M57764) (Lucy et al. 1993; Zhang et al. 1993).

Two patterns of restriction fragments were observed in the present study using *Alu*I restriction enzyme (restriction site 5'-AG | CT-3'). There were LL and LV genotypes (see, Figure 1 Lanes 3-6). A point mutation at position 52 that loses *Alu*I restriction site, could not find of restriction site (5'-AG | CT-3) that was known as V allele whereas L allele indicated of absence of mutation. So, LL genotype has one restriction site, yielding two fragments of 52 and 159 bp, LV genotype reveals 211, 159 and 52 bp fragments whereas VV genotype was undigested fragment and yields only one fragment of 211 bp.

Genotype and allele frequencies

The genotype and allele frequencies of Growth Hormone gene of beef cattle in Pesisir Selatan District were summarized in Table 1. Polymorphisms of Growth Hormone gene were found in SimPO (Simmental x PO) and SimPes (Simmental x Pesisir) breeds while Pesisir, PO, and Bali cattle were monomorphic populations. Monomorphic of GH-AluI in this study are similar to those reported in earlier studies (Mu'in 2008). It indicated that native cattle breeds (*Bos indicus*) have one variant allele in GH locus. Migration or introducing the other variant allele was not showed in this study.

Based on previous studies, *Bos indicus* cattle have 0.99-1.00 of L allele frequency, which were found in Gyr cattle (Kemenes et al. 1999; Pawar et al. 2007), Nelore cattle (Kemenes et al. 1999), Sahiwal cattle (Biswas et al. 2003), Kankrej cattle (Pawar et al. 2007), Madura cattle (Hartatik et al. 2013), Bali cattle/*Bos sondaicus* (Mu'in 2008). While its predominance in the taurine breed cattle with 0.642 – 0.80 of L allele frequencies such as Simmental cattle (Dybus et al. 2002), Limousine cattle (Hartatik et al. 2013), Charolais cattle (Kemenes et al. 1999), and Holstein cattle (Moravcikova et al. 2012; Arango et al. 2014; Hartatik et al. 2015). *Bos taurus x Bos indicus* crosses yielded L allele dominant in GH locus but lower than *Bos taurus*. It means that migration or introduction of V allele has been happened in population. Chanchim cattle, is synthetic cattle with 5/8 Charolais (*Bos taurus*) and 3/8 Nelore (*Bos indicus*), have L allele frequency (0.99) (Silveira et al. 2008).

LL genotype frequencies of GH gene in all of breeds were higher than LV genotype. The GH L allele was predominantly found in both of cattle populations This is same with previous studies in the Growth Hormone gene in cattle, Reis et al. 2001; Silveira et al. 2008; Mu'in et al. 2007; Hartatik et al. 2013; Volkandari et al. 2013; Moravcikova et al. 2012; Akcay et al. 2015).

Based on the observed versus expected genotype frequencies, the SimPO and LimPO population were in Hardy-Weinberg genetic equilibrium (see, Table 2).

Genotype effects

Growth Hormone acts directly by binding its receptors on the cells of bone, muscle, and fat tissue, and induces cell proliferation in these tissues. GH increases muscle protein synthesis and affects mammary growth in mammalians. Moreover, it plays an indirect role in cell growth (Ardiyanti et al. 2009; Akcay et al. 2015). Based on previous studies, polymorphism in GH locus affected weight gain, carcass weight, birth weight and marbling in cattle (Biswas et al. 2003; Tatsuda et al. 2008; Ardiyanti et al. 2009).

Genotypes of GH gene in SimPO and SimPes population showed no significant differences regarding any variables (Body weight, Body Length, High Shoulder, Hip Height and Heart girth). Production traits were controlled by many genes (polygenes) and interaction both of environment and genetic (Warwick et al. 1983).

Pereira et al. (2005) found that GH-LV genotype had positive effects on Yearling Weight in Chanchim cattle (synthetic cattle). The other hand, Aruna Pal et al. (2004) reported that genotype of GH-LL genotype had a significant effect on the average birth weight, 3 months body weight and daily body weight gains in Karan Fries bull populations.

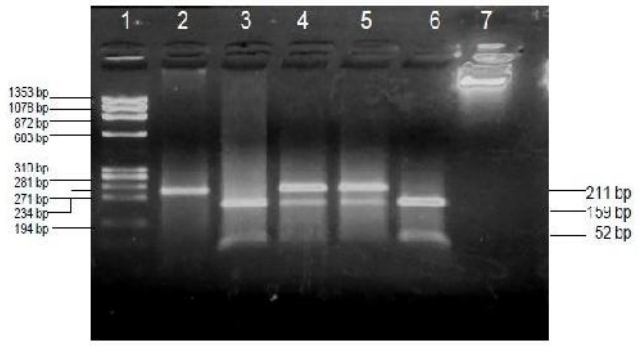

Figure 1. Representative result. Note: (1) Marker, (2) PCR product, (3-6) RFLP product, (7) Extracted DNA

Table 1. Allele and genotype frequencies of GH *AluI* loci

Breed	N	Genotype			Allele	
		LL (46)	LV (20)	VV (0)	L (57)	V (9)
Pesisir	15	1.000 (15)	0.000 (0)	0.000	1.000	0.000
PO	6	1.000 (6)	0.000 (0)	0.000	1.000	0.000
Bali	15	1.000 (15)	0.000 (0)	0.000	1.000	0.000
SimPO	15	0.267 (4)	0.733 (11)	0.000	0.634	0.366
SimPes	15	0.400 (6)	0.600 (9)	0.000	0.700	0.300

Table 2. Expected (He), observed (Ho) and HWE value for GH-*Alu*I

Breeds		Genotype *GH*			Allele frequencies		χ^2
		LL	LV	VV	L	V	
SimPO	*Observed*	4	11	0	0.6334	0.3666	5.029
	Expected	6.018	6.966	2.016			
SimPes	*Observed*	6	9	0	0.700	0.300	2.755
	Expected	7.350	6.300	1.350			

Note: $\chi^2_{0.05;2}$ = 5.99; *GH* = Growth Hormone; HWE = Hardy-Weinberg Equilibrium

Table 3. Body weight and body size of SimPO cattle and SimPes cattle based on genotype LL and LV by 18 months of age

Variables	Breeds			
	SimPO (LL)	SimPO (LV)	SimPes (LL)	SimPes (LV)
N	4	11	6	9
BW (kg)	270.50+63.07	260.00+49.26	176.17+34.89	176.61+26.73
BL (cm)	112.00+4.32	107.91+9.33	96.33+7.58	96.67+5.24
HS (cm)	117.00+12.36	111.36+7.57	98.50+6.35	99.11+3.66
HH (cm)	121.00+11.83	116.27+7.95	104.00+6.23	106.44+6.39
HG (cm)	155.5+22.23	148.55+12.41	130.00+6.78	131.33+8.12

Note: BW = Body weight; BL = Body Length; HS = High Shoulder; HH = Hip Height; CS = Heart Girth

In conclusion, by PCR-RFLP technique has been detected genotypes in the 5 Indonesian beef cattle. Polymorphisms were found in SimPO and SimPes populations. GH-L allele is dominant allele in GH locus. Non-significant effects of growth traits were found in this study.

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