ANALYSIS ON *Alu*-I GROWTH HORMONE (GH^{Alu-I}) GENE IN BALI CATTLE

Jakaria and R. R. Noor
Faculty of Animal Science, Bogor Agricultural University,
Darmaga Campus, Bogor 16680, West Java Indonesia
Corresponding E-mail: jakaria@ipb.ac.id

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ABSTRACT

The research was conducted to identify *Alu*-I locus of growth hormone (GH) gene in Bali cattle by using 232 blood samples collected from Bali and Lombok islands. PCR-RFLP and sequencing methods were used to detect the polymorphism and nucleotide sequence at *Alu*-I locus of GH gene. The result showed that Bali cattle from Bali island has one genotype (LL genotype), whereas Bali cattle originating from Lombok island has two genotypes, namely LL and VV genotypes, respectively. The L and V allele frequencies from Bali and Lombok islands were 1.00 and 0.00; 0.99 and 0.01, respectively. The sequencing result of Bali cattle LL genotype showed an AGCT sequence of enzyme *Alu*-I restriction site. Based on polymorphic informative content (PIC) value, it can be concluded that *Alu*-I locus of Bali cattle from Bali and Lombok islands were monomorphic and polymorphic, respectively.

Keywords: Bali cattle, growth hormone gene, monomorphic, polymorphic

INTRODUCTION

The Bali cattle breed is one of the existing indigenous cattle breeds (Aceh, Pesisir, Madura, Java and Bali) in Indonesia. Although no official historical records exists, it is generally accepted that the Bali cattle is the domesticated direct descendant of the wild Banteng (*Bos sondaicus, Bos javanicus, Bos bantinger*) still surviving as an endangered species in three National Wild Reservation Parks (Ujung Kulon, Baluran and Blambangan) in Java (Martojo 2003). As animal genetic resources, some molecular genetic studies have been reported in Indonesian cattle breeds including Bali cattle using microsatellite DNA (nuclear genome) and mitochondrial genome markers (Handiwirawan *et al*., 2003; Nijman *et al.*, 2003; Abdullah, 2008; Uggla, 2008; Mohammad *et al.*, 2009). However, molecular genetic marker based on coding sequence or candidate gene approach is limited and still need more in depth study of the existence of Indonesian cattle breeds especially Bali cattle.

The candidate gene approach is purposeful when a gene is known to function in such a way that it may explain genetic variation in traits of interest. The growth hormone (GH) gene is a candidate gene for body weight and weight gain in cattle since it plays a fundamental role in growth regulation (Silveira *et al.*, 2008). The GH gene is considered an attractive candidate gene for use as a beef and milk production marker due to its role in galactopoietic metabolism and the growth process. Bovine growth hormone (bGH) gene is localized in chromosome 19 (Hediger *et al.*, 1990), and consists of five exons separated by interval introns (Gordon *et al*., 1983).

The effects of some GH gene polymorphisms have been widely studied in beef cattle and the proximity between some of these polymorphisms, which can be characterized using different restriction enzymes, suggests a strong linkage between them. The presence of the *Alu*-I restriction site corresponds to the presence of the amino acid leucine (L) at position 127 in the polypeptide chain of cattle GH, whereas the absence of this site indicates the presence of valine (V) at the same position (Lucy *et al*., 1991; Switonski, 2002). The study of GH gene *Alu*-I locus have been reported in Bavarian Simmental cattle (Schlee *et al.*, 1994), Bali, Madura and Benggala cattle (Sutarno *et al*., 2002), Brahman cattle (Beauchemin *et al*., 2006), Angus and Shorthorn cattle (Barendse *et al*., 2006), Iranian cattle (Zakezadeh *et al*., 2006), and West Sumatra Pesisir cattle (Jakaria *et al.*, 2007).

The aim of this research was to identify the polymorphism of growth hormone (GH) gene *Alu*-I locus in Bali cattle from Bali and Lombok islands.

MATERIAL AND METHODS

Blood Sample and DNA Extraction

The 232 blood samples of Bali cattle were obtained from Bali Cattle Breeding Center (180 samples) and Center for Regional Artificial Insemination (20 samples) at Bali island, whereas 32 samples from Lombok island were collected from small holder farmers. Blood sampling were performed by veterinarians taken via jugular vein contain 5 ml blood samples and then preserved in ethanol absolute. Blood samples were extracted according Sambrook *et al.* (1989) and dissolved in TE solution. The quality and quantity of the total genome were analyzed using spectrophotometer and 1% agarose gel electrophoresis.

Amplification of GH Gene *Alu-I* Locus and Genotyping

The GH gene *Alu*-I locus was analyzed by using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method. PCR amplification conditions were as follows: 94°C for 2 min, followed by 30 cycles of denaturation at 94°C for 30 s, 60°C annealing for 45 s and extension at 72°C for 1 min, and final extension at 72°C for 5 min. A 211 bp fragment of partial exon 5^{th} in GH gene was amplified by using primer forward 5'-GCT GCT CCT GAG GGC CTT C-3' and reverse 5'-CAT GAC CCT CAG GTA CGT CTC CG-3' (Reis *et al.*, 2001). The PCR product was digested at 37°C for overnight by *Alu*-I enzyme (AGCT restriction site sequence) and then, the digestion product was separated by Mupid horizontal electrophoresis.

Sequence of GH *Alu-I* Fragment

The GH *Alu*-I fragment was done by sequencer machine of ABI Prims 3100-Avant Genetic Analyzer (PT Charoen Pokphan Indonesia). The sequence material used was PCR product of GH *Alu*-I fragment, primer *forward*, QIA-Quick PCR Purification Kit-Qiagen, 125 mM EDTA, ethanol absolute, 70% ethanol and Hi-Di *Formamide*.

Data Analysis

PCR-RFLP data was analyzed by allele frequency (Nei 1987). The allele frequency was calculated by formula as :

$$p = \frac{2(AA)+(Aa)}{2N}, q = \frac{2(aa)+(Aa)}{2N}$$

Where, p is the L allele frequency, q is the V allele frequency and N is the total number of individual tested.

Polymorphic Informative Content (PIC) value was estimated by calculated (Botstein *et al*., 1980) as :

$$PIC = 1 - \sum_{i=1}^{n} p_i^{\,2} - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2 p_i^2 p_j^2$$

Where p_i is the population frequency of the i^{th} allele and n is the number of alleles per marker. Sequences result were analyzed by Molecular Evolutionary Genetic Analysis (MEGA4) softwere program with alignment explorer/clustal method (Kumar and Tamura, 2006).

RESULTS AND DISCUSSION

Allele Frequencies of GH Gene *Alu-I* Locus

The following DNA restriction fragments result was obtained only one LL genotype of Bali cattle from Bali island, while Bali cattle from Lombok island was obtained two alleles, those were LL and VV, respectively. Based on genotyping data of GH *Alu*-I locus indicated 160 bp and 51 bp for the LL genotype, 211, 160 and 51 bp for the LV genotype and 211 bp (no digestion) for the VV genotype (Figure 1 and 2).

The genotype and allele frequencies of GH *Alu*-I locus for Bali cattle from Bali and Lombok islands are presented in Table 1. LL genotype and L allele frequencies of Bali cattle from Bali and Lombok islands were 1.00 and 0.97, respectively. The high L allele frequency was observed in both Bali cattle from Bali and Lombok islands. Based on the results obtained, the GH gene *Alu*-I locus is nomomorphic in Bali cattle from Bali island, whereas polymorphic in Bali cattle from Lombok island. Nei (1987) stated that monomorphic is allele frequency level equal or less than 0.99 (99%), whereas polymorphic is allele frequency level equal or less than 0.01 (1%). This means that the Bali cattle was found on the Bali island were uniform, unlike the Bali cattle on the Lombok island were various in GH *Alu*-1 locus. GH *Alu*-1 locus diversity (polymorphic) will give an importance subject in animal breeding program that can be used as a marker assisted selection (MAS) when association with the economic traits.

There was a tendency that L allele frequency of GH-*Alu*-I locus in hump cattle is (*Bos indicus*) higher than humpless cattle (*Bos taurus*) (Table 2). Base on result obtained that the L allele

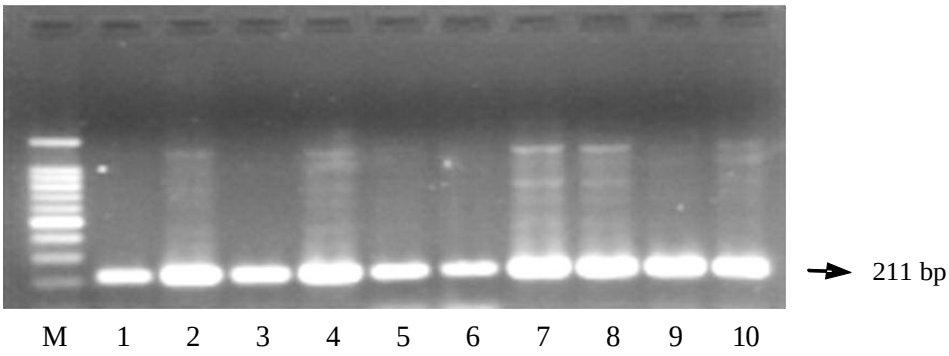

Figure 1. PCR Product of *GH Alu*-I Locus Detected by Agarose Gel Electrophoresis. M: marker (100 bp); line 1-10 : sample number

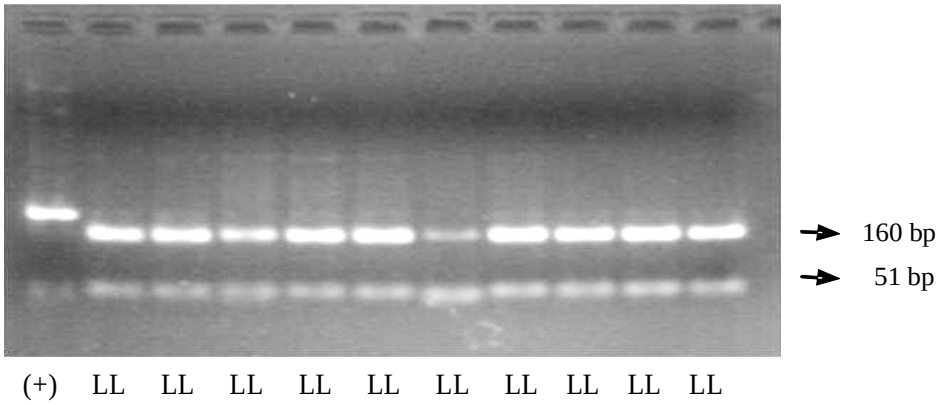

Figure 2. Genotyping Results of *GH Alu*-I Locus Detected by Agarose Gel Electrophoresis. (+): positive control (PCR product); LL: genotype

Table 1**.** Genotype Number and Allele Frequency of Bali Cattle from Bali and Lombok Islands

Originated	n	Genotype			Allele	
		LL	LV	VV	L	V
Bali island	200	200	0	0	1.00	0.00
Lombok island	32	31	0	1	0.97	0.03

n = number of animal

frequency is higher than the V allele in Bali cattle from Bali and Lombok islands. The highest L allele in Bali cattle population from Bali island is caused by natural selection, no crossing program and conservation area. While Bali cattle from Lombok island showed a crossbreeding program intensively. Crossbreeding programs introduced V allele, because the Limousine and Simmental cattle that used as artificial insemination (AI) semen carried V alleles. Jakaria *et al*. (2009) states that V allele frequency in Limousine and Simmental cattle were 0.182 and 0.306, respectively.

Polymorphic Informative Content (PIC) Value

PIC value result presented in Table 3. PIC value showed that the GH-*Alu*-I locus is no informative (monomorphic) in Bali cattle especially Bali cattle from Bali island, while Bali cattle from Lombok island is moderate informative (polymorphic). Based on the estimation PIC value, it could be concluded that GH *Alu*-I marker was not effective for genetic diversity information in Bali cattle from Bali island. Jakaria *et al*. (2007) also reported that PIC value was low in West Sumatra Coastal cattle using PCR-RFLP marker, in contrast on the PIC

Table 2. Allele Frequency Distribution of GH-*Alu-I* Locus in Humpless and Hump Cattle Breeds

Cattle breed	n	Allele Frequencies		Authors
		L	V	
Portuguese Beef cattle	195	0.76	0.24	Reis *et al*. (2001)
Mazandrani	97	0.91	0.09	Zakezadeh *et al*. (2006)
Angus	527	0.77	0.23	Barendse *et al*. (2006)
Shorthorn	500	0.76	0.24	Barendse *et al*. (2006)
Brahman	324	1.00	0.00	Beauchemin *et al*. (2006)
Nellore	79	1.00	0.00	Curi *et al*. (2006)
West Sumatra Pesisir	133	0.99	0.01	Jakaria *et al*. (2007)
Bali cattle from Bali island	200	1.00	0.00	Present study
Bali cattle from Lombok island	32	0.97	0.03	Present study

n = number of animal

Table 3. Estimation of Polymorphic Informative Content (PIC) Value in Bali Cattle from Bali and Lombok Islands

Originated	n	PIC Value
Bali island	200	0.000
Lombok island	32	0.333

n = number of animal

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        10          20          30          40          50          60
TTGTTTCCTC CTTGGCAGGA GCTGGAAGAT GGCACCCCCC GGGCTGGGCA GATCCTCAAG
        70          80          90         100         110         120
CAGACCTATG AACAATTTGA CACAAACATG CGCAGTGACG ACGCGCTGCT CAAGAACTAC
       130         140         150         160         170         180
GGTCTGCTCT CCTGCTTCCG GAAGGACCTG CATAAGACGG AGACGTACCT GAGGGTCATG
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Figure 3. Sequences Results of *GH Alu*-I Locus Detected by Sequencer Machine. A GCT= *Alu*-I enzyme restriction site

value estimation using microsatellite DNA marker where PIC value has high informative loci in Spanish sheep (Arranz *et al*., 2001). Thus, PCR-RFLP maker was less informative then microsatellite DNA marker.

Estimation of PIC value was a parameter that used as an informative marker. Botstein *et al*. (1980) reported that the criteria of PIC value divided in three group namely low ≤0.25, moderate 0.25<PIC<0.5, and high ≥0.5. Furthermore, explained that the PIC is not only can be used to determine an informative marker, but also can be used to determine the presence or absence of the polymorphic allele.

Sequensing of GH *Alu*-I locus

Sequencing results of GH *Alu*-I locus obtained *Alu*-I enzyme restriction site (AGCT) (Figure 3) that has a polymorphism in the GH gene. Zhang *et al*. (1993) and Lucy *et al*. (1993) reported that GH gene *Alu*-I locus occurred mutation between cytosine (AGCT) to guanine (AGGT) respectively. Jakaria *et al*. (2009) also reported a point mutation at GH gene of 5th exon from cytosine (C) to guanine (G) in Indonesian Limousine, Simmental and Bali cattle. Mutation of this gene have been described in dairy cattle (Lagziel and Soller, 1999) and beef cattle

(Barendse *et al*., 2006) to affect important production and reproduction traits.

CONCLUSION

This study can be concluded that high frequency of L allele on Bali cattle from Bali and Lombok islands. GH *Alu*-I locus was found monomorphic in Bali cattle from Bali island, while polymorphic in Bali cattle from Lombok island. Whereas AGCT sequence *Alu*-I enzyme restriction site was found in GH gene exon 5^{th} of Bali cattle from Bali island.

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