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Genotyping Growth Hormone-*Alu*I Locus of Bali Cattle in Pleihari, South Kalimantan

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Abstract

The study was conducted to genotyping growth hormone-*Alu*I (GH-*Alu*I) locus of Bali cattle in Pleihari, South Kalimanan. Blood samples were taken from 46 Bali cattle in Pleihari, South Kalimantan. The genotyping of this locus was conducted by digested the DNA fragment of 221 bp extended from the forth intron region (49 bp) to fifth of exon (162 bp) by *Alu*I enzyme. The resulted indicated that the Bali cattle population in Pleihari, South Kalimantan only L alelle was found, and no found V alelle and other alelle in this locus. Its concluded that the H-*Alu*I locus of Bali cattle in Pleihari, South Kalimantan was monomorphic.

(Key Words: genotype, growth hormone gene, monomorphic, *Alu*-I, Bali cattle)

Abstrak

Penelitian ini bertujuan untuk mengidentifikasi genotip dari lokus growth hormone-*Alu*I (GH-*Alu*I) pada populasi sapi Bali di Pleihari, Kalimantan Selatan. Sampel darah diambil dari 46 ekor sapi Bali (25 jantan dan 21 betina) di lokasi Pleihari, Kalimantan Selatan. Identifikasi genotip dari lokus ini dilakukan dengan mendigesti fragmen DNA berukuran 221 bp yang merentang dari daerah intron 4 (49 bp) hingga exon 5 (162 bp) menggunakan enzim restriksi *Alu*I. Hasil penelitian menunjukkan bahwa lokus GH-*Alu*I pada populasi sapi Bali di Pleihari, Kalimantan Selatan hanya memiliki satu macam alel, yaitu alel L, dan tidak ditemukan alel V atau alel lainnya pada lokus ini. Disuimpulkan bahwa lokus GH-*Alu*I pada populasi sapi Bali di Pleihari, Kalimantan Selatan bersifat monomorfik.

Kata kunci: sapi Bali, genotip, growth hormone gene, *Alu*-I, monomorfik.

Introduction

Growth hormone (GH) is the hormone which play role important in body growth control. GH or somatotrophin (STH) was protein content of 191 amino acids. Bovine GH was coding by gene at position of chromosome 19 (Hediger et al., 1990). Sequencing on bovine GH gene is conducted in 1980s, and it showed contained 1800 bp with 5 exons separated by 4 introns (Woychick et al., 1982; Gordon et al., 1983).

Polymorphism of bovine GH gene was first found in 1991 and occur in exon 5th using PCR-RLLP/*Alu*-I, then caused appearance two GH forms (Lucy et al., 1991). Variation of this locus was caused by nucleotide substitution, namely cytosine (C) on guanine (G), and made series changed of amino acid from leucine (Leu): CTG (L allele) into valine (Val): GTG (V allele) in position 127 (Zhang et al., 1992). The study previous has been found bovine GH-*Alu*I locus from somel cattle breeds was polymorphic with L allele frequence higher commonly, and some cattle breeds only showed that bovine GH was monomorphic (Table 1).

Polymorphism of GH-*Alu*I locus in some beef cattle breeds was found correlated with their growth characteristic. Schlee et al. (1994^{a}) reported that polymorphism of GH-*Alu*I locus affect GH concentration of male Germany Black and White cattle, male Baharian and Tyrolean Brown cattle, and male Simmental. Accordingly, male cattle GH concentration had LL genotype for three breeds was higher than LV genotype. Schlee et al. (1994^{b}) reprorted that LV genotype had correlated with breeding value of gains compared to the LL or VV genotypes of male Simmental. But Hereford breed was found V

allele had significant correlations by increased of gains from calving to 180 days of ages (Moody et al., 1996).

Explanation above indicated that GH gene polymorphism and their effect on growth aspect did not similarly among cattle breeds. The study was conducted to genotyping GH-*Alu*I locus of Bali cattle in Pleihari, South Kalimantan.

Material and Methods

Blood Sample and DNA Extraction

Blood sample was collected from 46 Bali cattle from Pleihari, South Kalimantan. The sampling of Bali cattle for research was randomly. The blood sample approximately 3 ml per head take from vena jugularis, was collected into K_3EDTA tube. Genomic DNA was extracted from the blood samples according Sambrook et al. (1989) and dissolved in TE solution. Quality of genomic DNA was checked by taking ratio of O.D. at 260 and 280 nm in the spectrophotometer. The samples having O.D. ratio between 1.6 to 2.2 were considered to be of good quality and used for PCR study. The quantity of DNA was estimated by spectrophotometry taking O.D.260 nm.

Genotyping GH-*Alu*I Locus

The GH-*Alu*I locus was analysed by using PCR-RFLP method. A 211 bp fragment of GH gene spanning from intron IV (49 bp) to exon V (162 bp) was amplified with a pair primers: GH-F: 5'-GCTGCTCCTGAGGGCCCTTC-3', and GH-R: 5'-CATGACCCTCA GGTACGTCTCCG-3' (Reis *et al*., 2001). The pair primers was provided from CyberGene AB. The position of primer forward and reverse in PCR product of GH gene shown in Figure 1.

Processing of DNA amplify was initiated by addition 19 µl dH_2O, 2 µl DNA solution (±50 ng) and a pair of primer 2 µl (16 pmol) into tube of 0.2 ml *Ready-To-Go PCR Bead* (Amersham Biosciences). PCR amplification conditions were as follow (Reis *et al*., 2001).: first denaturation of 95^{o}C for 5 minutes, followed by 30 cycles of denaturation at 95^{o}C for 30 seconds, annealing at 65^{o}C for 30 seconds, extension at 72^{o}C for 30 seconds, and final extension at 72^{o}C for 5 minutes.

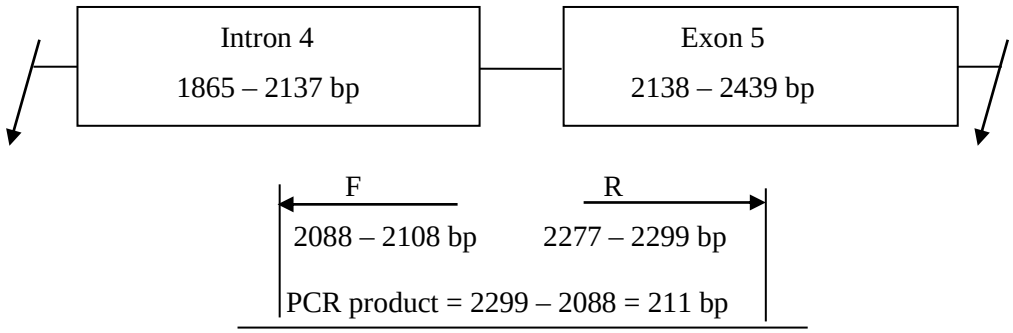

Figure 1. The position of primer forward and reverse in PCR product of GH gene

The PCR product (specific DNA fragment, size 211 bp) was digested by *Alu*-I enzyme (*Arhrobacter luteus*), production of Takara Bio Inc., its sequence and cut position was 5'-AG|CT-3'. Processing of DNA digestion was initiated into eppendorf tube 1,5 ml to added 10 µl PCR product, 2 µl 10X L bufer and 0,5 µl *Alu*I enzyme (10 unit/µl), then to added dH_2O until 20 µl. Incubated at 37^{o}C for 2 hours. The digestion product was separated by electrophoresed in 1% w/v agarose gel containing of etidium bromida into TBE buffer. The digestion product of respective DNA samples was taken 5 µl and mixed with 2 µl loading bufer, then pipetted into gel well. Running gel was regulated at 100 volt for 30 minutes and 100 bp DNA ladder used to identification the size of DNA bands. The result of electrophoresis was

examination under ultraviolet light, and followed to photo with Polaroid camera.

Genotype Identification of GH-*Alu*I Locus

Identification of genotype was done by comparison pattern of electrophoretic bands each sample on DNA marker band (DirectLoad^{TM} Wide Range DNA Marker, Sigma Production). The VV genotype of bovine GH-*Alu*I locus was appeared by existed of one band: 211 bp, LV genotype by three bands: 211 bp, 159 bp and 52 bp; and LL genotype by two bands: 159 bp and 52 bp (Reis et al., 2001).

Data Analysis

PCR-RFLP data was analysed by genotype and allele frequency (Nei and Kumar, 2000). The genotype and allele frequency was calculated by formula as:

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

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

Where, X_i is the i allele frequency, X_{ii} is the ii genotype frequency, n_{ii} is the number of cattle with the genotype of ii, n_{ij} is the number of cattle with the genotype of ij, n is the total number of Bali cattle tested.

Result and Discussion

GH-*Alu*I Genotype

Specific DNA fragment in size of 211 bp spanned from the forth intron region (49 bp) to fifth of exon (162 bp) of GH gene containing of polimorfic region (Lucy *et al*., 1991), had been amplified from genomic DNA of Bali cattle by using a pair of primers: GH-F dan GH-R. The visualization of amplification GH gene (211 bp) of Bali cattle using agarose gel 1.5%, and the position of primer forward and reverses in sequence product and the mutation position in 2141 bp (GenBank: M57764) can be seen in Figure 2 and 3, respectively.

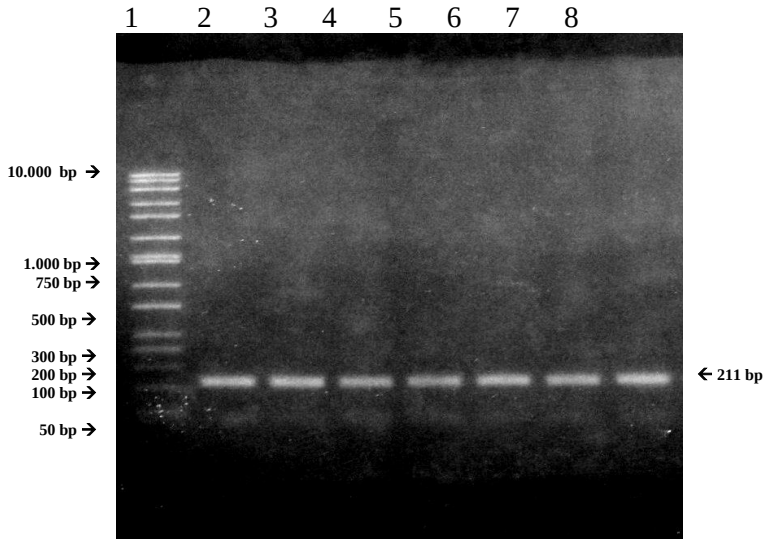

Figure 2. The visualization of amplification GH gene (211 bp) Bali cattle using agarose gel 1.5% (M: marker 100 bp, 2 – 11 research sampel.

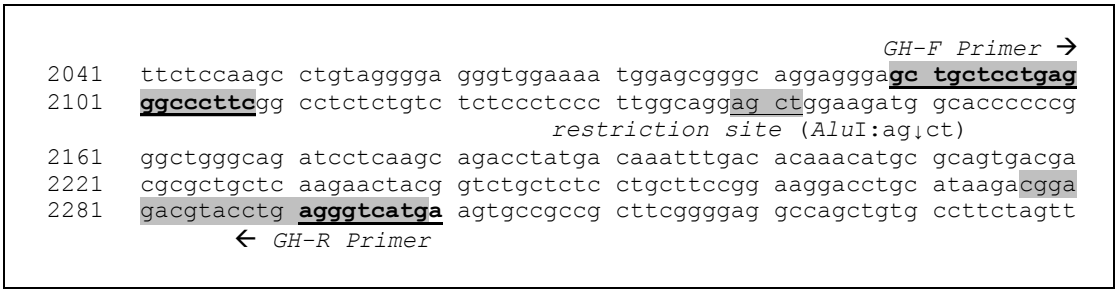

Figure 3. The position of primer forward and reverses in sequence product and the mutation position in 2141 bp (GenBank: M57764).

The result digestion with *Alu*I enzyme on PCR product (amplicon, size 211 bp) of all samples was found only one allele, namely L allele, while V allele and other allele did not found. This study, L allele was showed by successful *Alu*I enzyme (5'-AG|CT-3') found sequence was recognized of PCR product and successful to cut PCR product into two DNA fragment with size of 159 bp and 52 bp. According to Gordon et al. (1983) and Lucy et al. (1991), the successfully *Alu*I enzyme was found sequence recognized on along specific DNA fragment caused by sequence which to take the cut did not mutation (taken by nucleotide C), so that codon triplet serial was amino acid of leucine (Leu): CTG in position 127 of GH polypeptide sequence. On the contrary, V allele showed by failure of *Alu*I enzyme found these sequence, so that fail to cut PCR product. Consequence, the size of PCR product before and after digestion with *Alu*I was similarly, namely 211 bp. The failure *Alu*I enzyme found these sequence recognize caused by point which enzyme cutting was existed point mutation of nucleotide C into nucleotide G, so that cutting sequence changed from AGCT into AGGT. This point mutation caused change triplet codon series, namely CTG (Leu) change into GTG (Val) in position 127 of GH polypeptide sequence. However, V allele not found in Bali cattle population.

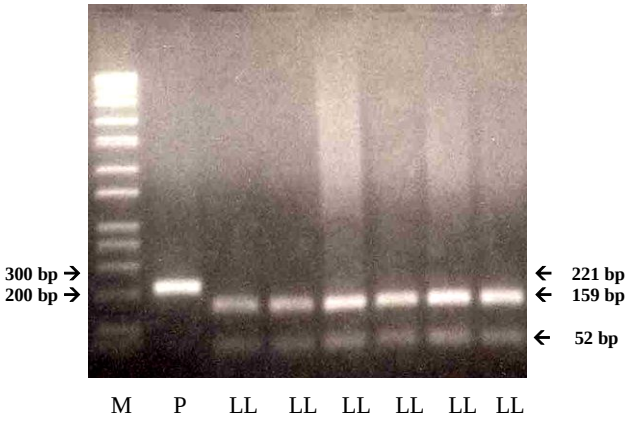

Figure 4. RFLPs of GH-*Alu*I locus of Bali cattle in South Kalimantan. Lane 1 is 100 bp DNA ladder (M); lane 2 is PCR product (P = 211bp); lane 3 until 8 are LL genotype (159 bp dan 52 bp).

The formation of DNA was double helix (diploid: 2N). If *Alu*I enzyme found sequence recognized in both helix of DNA, and both helix of DNA was cut into two L allele, then this individu was grouped into LL genotype. If *Alu*I enzyme only found sequence recognized in one of two helix of DNA in PCR product, then only one helix of DNA was cut, and resulted one L allele and one V allele. This individu was grouped into LV genotype. If *Alu*I enzyme did not found sequence recognized in both helix of DNA at PCR

product, then double helix of DNA did not cut. Therefore digesting PCR product resulted two V allele, and this individu was grouped into VV genotype. In this research, both genotypes: LV and VV, did not found in Bali cattle from Pleihari, South Kalimantan. Figure 4 showed genotype recognized in Bali cattle in Pleihari, South Kalimantan.

Alel dan Genotype Frequency

Frequency calculation of allele and genotype of GH-*Alu*I locus from 46 samples of Bali cattle in Pleihari, South Kalimantan according Nei and Kumar (2000). GH-*Alu*I locus of Bali cattle in this study only found one allele, namely L allele, and not found V allele or other allele. Therefore, GH gene found 100% has LL genotypes (Figure 4). The result indicated that GH-*Alu*I locus of Bali cattle (*Bos sondaicus*) was monomorphic. Therefore, GH-*Alu*I locus polymorphic commonly found in European cattle (*Bos taurus*) and their crossing, not found in Bali cattle from Pleihari, South Kalimantan. Jakaria and Noor (2011) also found that Bali cattle from Bali island has one genotype (LL genotype, monomorphic), where Bali cattle originating from Lombok island has two genotypes (polymorphic), namely LL and VV genotype. The L and V allele frequencies from Bali and Lombok island where 1.00 and 0.00; 0.99 and 0.01, respectively. Allele frequency distribution of GH-*Alu*I locus in some cattle breeds shown in Table 1.

The GH-*Alu*I locus monomorphic also found on Tharparkar cattle, with predominance of LL genotype (Biswas *et al.*, 2003). Some breeds of cattle as *Bos indicus* also had GH-*Alu*I locus were monomorphic, as found on Nelore (Ongole), Gyr, and Guzerath cattle breed (Vasconcellos *et al.*, 2003). Monomorphic of GH-*Alu*I locus of Bali cattle in Pleihari, South Kalimantan and in Bali island as reported by Jakaria and Noor (2011) indicated that as long as GH-*Alu*I locus of Bali cattle population never mutation and never contaminated by gene migration from other breeds. By neglected assumption gene mutation in future, that GH-*Alu*I locus which monomorphic of Bali cattle in Pleihari as well as Bali cattle in Bali island also usable as one of indicator to investigate crossing of Bali cattle with other breeds in certain region, especially with *Bos taurus*.

Conclusion

GH-*Alu*I locus was found monomorphic in Bali cattle from Pleihari, South Kalimantan. Hhowever, it usable as one of indicator for detecting purebreed of Bali cattle in certain region.

Table 1. Allele frequency distribution of GH-*Alu*I locus in *Bos taurus, Bos indicus* and *Bos sondaicus*

Cattle Breed	N	Alel		Source
		L	V	
Bali cattle (in Pleihari)	46	1,0000	0,0000	Result in this study
Bali cattle (in Bali island)	200	1.0000	0.0000	Jakaria & Noor, 2011
Nellore	63	1,0000	0,0000	Vasconcellos *et al.*, 2003
Nellore	79	1.0000	0.0000	Curi *et al*., 1006.
Guzerath	25	1,0000	0,0000	Vasconcellos *et al.*, 2003
Brahman	324	1.0000	0.0000	Beauchemin *et al*., 2006.
Madura	65	1,0000	0,0000	Hartatik et al., 2012.
Gyr	83	1,0000	0,0000	Vasconcellos *et al.*, 2003
West Sumatera Pesisir	134	0.9920	0.0080	Jakaria *et al.*, 2007.
Peranakan Ongole (PO)	52	0,9800	0,0200	Hartatik et al., 2012.
Bali cattle (in Lombok island)	32	0.9700	0.030	Jakaria & Noor, 2011
Santa Gertrudis	20	0,9700	0,0300	Vasconcellos *et al.*, 2003
Holstein (pejantan)	70	0,9600	0,0400	Lucy *et al*., 1993
Mertolenga	22	0,9550	0,0450	Reis *et al*., 2001
Karan Fries (Holstein FriesianxTharparkar)	26	0,9400	0,0600	Pal *et al*., 2004
Mirandesa	21	0,9285	0,0715	Reis *et al*., 2001
Nellore	211	0,9200	0,0800	Unanian, *et al*., 2000

Cattle Breed	N	Alel		Source
		L	V	
Limmousine x Madura	81	0,9100	0,0900	Hartatik et al., 2012.
Mazandrani	97	0.9100	0.0900	Zakezadeh *et al*., 2006
Benggala	115	0,9000	0,1000	Sutarno, 2004
Canchim	30	0,9000	0,1000	Vasconcellos *et al.*, 2003
Canchim (62,5% Charolais + 37,5% Zebu)	329	0,9000	0,1000	Pereira *et al.*, 2005
Baharian & Tyrolean Brown	20	0,9000	0,1000	Schlee *et al*., 1994[b]
Limmousine x PO	56	0,8900	0,1100	Hartatik et al., 2012.
Marinhoa	32	0,8750	0,1250	Reis *et al*., 2001
Holstein (induk)	142	0,8700	0,1300	Kratochvilova *et al*.,2000
Alentejana	22	0,8640	0,1360	Reis *et al*., 2001
Canchim (65,7% Charolais + 34,3% Zebu)	359	0,8500	0,1500	Pereira *et al.*, 2005
Simmental	52	0,8200	0,1800	Regitano *et al*., 2000
Caracu	30	0,8000	0,2000	Vasconcellos *et al.*, 2003
German Black & White	23	0,8000	0,2000	Schlee *et al*., 1994[b]
Angus	527	0,7700	0,2300	Barendse *et al.* (2006)
Aberdeen Angus	52	0,7700	0,2300	Vasconcellos *et al.*, 2003
Shorthorn	500	0,7600	0,2400	Barendse *et al.*, 2006
Preta	27	0,7595	0,2405	Reis *et al*., 2001
Charolais	36	0,7400	0,2600	Regitano *et al*., 2000
Charolais	30	0,7200	0,2800	Garcia, 2001 dalam Vasconcellos *et al.*, 2003
Simmental	41	0,7100	0,2900	Schlee *et al*., 1994[b]
Arouquesa	24	0,7085	0,2915	Reis *et al*., 2001
Polish Friesian	214	0,6900	0,3100	Grochowska *et al*., 2001[b]
Limousine	130	0,6730	0,3270	Dybus, et al., 2003
Polish Friesian (bulls)	155	0,6500	0,3500	Oprzadek et al., 2005
Barros	23	0,5870	0,4130	Reis et al., 2001
Maronesa	24	0,3960	0,6040	Reis et al., 2001

Keterangan: L = *Leu* (asam amino *leucine*); V = *Val* (asam amino *valine*)

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