Identification of Growth Hormone (Gh) Gene MspI and AluI Loci Polymorphism in Beef Cattles

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ABSTRACT

The research was conducted in order to identify the polymorphism of MspI and AluI locus of the growth hormone (GH) gene in Bali, Limousine and Simmental cattle. Total blood samples of 87 cattle were taken from population of Bali cattle were collected from Balai Pembibitan Ternak Unggul Sapi Bali in Bali island, whereas Limousine and Simmental cattle blood samples were collected from Balai Inseminasi Buatan (BIB) Singosari-Malang, West Java. PCR-RFLP and sequencing methods used to detect the polymorphism and mutation at MspI and AluI loci of GH gene. The results showed that at MspI locus, the Bali, Limousine and Simmental cattle had one genotype (-/-), three genotypes(+/+, +/-, -/-) and two genotypes (+/+, +/-), respectively whereas for AluI locus, the Bali, Limousine and Simmental cattles had one genotype (LL), two genotypes (LL, LV) and three genotypes (LL, LV, VV), respectively. The allele frequencies of + and – alleles in Bali, Limousine and Simmental cattle were 0.000 and 1.000; 0.636 and 0.364; 0.889 and 0.111 respectively, whereas the frequencies of L and V alleles in Bali, Limousine and Simmental cattle were 0.000 and 0.000; 0.818 and 0.182; 0.694 and 0.306 respectively. Based on polymorphic informative content (PIC) value, it can be concluded that MspI and AluI loci in Bali cattle are monomorphic, while in Limousine and Simmental cattle is polymorphic. Based on the sequencing results, the MspI (+/+ and -/- genotypes) and AluI (LL and VV genotypes) loci showed a occurrences of nucleotide base mutation from cytosine (C) to thymine (T) and cytosine (C) to guanine (G), respectively.

Key words: Indonesian cattle, growth hormone gene, PCR-RFLP, sequence, polymorphism

INTRODUCTION

Indonesia has some animal genetic resources that need more attentions to be utilized and developed sustainable. The Bali breed is one of the four existing indigenous cattle breeds (Aceh, Pesisir, Madura 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 still surviving as an endangered species in three National Wild Reservation Parks (Ujung Kulon, Baluran and Blambangan) in Java (Martojo, 2003).

Some molecular genetic studies have been reported in Indonesian cattle breeds using microsatellite DNA (nuclear genome) and mitochondrial genome markers (Handiwirawan et al., 2003; Nijman et al., 2003; Abdullah, 2008; Uggla 2008; Mohamad et al., 2009). However, molecular genetic marker based on coding sequence or candidate gene approach is limited and still needs more in depth study of its existence in Indonesian cattle breeds.

Molecular genetic markers in animal breeding programs could make selection more precise and efficient. Some of these markers are called candidate genes, e.g. the growth hormone genes, which are usually selected because of their biological significance on the quantitative traits of interest. Growth hormone has wide physiological activities, which include the regulation of growth, lactation and mammary gland development, gluconeogenesis, the activation of lipolysis, and the enhancement of amino acid incorporation into muscle protein (Burton et al., 1994). There is also evidence that growth hormone may be involved in the pubertal development and testicular function (Lin, 1996). Because of these important relationships, GH is a candidate gene for marker-assisted selection programs in cattle.

The GH gene is considered as an attractive candidate gene to be used as a marker due to its

role in galactopoietic metabolism and the growth process. Growth hormone gene is localized in chromosome 19 (Hediger et al., 1990), and consists of five exons separated by interval introns (Gordon et al., 1983). Several polymorphisms were identified in the *GH* gene. Cowan et al. (1989) and Hilbert et al. (1989) detected a polymorphic site for *Msp*I restriction endonuclease, the polymorphism being localized in the intron 3 of the *GH* gene in the position 1547 (Zhang et al., 1993), while a polymorphic site for AluI restriction endonuclease, has a genetic variant charac-terized by the substitution of one amino acid (leucine) for another (valine) at position 127, localized in the exon 5 in GH gene (Lucy et al., 1991).

The study of GH gene MspI and AluI loci have been reported in Bavarian Simmental cattle (Schlee et al., 1994), Hereford and Composite cattle (Sutarno et al., 1996; Sutarno 1998), Ongole Grade (PO) cattle (Sutarno et al., 2005), Brahman cattle (Beauchemin et al., 2006), Angus and Shorthorn cattle (Barendse et al. 2006), Iranian cattle (Zakezadeh et al., 2006), Indian Zebu cattle (Shodi et al., 2007) and West Sumatra Pesisir cattle (Jakaria et al., 2007).

The aim of this research was conducted in order to identify the polymorphism of growth hormone (GH) gene of MspI and AluI loci in Bali, Limousine and Simmental cattle breeds.

MATERIALS AND METHODS

Blood Sample and DNA Extraction

The total numbers of blood samples were taken from 87 samples consisting of Bali cattle 47 from Balai Pembibitan Ternak Unggul Sapi Bali in Bali Island, whereas Limousine and Simmental cattle 40 were collected from Balai Inseminasi Buatan (BIB) Singosari-Malang, West Java. Blood sampling were performed by veterinarians from the Faculty of Veterinary Medicine IPB Bogor. Bali, Limousin and Simmental cattle blood samples were taken via jugular vein contain 5 ml by venoject tube, and then preserved in ethanol absolute.

Total genome was extracted from blood samples by the phenol/chloroform method followed by ethanol precipitation (Sambrook et al., *1*989) and dissolved in TE solution. The quality of the total genome was analyzed using by 1% agarose gel electrophoresis.

Amplification of GH Gene *Msp*I and *Alu*I Loci and Genotyping

The GH gene *Msp*I and AluI loci were analyzed by using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method (Table 1). A 329 bp fragment of intron 3 and 211 bp fragment of exon 5 in *GH* gene was amplified by PCR using forward and reverse primers. The PCR products of GH gene MspI and AluI loci were digested at 37°C for overnight by *Msp*I and AluI enzyme respectively. The digestion products were separated by horizontal electrophoresis (85 volts, 50 min) in 2% agarose gels in 1 × TBE and 10% ethidium bromide.

Sequences of GH *Msp*I and *Alu*I Fragment

Sequences of GH MspI and AluI fragment was done by sequencer machine of ABI Prims 3100-Avant Genetic Analyzer to find nucleotide mutation in both fragments. The sequences of GH MspI and AluI fragment carry out in individual homozygot (+/+ and -/-) and (LL and VV) respectively. The sequences material used PCR product of GH MspI and AluI fragment, primer *forward*, QIA-Quick PCR Purification Kit-Qiagen, 125 mM EDTA, ethanol absolute, 70% ethanol and Hi-Di *Formamide*.

Table 1. Primer sequences were used in amplificates of GH *Msp*I and *Alu*I loci

Locus	Primer sequence	Annealing
GH *Msp*I[1)]	F 5'-CCC ACG GGC AAG AAT GAG GC-3' R 5'-TGA GGA ACT GCA GGG GCC CA-3'	53°C
GH *Alu*I[2)]	F 5'-GCT GCT CCT GAG GGC CTT C-3' R 5'-CAT GAC CCT CAG GTA CGT CTC CG-3'	55°C

Note: F = *Forward*, R = *Reverse*. [1)] Mitra et al. (1995), [2)] Reis et al. (2001)

Data Analysis

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

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

where, p is the (+) or (L) allele frequencies,
q is the (-) or (V) allele frequencies and
N is the total number of cattle tested.

Polymorphic Informative Content (PIC) value was estimated by calculated (Hildebrand et al. 1992) as :

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

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) packed program with *alignment explorer/clustal* method (Kumar and Tamura, 2006).

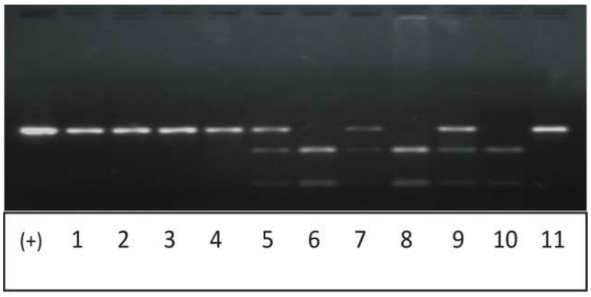
RESULTS AND DISCUSSION

Allele Frequencies of GH Gene MspI and *Alu*I Loci

The following DNA restriction fragments result was obtained three genotype for the GH gene MspI and AluI loci repectively. Based on genotyping data of GH MspI locus showed 222 bp and 105 bp for +/+ genotype, 327 bp, 222 bp and 05 bp for +/- genotype and 327 bp (no digestion) for -/- genotype (Fig. 1). Whereas genotyping data of GH AluI locus showed 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 (Fig. 2).

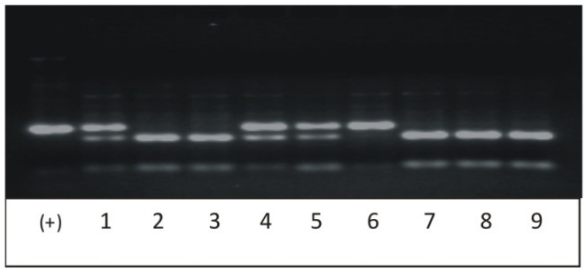
The genotype and allele frequencies of GH MspI and AluI loci for Bali, Limousine and Simmental cattles were presented in Table 1 and 2. The (-) allele with a high frequency in Bali cattle and low frequencies in Limousine and Simmental cattle breeds while (+) allele for GH-Mspl locus was high frequencies in Limousine and Simmental cattle breeds and low frequency in Bali cattle. The allele frequency of the GH-alul locus ranged from 0.694 to 1.000. The highest L allele frequency was observed in Bali cattle breed herd (1.000).

Study of the distribution of the GH gene MspI locus in several regions of the world's cattle reported that the frequency of (-) allele was higher in *Bos indicus* (hump) cattle group and lower in the Bos taurus (humpless) cattle group (Lagziel et al., 2000; Sodhi et al., 2007) (Table 2 and 3). The same research results also obtained that the frequency of (-) allele was high in West Sumatra Pesisir cattle (Jakaria et al., 2007) and Ongole Grade (PO) (Sutarno et al., 2005).



(+) ~ positive control (PCR product); lanes 1, 2, 3, 4, 11 ~ -/- genotype; lanes 5, 7, 9 ~ +/- genotype; lane 6, 8, 10 ~ +/+ genotype.

Figure 1. Genotyping Results of *GH-Msp*I Locus Detected by Agarose Gel Electrophoresis.



(+) was positive control (PCR product); lanes 2, 3, 7, 8, 9 were LL genotype; lanes 1, 4, 5 were LV genotype; lane 6 was VV genotype.

Figure 2. Genotyping Results of *GH-Alu*I Locus Detected by Agarose Gel Electrophoresis.

Table 2. Genotype and allele frequencies of GH MspI loci in Bali, Limousin and Simmental cattle breeds

No	Breed	n	Genotype			Allele	
			+/+	+/-	-/-	(+)	(-)
1.	Bali	47	0.000	0.000	1.000	0.000	1.000
2.	Limousine	22	0.409	0.454	0.136	0.636	0.364
3.	Simmental	18	0.773	0.222	0.000	0.889	0.111

Note: n = individual number.

Table 3. Genotype and allele frequencies of GH AluI loci in Bali. Limousin and Simmental cattle breeds

No.	Breed	n	Genotype			Allele	
			LL	LV	VV	L	V
1.	Bali	47	1.000	0.000	0.000	1.000	0.000
2.	Limousine	22	0.636	0.364	0.000	0.818	0.182
3.	Simmental	18	0.500	0.389	0.111	0.694	0.306

Note: n = individual number

Table 4. Distribution of the (-) allele frequency of GH-*Msp*I locus in humpless and hump cattle breeds

Cattle breeds	n	Breed types	Allele frequency (-)
Angus	65	Humpless	0.40
Charolais	7	Humpless	0.22
Gelbveih	15	Humpless	0.06
Limousine	18	Humpless	0.39
Simmental	23	Humpless	0.04
Brahman	23	Hump	0.65
Nellore	20	Hump	0.82
Siri	9	Hump	0.45
Grade Ongole (PO)*	114	Hump	0.26
West Sumatra Pesisir**	133	Hump	0.80
Indian zebu (17 breeds)***	750	Hump	0.67-0.94

Sources: Lagziel et al. (2000), *) Sutarno et al. (2005), **) Jakaria et al. (2007), ***) Shodi et al. (2007).

Table 5. Distribution allele frequency of GH-*Alu*I locus in humpless and hump cattle breeds

Cattle breed	n	Allele frequencies		Autors
		L	V	
Mazandrani	97	0.910	0.090	Zakezadeh et al. (2006)
Beef cattle Portugis	195	0.759	0.241	Reis et al. (2001)
Angus	527	0.770	0.230	Barendse et al. (2006)
Shorthorn	500	0.760	0.240	Barendse et al. (2006)
Brahman	324	1.000	0.000	Beauchemin et al. (2006)
Nellore	79	1.000	0.000	Curi et al. (2006)
West Sumatra Pesisir	133	0.993	0.007	Jakaria et al. (2007)

Based on the research results reported that the L allele frequency of GH-AluI locus was higher in hump (Bos indicus) cattle than humpless (Bos taurus) cattle (Table 5). There is a tendency that the L allele frequency higher than the V allele in Bos indicus cattle groups and including Indonesia native cattle. The low frequency of GH-AluI L allele of cattle population studied can be due to low number of samples, low actual allele frequency or the effect of severse natural selection at this locus.

Polymorphic Informative Content (PIC) Value

Based on the result analyzed showed that PIC values for GH-MspI and GH-AluI loci in Bali, Limousine and Simmental cattles were presented in Table 6.

Tabel 6. Estimation of polymorphic informative content (PIC) value in Bali, Limousin and Simmental cattle breeds

Cattle breed	n	PIC Value	
		GH *Msp*I locus	GH *Alu*I Locus
Bali	47	0.0000	0.0000
Limousine	22	0.2760	0.2598
Simmental	18	0.1779	0.3343

Note: n = individual number.

Based on the estimation of PIC value could be concluded that the GH-Mspl and GH AluI Loci were less (no) informative (monomorphic) in Bali cattle breed, on the other hand is more informative (polymorphic) in Limousine and Simmental cattle breeds.

Sequensing of GH-MspI and AluI Loci

Sequencing results showed that there are changes in nucleotide bases (mutation) from cytosine to thymine and cytosine to guanine for GH-MspI and AluI loci respectively (Table 7).

Table 7. Mutation of nucleotide basa in GH *Msp*I dan *Alu*I loci

Locus	Enzyme restriction site	Mutation	Position
GH-*Msp*I	C*CGG	Cytosine to Thymine	(1547)*
GH-*Alu*I	AG*CT	Cytosine to Guanine	(2141)*

*) based on sequences of Gordon *et al.* (1983).

Based on obtained result showed that same results reported by Zhang et al. (1993) and Lucy et al. (1993), GH gene MspI and AluI loci involved changes of nucleotide base between cytosine (C) to thymine (T) and cytosine (C) to guanine (G) respectively.

CONCLUSIONS

Based on our research results can be concluded that the frequency of (-) allele is high in Bali cattle, while low in Limousine and Simmental cattle breeds of GH-MspI locus. We also founded high frequency of L allele on all cattle breeds tested for GH-AluI locus. GH gene MspI and AluI loci founded monomorphic in Bali cattle, while polymorphic in Limousine and Simmental cattle breeds. The mutations occurred between cytosine (C) to thymine and cytosine (C) to guanine (G) for GH gene MspI and AluI loci respectively.

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