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POLYMORPHISM OF GROWTH HORMONE GENE IN INDONESIAN CATTLE

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ABSTRACT

The study was conducted to investigate the polymorphism of growth hormone (GH) gene in Indonesian cattle. If the polymorphic characteristic of this gene could be detected then its effect on growth could be studied and then it can be used as the genetic marker for selection of growth rate in Indonesian cattle. Blood samples were taken from three population of Bali cattle, Bali Improvement Project (P3Bali), Bali island, and South Kalimantan, and were 111, 85 and 46 samples respectively. Besides that blood samples were taken from population of Ongole grade (PO), Simmental x PO (SIMPO) and Limousine x PO (LIMPO) cattle in Special District of Yogyakarta, and were 43, 44 and 42 samples respectively. The identification of GH gene polymorphism was conducted by digested the DNA fragment of 221 bp extended from the fourth intron region (49 bp) to fifth of exon (162 bp) by *Alu*I enzyme. The results indicated that in the three Bali cattle populations only L allele was found, and no V allele or other alleles of GH gene, while the PO, SIMPO dan LIMPO cattle populations indicated that two alleles: L and V were found at the GH gene with higher allelic frequency for L allele. Its was concluded that the GH gene was not polymorphic in Bali cattle and the genotype of GH was homozygote LL, while the GH gene was found polymorphic in PO, SIMPO dan LIMPO cattle and the genotypes of GH were LL dan LV.

Key words: Indonesian Cattle, Growth Hormone Gene, Polymorphism

INTRODUCTION

Growth hormone (GH) was the hormone which play an important role in the control of body growth. The bovine growth hormone (bGH) is a single-chain polypeptide hormone produced in the anterior pituitary gland and consist of 191 amino acids. Bovine GH protein was coding by gene located at chromosome 19 (Hediger et al., 1990). Sequencing on bGH gene was conducted in 1980s, and containing 1800 bp with five exons separated by introns (Woychick et al, 1982; Gordon et al., 1983).

A polymorphism of bGH gene was occur in the fifth exon, that caused two variants of GH (Lucy et al., 1991). Variation of this GH was caused by single nucleotide substitution, namely a cytosine (C) for a guanine (G) that causes an amino acid change from leucine (L allele, codon CTG) to a valine (V allele, codon GTG) at the residue 127 (Zang et al., 1992). The previous study showed that bGH gene from most of cattle breeds were polymophic with the frequency of L allele commonly high (Lucy et al., 1993; Schlee et al., 1994[a]; 1994[b]; Reis et al., 2001; Grochowska et al., 2001;

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Vasconcellos et al., 2003; Regitano et al., 2000 in Vasconcellos et al., 2003). Only several breeds showed that bGH did not polymorphic, such as in Tharparkar (Biswas et al., 2003), Nelore, Gyr, and Guzerath cattle (Vasconcellos et al., 2003).

Recently several studies have investigated the associations between genetic polymorphism at the bGH locus with production traits. Schlee et al. (1994[a]) reported that polymorphism of GH affected GH concentration in male Germany Black and White cattle, male Baharian and Tyrolean Brown cattle, and male Simmental. GH concentration of LL genotype was higher than LV genotype. Schlee et al. (1994[b]) reported that LV genotype showed the higher breeding value of gains compared of LL or VV genotypes in male Simmental. But in Hereford breed V allele had significant correlation with the increased of gains from calving to 180 days of age (Moody et al., 1996). Some reported also mentioned that genotype of GH gene was associated with birth weight in dairy cattle (Biswas et al., 2003), weaning weight in beef cattle (Marshall and Kim, 2000) dan mature live weight in beef cattle (Zwierzchowski et al., 2001). Explanation above indicated that bGH gene polymorphism and their effect on growth aspect was not similar among cattle breeds. The study was conducted to evaluate the polymorphism of GH gene in Indonesian cattles.

MATERIALS AND METHODS

Animals and blood sample

The present study was carried out in 371 Indonesian cattle, consisted of 111 selected Bali cattle from Bali Improvement Project (P3Bali), 85 unselected Bali cattle outside of P3Bali in Bali island, and 46 unselected Bali cattle in outside of Bali island (South Kalimantan). Besides that samples were taken from population of Ongole grade (PO), Simmental x PO (SIMPO) and Limousine x PO (LIMPO) cattle in Special District of Yogyakarta, and amounting to 43, 44 and 42 samples respectively. About 3 ml venous blood was collected under sterile conditions from the jugularis vein of the animals into a sterile tube containing K_3EDTA as anticoagulant, and brought to Biochemistry Laboratory, Biotechnology Study Centre, Gadjah Mada University, Yogyakarta, for DNA analysis.

DNA preparations

The DNA genome was isolated from the blood samples following phenol-chloroform extraction method described by Sambrook et al. (1989). The quality of 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.

PCR-RFLP

A 211 bp fragment of bGH gene spanning from intron IV (49 bp) to exon V (162 bp) was amplified with a couple of primers, namely GH-forward: 5'-GCTGCTCCTGAGGGCCC TTC-3', dan GH-reverse: 5'-CATGACCCTCAGGTACGTCTCCG-3' (Reis et al., 2001). The sample for amplification was prepared from 19 µl dH_2O, 2 µl DNA solution (50 – 100 ng) and a pair of primer accordingly 2 µl (16 pmol) into tube of 0,2 ml Ready-To-Go PCR Bead (Amersham Biosciences). The amplification of DNA fragment were done by using

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Thermal Cycler machine. The PCR machine was programmed for first denaturation at 95°C for 5 minutes, then 35 cycles of amplification (denaturation at 95 °C for 30 seconds, annealing at 65 °C for 30 seconds, extention at 72 °C for 30 seconds), then followed by final extention at 72 °C for 5 minutes.

The 211 bp amplicon was digested with *Alu*I (Arhrobacter luteus) enzyme to identify polymorphism at GH gene. The PCR product of 10 µl was put into 1,5 tube, then 2 µl of 10X L Buffer, 0,5 µl AluI enzyme (10 unit/ µl), and dH_2O was added until the volume reached 20 µl. Then the tube was incubated at 37°C for 2 hours. The digested product was electrophorized in 1% w/v agarose gel in TBE buffer at 100 V for 30 minutes. A 100 bp DNA ladder was loaded as DNA marker. The result of electrophoresis was visualized under the ultraviolet light, then photographed using Polaroid film. The VV genotype of bGH was identified by one band of 211 bp, the LV genotype by three bands of 211 bp, 159 bp and 52 bp, and the LL genotype by two bands of 159 bp and 52 bp (Reis et al., 2001).

RESULTS AND DISCUSSION

Genotyping

The specific DNA fragment of 211 bp spanned from the fourth intron region (49 bp) to fifth of exon (162 bp) of bGH gene, containing polymorphic region (Lucy et al., 1991), was obtained by amplification using a pair of primer: GH-forward and GH-reverse. Identification of genotype was done by comparing the pattern of electrophoretic bands in each sample with DNA marker.

The result of the digestion with *Alu*I enzyme on PCR product of all samples of Bali cattle indicated that only one allele, namely L allele, but not V allele or other allele was found. In this study, L allele was showed by two DNA fragment of size 159 bp and 52 bp as the two restriction site was recognized and cut by the *Alu*I. This result also indicated no mutation of amino acid leucine (CTG) in position 127 of GH polypeptide sequence. On the other hand, there were two alleles: L and V were found at the GH gene in the PO, SIMPO dan LIMPO cattle populations. V allele was showed by one DNA fragment of size 211 bp as the no restriction site was recognized, so that no cut by the *Alu*I. This result indicated that occured mutation that caused an amino acid change from leucine (L allele, codon CTG) to a valine (V allele, codon GTG) at the residue 127 (Lucy et al., 1991; Zang, et al., 1992).

The formation of DNA was double helix (diploid: 2n). If *Alu*I enzyme found recognized the sequence both helix of DNA, and cut at the two restriction site, then the individu was LL genotype. If *Alu*I enzyme only recognized the sequence in only one of two helix of DNA , then the individu was LV genotype, and if no sequence was recognized in both helix of DNA , then both helix of DNA was not cut, the individu was VV genotype.

In this study, both the LV and VV genotype were not found in Bali cattle, while in PO, SIMPO and LIMPO cattle only the VV genotype was not found. Figure 1 showed the LL and LV genotype of GH in some samples in this study.

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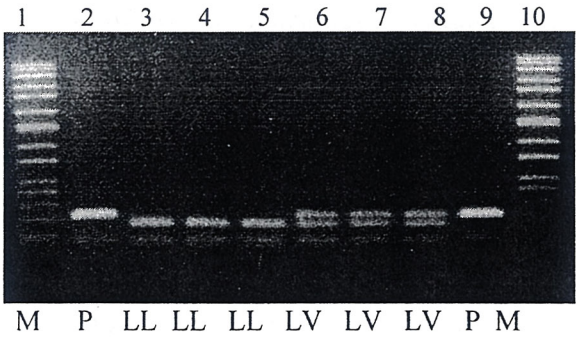

Figure 1. Electropherogram of the PCR product after digestion with AluI Lanes 1 and 10: DNA markers (50 bp until 10.000 bp); lanes 2 and 9: PCR product (211 bp); lanes 3, 4, and 5: LL genotypes (159 bp and 52 bp); lanes 6, 7, and 8: LV genotypes (211 bp, 159 bp, and 52 bp.

Allele and genotype frequency

The allele and genotype frequencies of GH of selected Bali cattle population at P3Bali, unselected Bali cattle population from Bali island, and unselected Bali cattle population from South Kalimantan were similar (Table 1). The frequency of GH allele was 100% L, and so the frequency of LL genotype was 100 %. The results indicated that GH gene of Bali cattle were non polymorphic. The non polymorphic GH gene also was found on Tharparkar cattle, with predominance of LL genotype (Biswas et al., 2003), besides that several breeds of *Bos indicus* cattle were reported non polymorphic, such as Nelore (Ongole), Gyr, and Guzerath cattle breed (Vasconcellos et al., 2003). Non polymorphic GH gene of Bali cattle (Indigenous) indicated that there is no mutation of GH gene and there is no migration from other breeds. By neglected the possibility of gene mutation in future, the non polymorphic of GH gene in Bali cattle could be used as an indicator that population crossing with other breed, especially with Bos taurus has never been practiced.

In this study, the local Indonesian cattles, represented by the PO, SIMPO and LIMPO cattles, showed the polymorphic of GH. The polymorphism of GH gene at third breeds indicated by the finding of V allele beside L allele, and the frequency of L allele was found higher than V allele (Table 1). The frequency of L allele was the highest in PO cattle, and lowest in SIMPO cattle population. The previous study also showed that GH gene from most of cattle breeds were polymophic with the frequency of L allele commonly high (Lucy et al., 1993; Schlee et al., 1994[a]; 1994[b]; Reis et al., 2001; Grochowska et al., 2001; Vasconcellos et al., 2003; Regitano et al., 2000 in Vasconcellos et al., 2003).

The result of this study showed that most of PO cattle population (97,7%) have LL genotypes, and only 2,3% (1 animal) which was LV genotype. The finding of LV genotype at PO population in this research is anticipated caused by migration of alel V of other breed, which probably from *Bos taurus*. It's caused, GH gene at Ongole (Nellore) breed and some *Bos indicus* other (Gyr and Guzerat) found non-polimorfik with predominance of LL genotype (Vasconcellos Et al., 2003). This anticipation is strenghtened by SIMPO and LIMPO found have V alel with high frequency. This situation prove that there is migration of alel V from Bos taurus, especially at SIMPO cattle population.

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Table 1. Allele and Genotype Frequencies of GH Gene in Indonesian Cattle

Breed of Cattle*)	n	GH gene				
		Allele Frequency		Genotype Frequency (%)		
		L	V	LL	LV	VV
Bali[1)]	111	1,00	0,00	100,0	0,0	0,0
Bali[2)]	85	1,00	0,00	100,0	0,0	0,0
Bali[3)]	46	1,00	0,00	100,0	0,0	0,0
PO	43	0,99	0,01	97,7	2,3	0,0
SIMPO	44	0,75	0,25	50,0	50,0	0,0
LIMPO	42	0,89	0,11	78,6	21,4	0,0

*) Bali[1)]: Selected Bali cattle at P3Bali; Bali[2)]: Unselected Bali cattle outside of P3Bali in Bali island; Bali[3)]: Unselected Bali cattle in outside of Bali island (South Kalimantan); PO: Ongole grade; SIMPO: Simmental x PO; LIMPO: Limousine x PO.

It could be expected that most of Simmental or Limousine bulls which used for crossing have LV genotype. The frequency of the V allele at Simmental were reported to be 0,18 – 0,29 (Regitano et al., 2000 in Vasconcellos, et al., 2003 and Schlee et al., 1994[b]).

CONCLUSION

The GH gene of Bali cattle were non polymorphic, only L allele of the GH was found. The non polymorphic of this gene in the population could be used as an indicator for detecting the purity of this breed as there is no migration of this gene from other breeds. The polymorphism of GH gene found at PO cattle population and also of the PO cross with Simmental and Limousine may indicated that probably there is an association between the polymorphism of GH with some production traits in cattle.

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