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Full Length Research Paper

Identification of single nucleotide polymorphism of growth hormone gene exon 4 and intron 4 in Pesisir cattle, local cattle breeds in West Sumatera Province of Indonesia

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The pupose of this study was to identify genetic polymorphisms of bovine growth hormone gene exon 4, and intron 4 in local cattle breeds in West Sumatera Province of Indonesia. DNA was isolated from 60 blood samples and polymerase chain reaction (PCR) product of GH5 fragment (366 bp) were directly sequenced. Multiple alignments, including 60 bGH DNA sequences obtained by direct sequencing and bGH sequences from a public database (National Center for Biotechnology Information, acces number M57764), revealed 15 polymorphisms (five SNP, eight deletion, and two insertion). Eight deletions were detected in position 1740, 1743, 1745, 1747, 1749, 1750, 1753, and 1754 with frequency allele of 0.50, 0.22, 0.125, 0.53, 0.06, 0.06 and 0.83, respectively. Besides, two insertions C were detected in position 1790 and 1895 with frequency allele of 0.06 and 1.00, respectively. Five mutations were detected in position 1914, 1930, 1947, 1980, and 2025 with genotypes C → G, G → A, T → G, T → C, and A → G respectively with frequency allele of 0.40, 0.48, 1.00, 0.42, and 0.38, respectively. Another deletion at position 1740, 1743, and 1754 changed codon CAG, TCG, TGG, CTT, GGG, CCC to codon CGT, GTG, GCT, TGG, GCC and new deletion at position 1745, 1749, and 1754 changed the codon TCG, TGG, CTT, GGG, CCC, CTG to TCG, GGC, TGG, GCC, CTG. Deletion at position 1749 and 1754 changed the codon TCG, TGG, CTT, GGG, CCC, and CTG to TCG, TGG, CTG, GGC, CCT. These data provide evidence that *GH* gene of this breed is slightly different from other breeds. This polymorphic source can be used to refer to performance and to investigate whether these polymorphics are responsible for quantitative variation in growth.

Key words: Sapi Pesisir, direct sequencing, growth hormone gene, polymorphism.

INTRODUCTION

The Pesisir cattle breed is one of the existing indigenous cattle breeds in Indonesia that has been adapted to relatively harsh environment, especially to hot and humid climate and low-quality feed to produce meat. Demand for this cattle is high because it is relatively cheaper compared to superior cattle. In the last 20 years, decreasing live weight occur in this cattle due to negative selection, where farmer maintains the small cattle and sold the big one to get higher price. Recently, selection for better performance of such important indigenous breed has got more attention, especially from the advancement of genetically molecular biotechnology.

Bovine growth hormone gene (*bGH*) plays a vital role in post-natal growth and general metabolism, reproduction and milk production (Kish, 2008). Investigation concerning this gene are important by considering

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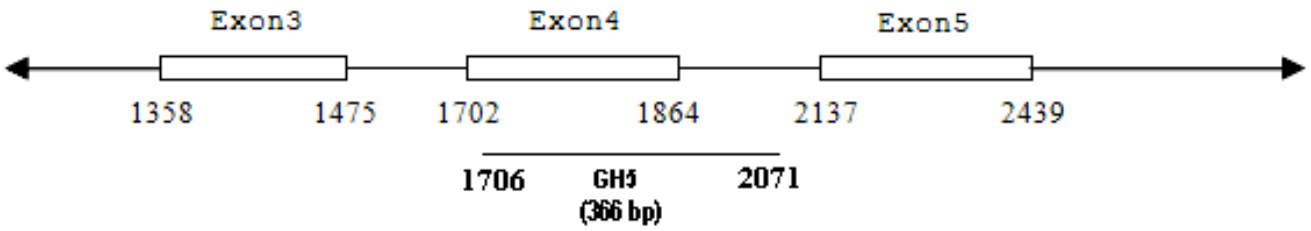

Figure 1. Map of bovine growth hormone gene with the position and length of the PCR amplified fragment from part exon 4 and intron 4 (Gordon et al., 1983).

improvements in cattle production. The bGH is found in chromosome 19, in the bovine genome (Hediger et al., 1990), it is approximately 1800 bp in size and is composed of five exons and four introns (Woychik et al., 1982). The complete DNA sequence has been determined (Gordon et al. 1983).

The candidate gene approach is benefecial when a gene is known to function in such a way that it may explain genetic variation in traits of interest. The *bGH* gene is considered as a promising candidate gene for improving growth, meat and milk production marker due to its role in galactopoietic metabolism and the growth process. Several polymorphic regions have been reported at different region of *bGH* gene. Polymorphism in introns or flanking sequences have potential usefulness as genetic marker. This gene has been widely studied because of its effect on important biological function including growth, body composition and development of mammary cell. A considerable number of studies seeking an association between variants of this gene and productive traits have been reported (Hoj et al., 1993; Lucy et al., 1993; Lagziel et al., 1996, 1999; Dybus, 2002; Zhou et al., 2005; Khatami et al., 2005; Ferraz et al., 2006; Pawar et al., 2007; Hasret et al., 2009; Tatsuda et al., 2008; Yardibi et al. 2009; Komisarek et al., 2011).

This research was aimed at identifying the polymorphism of growth hormone (*GH*) gene in exon 4 and intron 4 by direct sequencing in Pesisir cattle from West Sumatera, Indonesia.

MATERIALS AND METHODS

Animals

The animal subjects used in this study were 60 local cattle breeds (Sapi Pesisir) randomly selected from farmer.

Preparation of DNA

Blood sample are collected by jungular vein puncture with a 14 gauge needle into a vacutainer containing 0.5 ml of 0.5 M ethylenediaminetetraacetic acid (EDTA). The blood samples were kept on ice and then stored at -20°C refrigerator. Total DNA was extracted from the whole blood samples using a commercial DNA extraction kit Promega Wizard® Genomic DNA purification kit according to the manufacture protocol. The 366-bp GH gene fragment, covering a part of the fourth exon and part of the adjacent fourth intron was amplified using pure Tag ready-to-go PCR Beads from GE Healthcare. For each beads, 2 µl (20 ng) genomic DNA, 2 µl (20 nM) of each primer, and 19 µl ddH_2O was added. The primer sequences were as follows: GH5 (forward): 5'-TTGGAGCTGCTTCGCATCTCA-3''; GH5 (reverse): 5'-ATTTTCCACCCTCCCCTACAG3'' (Yao et al., 1996). PCR condition was 5 min at 94°C, 60 s at 94°C, 90 s at 62°C, 80 s at 72°C, 40 cycles and 5 min at 72°C. The resulting products were loaded to 1% agarose gel within 1 x Tris-Borate-EDTA (TBE) and then run with 50 V for 60 min for separation of the DNA fragments. The bands were stained with ethidium bromide prior to visualization by UV light. Following electrophoresis, PCR products were gel-purified using Ultraspine II according to the instruction of the supplier and DNA was stored at -20°C. Then the amplified 366 bp product was sequenced in Seqlab Laboratories Gottingen Germany.

RESULTS AND DISCUSSION

The PCR amplified a 366 bp fragment from part exon 4 and intron 4. The position and the length of the fragment are illustrated in Figure 1. Using the sequence of Gordon et al. (1983) as basic analysis, we mapped 15 polymorphisms (eight deletions, two insertions and five mutation) (Table 1).

Five new polimorphism were detected in this fragment : transversion C → G at position 1914, transition G → A at position 1930, transversion T → G at position 1947, transition T → C at position 1980, and transition A → G at position 2025 with frequency 0.40; 0.48; 1.00; 0.42; dan 0.38 respectively. Insertion C at position 1895 and tranversion T → G at position 1947 occured in all samples and was not found in other breeds. So, can be use as marker for this breed.

Insertion A at position 1752 that changed the sequence from GCTTGGGCC to GCTTGAGCCC changed the recognition site of restriction enzyme *Ppe*I (GGGCC↓C) or enzyme *PspOM*I(G↓GGCCC) or enzyme *EciE*I (GGGCCC). Deletion G at position 1753 that changed the sequence from TGGGCCCT to TGGCCCT changed the recognition site of restriction enzyme *Ppe*I (GGGCC↓C) or enzyme *PspOM*I(G↓GGCCC) or enzyme *EciE*I (↓GGGCCC). Insertion C at position 1790 changed the

Table 1. Fifteen (15) polymorphisms in bovine GH gene exon 4 intron 4 identified by direct sequencing.

Number	Sample	Mutation	Position	Frequency
1	2	Deletion A	1740	0.50
2	11	Deletion C*	1743	0.22
3	13	Deletion T*	1745	0.20
4	17	Deletion G*	1747	0.125
5	21	Deletion T*	1749	0.53
6	34	Deletion T	1750	0.06
7	36	Deletion G*	1753	0.06
8	36	Deletion C*	1754	0.83
9	46	Insertion C	1790	0.06
10	53	Insertion C	1895	1.00
11	51	C → G*	1914	0.40
12	49	G → A*	1930	0.48
13	43	T → G*	1947	1.00
14	30	T → C*	1980	0.42
15	25	A → G*	2025	0.38

*New polymorphism.

sequence from ACAGCTTGGT to ACAGCCTTGGT. This can be detected by restriction enzyme *Alu*I(AG↓CT) or enzyme *Bsal*l(↓AGCT). Mutation at position 1914 will changed the sequence GCCCTCTCCT to GCCCTGTCCT and changed the recognition site of restriction enzyme *Mnl*I(CCTCNNNNNNN↓), and transition G → A at position 1930 that changes the sequence from GCCAGGAGAAT to GCCAGAAGAAT will create the binding site enzyme *Ncu*I(↓GAAGA). Transversion T → G at position 1947 was also found at Bos Taurus gen access number AF117348.1 it can be detected only by seqencing and the mutation T → C at position 1980 changed the recognition site of restriction enzyme *Bfa*I(C↓TAG) or enzim *Mja*I(↓CTAG). Finally, the mutation A → G at position 2025 can be only detected by sequencing.

From our sequences, no polymorphism was found at position 2141, showing that the fragment harbors a non polymorphic *Alu*I-RFLP site. Polymorphism at this position was reported by Zhang et al. (1992, 1993) and Lucy et al. (1993). The polimorphism at this position was caused by the mutation C to G, that changed the codon CTG → GTG (Leu to Val). Non polymorphic site at position 2141 was obtained for Pesisir Cattle in this study which is in accordance with those of Brown Swiss (Lucy et al., 1993), Gyr Cattle (Kemenes et al., 1999), Gyr Brazilia (de Mattos et al., 2004), Guzera and Nelore (Kemenes et al., 1999), Iranian Sistani and Dashtyari (Masoudi et al., 2002), Angus, Brahman, and FH (Sumantri et al., 2011).

Deletion at position 1740, 1743, and 1754 (genotypes A) changed the sequence from CAGTCGTGGCTTGGGCCC to CGTGTGGCTTGGGCC and from the codon CAG, TCG, TGG, CTT, GGG, CCC to codon CGT, GTG, GCT, TGG, GCC. These deletions changed the structure of amino acid from Glisine, Serine, Tryptophan, Leusine, Glisine, Proline to Arginin, Valine, Valine, Tryptophan, Alanine. Deletion at position 1745, 1749, dan 1754 (genotypes B) changed the sequence from TCGTGGCTTGGGCCCCTG to TCGGGCTGGGCCCTG and from the codon TCG, TGG, CTT, GGG, CCC, CTG to TCG, GGC, TGG, GCC, CTG. These deletions changed the amino acid Serine, Tryptophan, Leusine, Glysine, Proline, and Leusine to Serine, Glysine, Tryptophan, Alanine, and Leusine. Deletion at position 1749 and 1754 (genotype C) changed the sequence from TCGTGGCTTGGGCCCCT to TCGTGGCTGGGCCCT and from the codon TCG, TGG, CTT, GGG, CCC, and CTG to TCG, TGG, CTG, GGC, and CCT. These deletions changed the amino acid serine, tryptophan, leusine, glysine, proline, and leusine to serine, tryptophan, leusine, glysine, proline and alanine (Figure 2).

In summary, our study reveals that there were 15 polymorphisms in this bovine *GH* gene fragment, 11 of which had not been reported previously. These data provide evidence that *GH* gene in Pesisir cattle is a good polymorphic source and can be used to refer to performance and to investigate whether this polymorphic might be responsible for quantitative variation in growth.

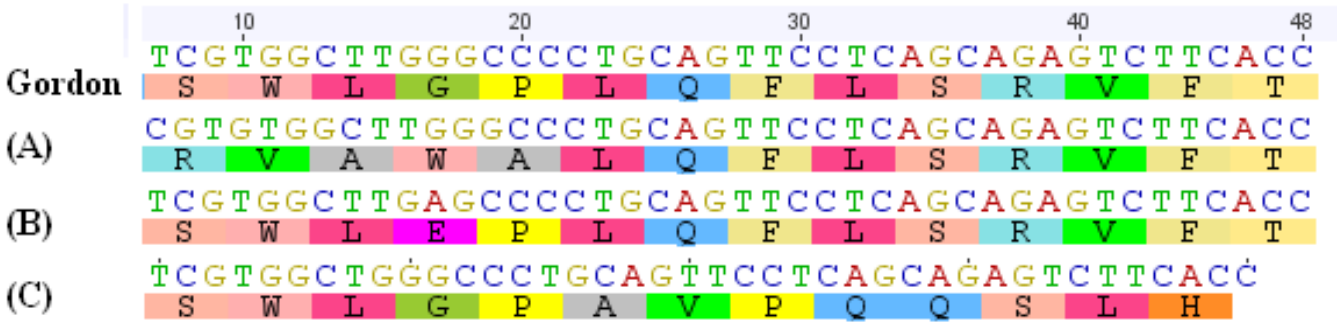

Figure 2. Amino acid sequence compared to Gordon et al. (1983). A) Sequence with deletion at positiona 1740, 1743 and 1754. B) Sequence with deletion at position 1745, 1749 and 754. C) Sequence with deletion at positions 1749 and 1754.

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