

*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

Yurnalis<sup>1\*</sup>, Sarbaini<sup>1</sup>, Arnim<sup>1</sup>, Jamsari<sup>2</sup>, and Wolfgang Nellen<sup>3</sup>

<sup>1</sup>Faculty of Animal Husbandry, Andalas University, Indonesia.

<sup>2</sup>Faculty of Agriculture, Andalas University, Indonesia.

<sup>3</sup>Genetics Department, Kassel University, Germany.

Accepted 16 November, 2012

The purpose 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, access 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 polymorphisms are responsible for quantitative variation in growth.

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

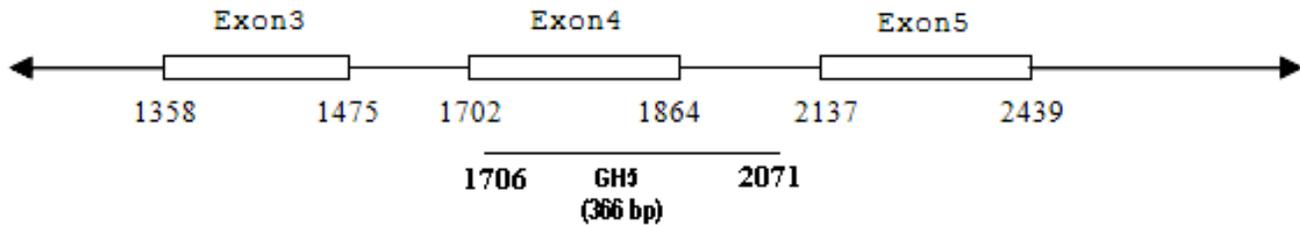
## INTRODUCTION

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

\*Corresponding author. E-mail: [yurnalisunand@yahoo.com](mailto:yurnalisunand@yahoo.com).



**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 beneficial 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 jugular 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<sub>2</sub>O 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 polymorphism 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 transversion T → G at position 1947 occurred 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 *PpeI* (GGGCC↓C) or enzyme *PspOMI*(G↓GGCCC) or enzyme *EciEI* (GGGCC). Deletion G at position 1753 that changed the sequence from TGGGCCCT to TGGCCCT changed the recognition site of restriction enzyme *PpeI* (GGGCC↓C) or enzyme *PspOMI*(G↓GGCCC) or enzyme *EciEI* (↓GGGCC). 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

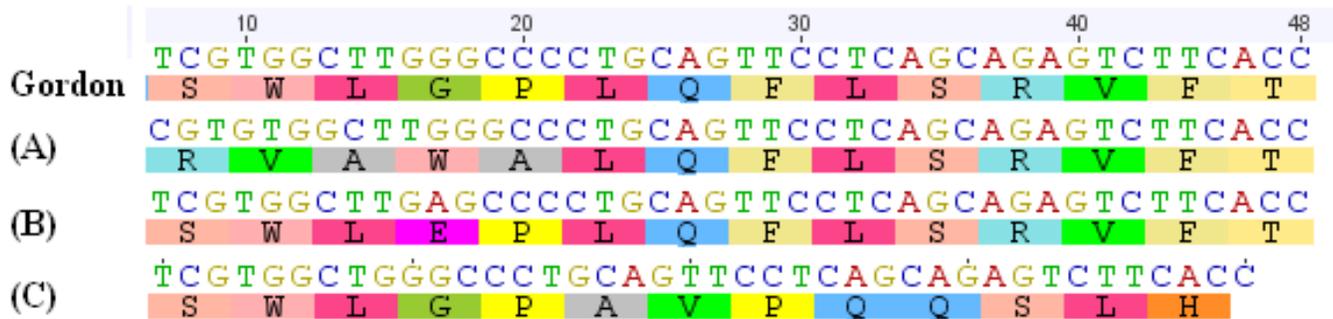
\*New polymorphism.

sequence from ACAGCTTGGT to ACAGCCTTGGT. This can be detected by restriction enzyme *AluI*(AG↓CT) or enzyme *BsaI*(↓AGCT). Mutation at position 1914 will change the sequence GCCCTCTCCT to GCCCTGTCCT and change the recognition site of restriction enzyme *MnI*(CCTCNNNNNN↓), and transition G → A at position 1930 that changes the sequence from GCCAGGAGAAT to GCCAGAAGAAT will create the binding site enzyme *NcuI*(↓GAAGA). Transversion T → G at position 1947 was also found at *Bos Taurus* gen access number AF117348.1 it can be detected only by sequencing and the mutation T → C at position 1980 changed the recognition site of restriction enzyme *BfaI*(C↓TAG) or enzyme *MjaI*(↓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 *AluI*-RFLP site. Polymorphism at this position was reported by Zhang et al. (1992, 1993) and Lucy et al. (1993). The polymorphism 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 CAGTCGTGGCTTGGGCC 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 TCGTGGCTTGGGCCCTG 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, Glycine, Proline, and Leusine to Serine, Glycine, Tryptophan, Alanine, and Leusine. Deletion at position 1749 and 1754 (genotype C) changed the sequence from TCGTGGCTTGGGCCCT 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, glycine, proline, and leusine to serine, tryptophan, leusine, glycine, 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 positions 1740, 1743 and 1754. B) Sequence with deletion at position 1745, 1749 and 754. C) Sequence with deletion at positions 1749 and 1754.

## REFERENCES

- de Mattos K, Nassif Del Lama S, Martinez ML, Freitas AF (2004). Association of bGH and Pit-1 gene variants with milk production traits in dairy Gyr bulls. *Pesquisa Agropecuária Brasileira*. 39(2).
- Dybus A (2002). Association of Growth Hormone and Prolactin genes polymorphisms with milk production traits in Polish Black and White Cattle. *Anim. Sci.* 20:203-212.
- Ferraz ALJ, Bortolossi JC, Curi RA, Ferro MIT, Ferro JA, Furlan LR (2006). Identification and characterization of polymorphisms within the 5' flanking region, first exon and part of first intron of bovine GH gen. *Anim. Breed. Genet.* 123:208-212.
- Gordon DF, Quick DP, Erwin CR, Donelson JE, Maurer RA (1983). Nucleotide sequence of the bovine growth hormone chromosomal gene. *Mol. Cell. Endocrinol.* 33:81-95.
- Hasret Y, Hosturk GT, Paya I, Kaygisiz F, Ciftioglu G, Mengi A, Oztabak K (2009). Association of Growth Hormone Gene Polymorphism with Milk Production Traits in South Anatolian and East Anatolian Red Cattle. *J. Anim. Vet. Adv.* 8(5):1040-1044.
- Hediger R, Johnson SE, Barendse W, Drinkwater RD, Moore SS, Hetzel J (1990). Assignment of the growth hormone gene locus to 19q26-qter in cattle and to 11q25-qter in sheep by *in-situ* hybridization. *Genomics* 8:171-174.
- Hoj S, Fredholm M, Larsen NJ, Nielsen VH (1993). Growth hormone gene polymorphism associated with selection for milk fat production in lines of cattle. *Anim. Genet.* 24(2):91-95.
- Kemenes PA, Regitano ICA, Rosa AJM, Paker IU, Razook AG, Figueiredo LA, Silva NA, Etchigaray MAI, Coutinho ILL (1999).  $\alpha$ -casein and  $\beta$ -lactoglobulin and growth hormone allele frequencies and genetic distances in Nelore, GYR, Guzera, Caracu, Charolais, Canchim and Santa Gertrudis cattle. *Genet. Mol. Biol.* 22(4):539-541.
- Khatami SR, Lazebnvi OE, Maksimenko VF, Sulimova GE (2005). Association of polymorphisms of the growth hormone and prolactin genes with milk productivity in Yaroslavl and black-and white cattle. *Genetika* 41(2):229-236.
- Kish S (2008). Exploring How Growth Hormones are Released in Animals. National Institute of Food and Agriculture. USDA
- Komisarek J, Michalak A, Walendowska A (2011). The effects of polymorphisms in *DGAT1*, *GH* and *GHR* genes on reproduction and production traits in Jersey cows. *Anim. Sci. Pap. Rep.* 29(1):29-36.
- Lagziel A, Lipkin E, Soller M (1996). Association between SSCP haplotypes at the bovine growth hormone gene and milk protein percentage. *Genetics* 142:945-951.
- Lagziel A., Lipkin E, Ezra E, Soller, M, Weller JI (1999). An MspI Polymorphism at the bovine growth hormone (bGH) gene is linked to a locus affecting milk protein percentage. *Anim. Genet.* 30:296-299.
- Lucy M, Hauser CSD, Eppard PJ, Krivi GG, Collier RJ (1991). Genetic polymorphism within the bovine somatotropin (bST) gene detected by polymerase chain reaction and endonuclease digestion. *J. Dairy Sci.* 74(Suppl. 1):284.
- Masoudi AK, Omrani H, Torkamanzahi A (2002). Study of growth hormone gene polymorphism in Iranian Bos indicus cattle by PCR RFLP technique: Proceeding of the First Seminar on Genetics and Breeding Applied to Livestock Poultry and Aquatics at Tehran, Iran. pp.141-145.
- Pawar RS, Tajane KR, Joshi CG, Rank DN, Bramkshtri BP (2007). Growth hormone gene polymorphism and its association with lactation yield in dairy cattle. *Indian J. Anim. Sci.* 77(9):884-888.
- Sumantri C, Imron M, Sugyono, Andreas E, Misrianti R, dan Ishak ABL (2011). Keragaman Grup Gen Hormon Pertumbuhan (GH, GHR, GHRH dan Pit-1) dan Hubungannya dengan Respon Superovulasi, Tingkat Ovulasi, Tingkat Fertilisasi dan Kualitas Embrio Sapi di Balai Embrio Ternak (BET) Cipelang. *JITV* 16(2):126-139.
- Tatsuda K, Oka A, Iwamoto E, Kuroda Y, Takeshita H, Kataoka H, Kouno S (2008). Relationship of the Bovine Growth Hormone Gene to Carcass Traits in Japanese Black Cattle. *Anim. Breed. Genet.* 125:45-49.
- Woychik RP, Camper SA, Lyons RH, Horowitz S, Goodwin EC, Rottman FM (1982). Cloning and nucleotide sequencing of the bovine growth hormone gene. *Nucleic Acids Res.* 10:7197-7210.
- Yao JB, Aggrey SE, Zadworny D, Hayes JF, Kuhnlein U (1996). Sequence variations in the bovine growth hormone gene characterized by single-strand conformation polymorphism (SSCP) analysis and their association with milk production traits in Holsteins. *Genetics* 144:1809-1816.
- Yardibi H, Hosturk HT, Paya I, Kaygisiz F, Ciftioglu G, Mengi A, Oztabak K (2009). Associations of Growth Hormone Gene Polymorphisms with Milk Production Traits in South Anatolian and East Anatolian Red Cattle. *J. Anim. Vet. Adv.* 5:1040-1044.
- Zhang HM, Brown DR, Denise SK, Ax RL (1992). Nucleotide sequence determination of a bovine somatotropin allele. *Anim. Genet.* 23:578.
- Zhang HM, Brown DR, Denise SK, Ax RL (1993). Rapid communication: polymerase chain reaction-restriction fragment length polymorphism analysis of the bovine somatotropin gen. *Anim. Genet.* 71:2276.
- Zhou GL, Jin HG, Liu C, Guo SL, Zhu Q, Hou, WY (2005) Associated of genetic polymorphism in GH gene with milk production traits in Beijing Holstein cows. *J. Biosci.* 30:595-598.