Association of a novel single nucleotide polymorphism in growth hormone receptor gene with production traits in Bali cattle

Maskur, Rodiah, Chairussyuhur Arman
Faculty of Animal Science, University of Mataram, Indonesia

Abstract

The growth hormone (GH) is the main regulator of postnatal growth and metabolism in mammals. The action of GH on target cells depends on the growth hormone receptor (GHR). This is mediated through induced transcription of other genes. GHR gene is one of the candidate genes employed in selection strategy using DNA markers (marker assisted selection). This study was designed to identify the novel single nucleotide polymorphisms (SNPs) in exon 8 and intron 8 of GHR gene that may affect production traits in Bali cattle. A SNP was identified by the direct sequencing technique. Genotypes of the SNPs were identified using PCR-RFLP. The SNP was located in intron 8 of the GHR gene and was caused by A/G transition. It was identified using the HpyCH4III restriction enzyme. Polymorphism of GHR/HpyCH4III has a significant influence on weaning weight and average daily gain, but not on birth weight of Bali cattle.

Introduction

Growth hormone (GH) is an anabolic hormone which promotes growth and reproduction of cells in humans and other animals. The growth hormone exerts its effects on growth and metabolism by interacting with a growth hormone receptor (GHR) on the surface of the target cells (Listrat et al., 2005). Mutation of GHR gene might affect its binding capacity and signaling pathway, thereby altering the GH activity in the target tissues (Di Stasio et al., 2005).

In cattle, GHR is encoded by a single gene located in chromosome 20 (Moisio et al., 1998). The gene coding for bovine GHR consists of nine exons (from 2 to 10) in the translated part and of a long 5’-noncoding region that includes nine untranslated exons - 1A, 1B, 1C, 1D, 1E, 1F, 1G, 1H, 1I (Jiang and Lucy, 2001).

Variation in the GHR gene sequence is associated with a number of performance traits in cattle (Viitala et al., 2006; Garrett et al., 2008). The single nucleotide polymorphism (SNP) in exon 8 has been shown to be associated with milk yield and composition (Blott et al., 2003; Viitala et al., 2006), as well as feed intake, feed conversion efficiency and body energy traits (Banos et al., 2008). The association between GHR gene polymorphisms with production and carcass trait has been reported in several cattle breeds. For example, SNP in 5’-noncoding regions have been reported to be associated with the rate of growth and carcass trait in some breed of beef cattle such as Limousin, Charolais and Hereford (Maj et al., 2004). Similarly, Ge et al. (2003) reported that a GHR gene polymorphism caused by A/G transitions in the promoter region was associated with the concentration of insulin like growth factor-1 (IGF-1) serum in Angus cattle. This study aims to discover novel single nucleotide polymorphisms (SNPs) in exon 8 and intron 8 of the GHR gene and to determine their effects on birth weight, weaning weight and average daily weight gain in Bali cattle.

Materials and methods

The University of Mataram, Faculty of Medicine, Ethical Committee for Medical Research, Mataram, Indonesia approved all animal procedures for this experiment (Register No. 4/UN18.8/ETIK/2013).

Phenotyping: measurement of the production traits

Two hundred and fifty Bali cattle (aged 1.0-1.5 years) for which phenotypic records were available with respect to body weight and body conformation, were reared at University Field Stations. All animals were ear-tagged. The average daily gain was measured weekly for a period of 3 months. The data on the birth weight and weaning weight of the calves at 6 months old were previously recorded and were used to complement the quantitative data.

Single nucleotide polymorphism discovery

Blood samples for DNA analysis were collected from the jugular vein of each animal. Blood was collected on K$_2$EDTA and stored at 25°C for few weeks or at 75°C up to several months. The isolation of DNA from whole blood of the 250 Bali cattle was performed by the method of Sambrook et al. (1989). Amplification was carried out following the method of Oikonomou et al. (2008). The PCR reaction contained 100 ng DNA, 0.5 μM of each primer, 1xPCR buffer (10 mM Tris-HCl pH 9.0), 1.5 mM MgCl$_2$ and 50 mM KCl, 5% deionized formamide, 200 μM dNTPs, and 0.025 UTaq DNA polymerase (Pharmacia) in a volume of 25 μL. The amplification was performed for 35 cycles using DNA thermal Cycler (Perkin Elmer Cetus Corp.). The first cycle was at 95°C for 5 min followed by 33 subsequent cycles of 94°C x 45 s, then 56°C x 45 s, then 72°C x 60 s and the last cycle at 72°C for 5 min. Sequence and position of the primer can be seen in Table 1. The identification of SNPs in the gene fragments was conducted by means of the direct sequencing method (Kwok and Duan, 2003). DNA samples (PCR products) were grouped in the form of a DNA Pool for each sub population of cattle. Sequencing of DNA fragments was carried out by the ABI3730xl sequencer machine. Sequence alignment was performed using the BioEdit (http://www.mbio.ncsu.edu/bioedit) and Mega4 programme (http://www.megasoftware.net/mega4). The alignments of reverse and forward sequences were applied to produce consensus sequences. The sequences of each individual DNA fragments were aligned with original sequences to identify the presence of SNPs.

Genotyping of the candidate gene

The determination of the genotype of each individual animal was conducted based on the SNPs that were found at the SNPs discovery stage. Genotyping was performed by the PCR-
RFLP techniques. PCR reaction used in this step is equal to PCR reaction used at the SNPs discovery stage. A specific restriction enzyme was identified by the webcutter (http://rna.ulundberg.gu.se/cutter2) and nebcuter programme (http://tools.neb.com/NEBcuter). The gene fragments containing the SNPs were amplified using PCR, then the gene fragments were digested using a specific restriction enzyme. The size and number of alleles were determined using agarose gel electrophoresis.

Statistical analysis
Genotyping and allele frequency within and among genetic groups were determined by the method of Goodman adapted by Curi et al. (2005). The association analysis was performed using General Linear Model (GLM) and the least square means of the genotypes were compared by t-test (as implemented in the SAS programme). The linear model used was as follows:

\[ Y_{ij} = A + G_i + e_{ij} \]

where, \( Y_{ij} \) = production trait, \( A \) = overall mean, \( G_i \) = fixed effect of the i genotype, and \( e_{ij} \) = random error.

Results and discussion
Single nucleotide polymorphism discovery in intron 8 - exon 8 of the GHR gene

The GHR SNP has been studied in various cattle breeds, including dairy and beef breeds. Blot et al. (2003) found 10 new SNPs in the GHR gene from the Friesian Holstein and Jersey breeds: one transition in 3’UTR, one indel and one transition in intron 2, one transversion in exon 8 and one in intron 8, one transition in intron 9, and four mutations in exon 10 (one transversion and three transitions). Waters et al. (2010) reported six new SNPs in 5’non coding region and 1 new SNP in intron 2 of GHR gene from the Friesian Holstein dairy cattle. The data reported in this paper add to these reports and extend the work to Bali cattle.

Allelic and genotypic frequencies of the GHR/HpyCH4III gene in Bali cattle

Allelic and genotypic identification of intron 8 GHR gene using the RFLP-HpyCH4III technique produces two alleles namely A and G allele with three genotypes AA, AG and GG (Figure 2). The distribution of allelic frequency of the A allele was slightly higher than G allele, respectively 0.532 and 0.468, while the frequency distribution of the genotypes AA, AG and GG were respectively: 0.156; 0.752 and 0.092 (Table 2). The Chi-square (\( \chi^2 \)) test showed that the genotype distributions of intron 8 GHR gene were not at Hardy-Weinberg equilibrium (H-WE) in Bali cattle. The genotype frequencies at polymorphic loci of intron 8 GHR gene showed a highly significant difference (\( P<0.01 \)). This contrasts with the exon 10 GHR gene in Polish Holstein Friesian cattle, as reported by Olenski et al. (2010) where the allelic frequencies distributions between the A and G allele differed, the G allele (0.832) being higher than that of the A allele (0.168).

Data in Table 3 show the results of genetic index measurements in Bali cattle population. These data indicate a genotypic imbalance in the population where genotype heterozygote frequencies are higher than the Hardy-Weinberg expectation. This could be due to intensive selection, resulting in a tendency...

### Table 1. Sequence and position of the primer.

| Gene | DNA sequence       | Position of the primer |
|------|-------------------|------------------------|
| GHR  | F : 5’-GGCTCTGCAGTGAGCTATT-3’ | Exon 8 and Intron 8 |
|      | R : 5’-ACCTCTGGGTCTGGATTTA-3’ | 170271 bp – 170611 bp |

### Table 2. Allele and genotype frequencies of GHR/HpyCH4III gene in Bali cattle.

| Gene | N     | Allele frequencies | Genotype frequencies | \( \chi^2 \) (H-WE) |
|------|-------|--------------------|----------------------|---------------------|
|      | 250   | 0.468              | 0.532                | 0.092               | 0.752 | 0.156 | 65.072** |

H-WE, Hardy-Weinberg equilibrium. The number of individuals for the GG, AG and AA genotypes are given in Table 4. **P<0.01.

![Figure 1. Sequence alignment of exon 8 and part of intron 8 GHR gene.](Image)
towards the accumulation of certain genotypes (Tambasco et al., 2003) and the possibility of inbreeding (Machado et al., 2003).

The effective population size (Ne) (Table 3) illustrates that the GHR gene alleles in the population have very different frequencies in which one allele is dominant allele frequencies. Based on the polymorphic information content (PIC) value of 0.37, it can be stated that the genetic diversity of GHR gene within Bali cattle population is at the medium level. This statement is based on PIC levels of polymorphism as determined by Botstein et al. (1980) in which levels of ≤0.25 are classified as low, 0.25 ≤ PIC ≤ 0.5 are classified as medium and PIC ≥ 0.5 are classified as high polymorphism. Zulkharnaim et al. (2010) also reported that Bali cattle have a low genetic diversity at GHR/Au1 loci in exon 10 of GHR gene.

**Association of GHR gene polymorphism with production traits**

The association between the GHR gene genotype and birth weight, weaning weight and average daily gain in Bali cattle can be seen in Table 4. The effect of genotype of GHR gene was found to be significant (P<0.01) for weaning weight and average daily gain (ADG) and not significant for birth weight (P>0.05) in Bali cattle. One explanation could be that GHR is a mediator of GH biological activity in target cells through stimulating myogenic signal transduction, while GH is a regulator of growth and metabolism after birth.

The role of GH on target cells mainly depends on the expression of GHR. GH secretion, binding to the receptor and the expression of GHR at the beginning of growth is an important factor in determining the optimal metabolic function in muscle (Katsumata et al., 2000). The action of GH precedes the formation of a bond with the transmembrane GHR which is present on the surface of most cells and encourages the activation of GHR. Furthermore, the expression of GHR stimulates the expression of an IGF-1 which is a mediator of GHR activity on the target cell (Locatelli and Bianchi, 2014).

The association between weaning weight, ADG and birth weight in Bali cattle and the GHR gene polymorphisms due to a mutation in intron 8 region needs to be explained. Mutations in intron regions may not lead to changes in the protein sequence of the GHR gene to produce a protein with an unchanged amino acid sequence but it could nonetheless result in a change in structural and functional properties (Komar, 2007). Mutations in intron 8 regions of GHR gene in Bali cattle may also lead to changes in the amino acid sequence of protein if it occurs at the initial site of the mRNA splicing after transcription. In conclusion this study shows that the AG SNP in intron 8 of the GHR gene in Bali cattle significantly affects production traits, in particular postnatal growth. It remains to be seen if the findings we report also apply to other breeds of cattle.

**Conclusions**

The SNP analysis of intron 8 of GHR gene reveals AG transition in the base position of 241 bp. The polymorphisms of nucleotide sequence caused by these mutations were identified using the restriction enzyme *HpyCH4III*. The AG transition resulted in three genotypes SNP of GG, AG and AA with a frequency of 0.092, 0.752 and 0.156 respectively and two alleles of A and G with almost equal frequency distribution of 0.532 and 0.468. Genotypic polymorphism of GHR/*HpyCH4III* has a significant influence on weaning weight and average daily gain, but no significant influence on birth weight of Bali cattle. GG genotype had a weaning weight and average daily gain of 287 kg/day respectively, higher than the AG and AA genotypes.

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