Detection of growth hormone (GH|MspI,GHR|AluI, Pit1|HinfI) genes polymorphism and its association with body weight of Grati-Bali Cattle (Bos sondaicus)

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Abstract. Bali cattle is the only native Indonesian cattle that still exist and well developed. Bali cattle are known to have adaptability and a higher percentage of carcasses than other local cattle. Molecular selection related to growth traits can be done by exploration of growth hormone genes in the population of Bali cattle. This study aims to detect the level of polymorphism of GH, GHR and Pit1 genes and their association with the body weight of Grati-Bali cattle as a first step to produce a MAS (marker assisted selection). A total of 171 DNA samples Grati-Bali cattle were collected from the Beef Cattle Research Station experiment cages and its body weight data of 2014-2018 include birth weight (BW), weaning weight (WW) and yearling weight (YW). Blood samples were isolated using a zymo extraction kit. Detection of the diversity of growth hormone genes (GH, GHR and Pit1) using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method with MspI, AluI, HinfI restriction enzymes. Data analysis used Chi-Square test for genotype, allele frequencies and Hardy-Weinberg Equilibrium (HWE). Association analysis used the GLM model. The results showed that the average BW, WW and YW of Grati- Bali cattle in the study were 13.3±0.3 kg, 73.7±2.4 kg and 121.3±4.2 kg, respectively and there was no statistical difference in body weight for male and female calves. The analysis showed that GH and Pit1 genes were detected by 1 genotypes each with an allele frequency > 99% and monomorphic. GHR gene was detected 3 genotypes namely AA, AG, GG, with allele frequencies A were 0.579 and G were 0.421. The GHR gene PIC value was in the moderate category. It was concluded that the GHR|AluI gene in Bali cattle is polymorphic but has not been significantly associated with the body weight of Grati-Bali cattle.

1. Introduction
Bali cattle is the only indigenous cattle from Indonesia which still exist and thrive in tropical Indonesia. DGLS [1] reports that Bali cattle is the only cattle in the world whose ancestors (Banteng) are still alive today and Bali cattle have been included in the FAO list of cattle breeds in the world. Based on its history, Bali cattle were formed from the domestication of wild Banteng which occurred thousands of years ago [2]. Bali cattle was known to have high adaptability and able to survive and produce in areas with low feed quality. The adaptability of Bali cattle causes it to be widely distributed and developed outside of its purification areas such as Sumatra, Java, Kalimantan and Sulawesi, so that their productivity performance is also very diverse. The performance of livestock production is largely...
determined by the influence of genetics, the environment and their interactions [3]. One of the economic
erformance of livestock is growth trait. This trait is controlled by many genes. Some of the main genes
related to growth traits that were widely used in the study of candidate genes and associations in beef
cattle are the GH, GHR and Pit-1 genes.

The GH gene is a protein hormone that synthesized and secreted by the pituitary gland [4]. The GH
gene is needed for tissue growth, fat metabolism, reproduction regulation, lactation, normal body growth
[5]. The gene coding for GH in cows was found on chromosome 19 at the q26-qtr position [6]. Growth
hormone receptors (GHR) act as cell surface receptors that help mediate the effects of GH on somatic
cell growth. GH affects growth and metabolism by interacting with a specific receptor called growth
hormone receptor (GHR) on the surface of target cells so that GHR affects the biological action of GH
on target cells [7]. The GHR gene was located on chromosome 20 [8]. Pit-1 is a specific factor for
pituitary cells that binds to elements required for GH and prolactin (PRL) expression [9, 10]. The Pit 1
gene in cattle was located at the centromere of chromosome 1 [10]. This gene was generally found in
polymorphic conditions in several Bos taurus and Bos indicus cattle [11].

Regarding the function of these three candidate genes, several research results have recommended
that these three genes can be used as part of strong candidate genetic markers for growth traits in cattle.
Polymorphic GH gene in SO cattle [12], PO cattle [12-14], Madura cattle [15] was also polymorphic in
Pesisir cattle [12,16] but in Bali cattle are monomorphic [12]. Analysis of the diversity of the GH gene
was also reported as polymorphic in PO cattle [13], Pasundan cattle [17] and Pesisir cattle but was
monomorphic in Bali cattle [18]. The Pit-1 gene was reported to have low genetic diversity in
populations of Bali, Madura, Pesisir, Aceh and Katingan cattle [19] as well as PO Grati cattle [20] and
Pasundan cattle [21].

Studies on the genetic diversity of GH, GHR and Pit1 genes in the same population have not been
widely reported. This study aims to detect the polymorphisms of GH, GHR and Pit1 genes and their
association with body weight in the Grati-Bali cattle population. The use of these three candidate genes
was carried out on the basis that in general economical quantitative traits were influenced by many genes
(polygens) and as an anticipation when there was one gene that did not affect growth traits in Bali cattle,
it could be observed for other genes. In addition, the interaction of the three genes can be observed more
deeply because in their biological function the three candidate genes were interrelated.

2. Materials and methods

2.1. Performance data of Grati-Bali cattle
Grati-Bali cattle performance data were collected from 171 calves in the experimental cage at the Beef
Cattle Research Station including birth, weaning and yearling weight. Grati-Bali cattle blood samples
were collected from the jugular vein using a vaccutainer tube containing K3 EDTA. DNA was extracted
using a zymo extraction kit and then stored at -20°C for further use.

2.2. PCR-RFLP analysis
The method used to detect DNA variations was PCR-RFLP. The primers used for the GH gene refers to
Sutarno et al (2005), GHR refers to Di Stasio et al (2005) and Pit-1 refers to Moody et al (1995)
[7,10,14]. The primary sequences of forward and reverse were shown in table 1. The PCR reagent
composition consisted of PCR kit MyTaq (MyTaq, Bioline), forward and reverse primers (200 ng/μL),
DNA samples (5–50 ng/μL) and ddH2O to a final volume of 20 μL. PCR was performed using a
Thermocycler (AB System). The 3 gen PCR program was set at temperature: pre denaturation 95°C for
1 minute; 35 cycles for denaturation of 95°C for 15 seconds, 53.8 each; 65.7 and 54.6 (for genes GHR,
GH and Pit-1) for 15 seconds, extension at 72°C for 10 seconds and final extension at 72°C for 5 minutes.

The restriction enzymes used to detect variations in the GH, GHR and Pit1 genes were Mspl, Alul and
HinfI. The PCR and RFLP products were visualized by 2% agarose gel and followed the GelRed™
Nucleic Acid Gel Stain and visualized on geldoc (Infinity VX2).
Table 1. Accession number of genbank, length, location and primary sequences of 3 growth hormone genes in Grati-Bali cattle

| Gene | Assesion number of GenBank | Length (pb) | Location | Primary Sequences |
|------|---------------------------|-------------|----------|-------------------|
| GH-L2 | JQ711182.1                | 327         | Exon 3 and 4 | F: 5’-CCCACGGGCAAGAATGAGGC-3’  
          |                       |             |          | R: 5’-TGAGGAACGTGCAGGGCACA-3’  |
| GHR   | AF140284.1                | 342         | Exon 10  | F: 5’-GCT AAC TTC ATC GTG GAC AAC-3’  
          |                       |             |          | R: 5’-CTG GAT TTT GAT CAG CAG-3’  |
| Pit-1 | Y15995.1                  | 1301        | Exon 5 and 6 | F: 5’-CAATGAGAAAGTTTGCTGC-3’  
          |                       |             |          | R: 5’-TCTGCATTGCAGATGCTC-3’  |

2.3. Data Analysis

2.3.1. Allele frequency. Individual genotypes were determined based on differences in the number and size of RFLP bands. The genotype and allele frequencies were calculated according to the formula Nei and Kumar [22] with the following statistical model formula:

\[
X_i = \frac{(2N_{ii} + \sum_{j 
eq i} N_{ij})}{2N} 
\]

\(N_{ij}\) = Number of individual with ii genotype  
\(N_{ij}\) = Number of individual with ij genotype  
\(N\) = Number of sample

2.3.2. H-W Equilibrium. Hardy-Weinberg (H-W) equilibrium was calculated according to the formula Kaps & Lamberson (2004) with the following model formula [23]:

\[
X^2 = \sum \frac{(o-e)^2}{e} 
\]

\(X^2\) = Chi-square test  
\(o\) = Number of observed in category i  
\(e\) = Number of expected in category i

2.3.3. Heterozigosity. Data of the heterozigosity in the Grati-Bali cattle were calculated according to formula Weir (1991) and the Polymorphism Information Content (PIC) value of GH, GHR and Pit1 gene were calculated according to formula [24,25].

2.3.4. Statistical Analysis. Analysis of variance on the genotype data of GH, GHR and Pit1 gene with the birth weight, weaning weight and yearling weight were performed by general linear model (GLM) model by IBM SPSS ver 20.0 software.

3. Results and discussion

3.1. GH, GHR and Pit1 gene polymorphisms
The genotype and allele frequencies and heterozygosity of GH, GHR and Pit1 genes in Grati-Bali cattle are shown in table 2.
Table 2. The genotype and allele frequencies and heterozygosity of GH, GHR and Pit1 genes

| Gene | Sex | n   | Genotype Frequencies | Allele Frequencies | Heterozygosity |
|------|-----|-----|----------------------|--------------------|----------------|
|      |     |     |                      |                    |χ²test | He  | Ho  | PIC | Ne  |
| GH   | M   | 41  | 0.000                | 0.000              | 1.000 | 0.000 | 0.000 | 1.000 |
|      | F   | 130 | 0.000                | 0.000              | 1.000 | 0.000 | 0.000 | 1.000 |
|      | Total | 171 | 0.000                | 0.000              | 1.000 | 0.000 | 0.000 | 1.000 |
| GHR  | M   | 41  | 0.390                | 0.610              | 0.000 | 0.695 | 0.305 | 7.887 | 0.424 | 0.429 | 0.334 | 1.736 |
|      | F   | 130 | 0.092                | 0.900              | 0.008 | 0.452 | 0.458 | 85.920 | 0.496 | 0.498 | 0.373 | 1.986 |
|      | Total | 171 | 0.164                | 0.830              | 0.006 | 0.579 | 0.421 | 84.578 | 0.488 | 0.489 | 0.369 | 1.951 |
| Pit1 | M   | 41  | 0.000                | 0.000              | 1.000 | 0.000 | 0.000 | 1.000 |
|      | F   | 130 | 0.000                | 0.000              | 1.000 | 0.000 | 0.000 | 1.000 |
|      | Total | 171 | 0.000                | 0.000              | 1.000 | 0.000 | 0.000 | 1.000 |

\( n = \) number of samples; \(Ho = \) heterozygosity of observations; \(He = \) Heterozygosity of expectations; \(Ne = \) number of effective alleles; \(PIC = \) Polymorphism Information Content ; if \(\chi^2_{\text{test}} < \chi^2_{\text{tab}} (\alpha=0.05)\) means the genotype frequency is in HWE

Based on table 2. The result of analysis showed that the GH|MspI gene in Grati-Bali cattle was monomorphic. Several research results related to the diversity of GH genes in Bali cattle used more AluI enzymes than MspI. Agung et al (2017) reported that the Bali cattle population in Nusa Penida using the GH|AluI gene was also monomorphic with the allele frequency A = 1.00 and the allele B = 0.00 [12]. The same thing was also found in the GH|AluI gene in Bali cattle in Bali island [26] and Bali cattle on the South Pesisir [27] with allele frequencies L = 1.00 and V = 0.00, respectively. The diversity of growth genes in several other local cattle has also been reported by Hartati and Soewandi (2020) that the GH|MspI gene in Grati-Madura cattle is polymorphic with the genotype frequency \( MspI^{+/-} = 0.00, MspI^{+/-} = 0.16\) and \( MspI^{-/-} = 0.884\) and the allele frequency of \( MspI^{+} = 0.058\) and \( MspI^{-} = 0.942\) [15]. In Grati-PO cattle, the GH|MspI gene was also reported as polymorphic with the genotype frequency \( MspI^{+/-} = 0.011, MspI^{+/-} = 0.188\) and \( MspI^{-/-} = 0.801\) while the allele frequency \( MspI^{+} = 0.105\) and \( MspI^{-} = 0.895\) [13]. The PO Crossbred cattle were also reported to be polymorphic with genotype AA = 0.375, AB = 0.625 and BB = 0.000 and allele frequencies A = 0.714 and B = 0.286 [28]. In Pesisir cattle, 3 genotypes were also detected, namely \( MspI^{+/-} = 0.333, MspI^{+/-} = 0.400\) and \( MspI^{+/-} = 0.267\) with the allele frequency \( MspI^{+} = 0.533\) and \( MspI^{-} = 0.467\) [29].

The results of statistical analysis of the Pit1|HinfI gene in Bali Grati cattle had 3 genotypes, namely AA, AB and BB with a frequency of 0.00, 0.00 and 1.00 respectively and the allele frequency of A = 0.00 and allele B = 1.00. Thus, the Pit1 gene in Bali Grati cattle is monomorphic. The same result was also reported by Jakaria and Noor (2015) that the genetic diversity of the Pit1|HinfI gene in Bali cattle was very low with the allele frequencies A = 0.018 and B = 0.982, while the Ho and He values were 0.037 and 0.036, respectively [19]. The diversity of the Pit1|HinfI gene in several other local cattle was also reported to have low diversity such as PO Grati [20], Madura, Pesisir Aceh and Katingan cattle [19] and Pasundan cattle [30].

The result of GHR gene polymorphism analysis in Bali Grati cattle detected 3 genotypes, namely AA, AG and GG with genotype frequencies 0.164, 0.830 and 0.006, respectively, while the A and G allele frequencies were 0.579 and 0.421, respectively. Based on the results of this analysis, the GHR|AluI gene in Bali Grati cattle is polymorphic and has the potential to be associated with growth traits in Grati-Bali cattle. This is based on the opinion of Nei (1975) and Allendorf and Luikart (20070 that a locus to be polymorphic if the frequency of the most common allele is less than 0.99 (99%) while monomorphic
if only one allele is known at a locus or the most common allele which has a high frequency (>99%) [31,32]. The results of this study are different from those reported by Zulkhairnaim et al (2010) that the GHR|AluI gene in Bali cattle from P3Bali and Bali BIBD were monomorphic with genotype AA, AG and GG frequencies of 0.988, 0.006 and 0.006 respectively and allele frequencies A = 0.991 and B = 0.009 [18]. Research related to the diversity of the GHR|AluI gene has also been reported in Pasundan cattle and it was detected to be polymorphic with three types of genotypes, namely: AA (0.412); AG (0.429) and GG (0.160). The allele frequencies of A and G were 0.626 and 0.374, respectively [17].

The results of statistical analysis showed that the GHR gene was not in the Hardy-Weinberg Equilibrium, this explains that the genotype and allele frequencies of GHR genes in the Grati-Bali cattle population are not constant. Mating managements and limited population numbers cause change of gene frequencies. Factors that can change the frequency of genes in a population are selection, mutation, migration, genetic drift and evolution [33,34].

3.2. Heterozygosity

The value of genetic diversity within a breed is expressed as mean heterozygosity. The genetic diversity of a gene can be evaluated by looking at other indicators of genetic diversity such as heterozygosity of observation (Ho), heterozygosity of expectation (He), number of effective allele (Ne) dan Polymorphism Information Content (PIC). Based on table 2 it can be seen that Ho and He in the GH and Pit1 genes have a value of 0.000, as well as the PIC value of 0.000, this shows that the genetic variation in Grati-Bali cattle is very low. The number of effective alleles (Ne) in the GH and Pit1 genes also shows a value of 1.000, this means that only one allele is effectively present and has a high frequency. Russel (2010) stated that the decreasing heterozygosity value can cause the loss of genetic variation in a population and even get a fixed allele[35]. The heterozygosity value is highly dependent on the number of samples, the number of alleles and the frequency of alleles [31].

The results of the GHR gene diversity analysis in Grati-Bali cattle showed that the Ho and He values were 0.489 and 0.488 respectively, while the PIC value was 0.369. It was shown that the genetic variation of the GHR gene in Grati Bali cattle is very high. Determination of the informative level of a marker can be seen from the PIC value with three categories, namely low (<0.25), moderate (0.25 <PIC <0.5), and high (≥0.5) [25]. Based on this classification, the GH|MspI and Pit1|HinfI genes were in the very low category while the GHR genes were in a moderate category. A very low of PIC value indicates that the markers on GH|MspI and Pit1|HinfI are not informative so that they cannot be associated with certain traits in Grati-Bali cattle population, meanwhile the GHR gene is very informative and can be associated with growth traits in Grati-Bali cattle.

3.3. Association of GHR |AluI genes with body weight of Grati-Bali cattle

The descriptive analysis results of Grati-Bali cattle body weight performance are presented in table 3 below:

| Sex    | N  | BW±SE | WW±SE | YW±SE |
|--------|----|-------|-------|-------|
| Male   | 26 | 13.0±0.3 | 73.9±3.3 | 121.4±5.9 |
| Female | 25 | 13.5±0.5 | 73.5±3.5 | 121.1±5.9 |
| Total  | 51 | 13.3±0.3 | 73.7±2.4 | 121.3±4.2 |

n = number of samples; BW = birth weight; WW = weaning weight; YW = yearling weight; SE = standard error

Based on table 3, it can be seen that the average birth weight, weaning weight and yearling weight of Bali cattle are 13.3±0.3 kg, 73.7±2.4 kg and 121.3±4.2 kg, respectively. The results of statistical analysis showed that sex had no significant effect (P> 0.05) on birth weight, weaning weight and yearling weight of Grati-Bali cattle. Birth weight, weaning weight and yearling weight in this study are lower than Bali
cattle in BPTU HPT Denpasar, namely 17.8±1.08 kg, 88.59±16.15 kg and 131.12±25.50 kg [36] as well as the results of research [37] at the location which is the same as birth and weaning weight 17.91±1.26 kg and 85.06±16.55 kg respectively, but yearling weight is lower than Gati-Bali cattle, namely 117.56±19.40. The difference of mean value can be caused by differences in data and time of observation. Furthermore, it may also be due to differences in feed and maintenance management [38,39].

The analysis results of the genotype association of the GHR|AluI gene with birth weight, weaning weight and yearling weight for Grati-Bali cattle are presented in Table 4 below:

**Table 4. Association analysis of GHR|AluI genotype with body weight of Grati-Bali cattle**

| Genotype | n | BW±SE | n | WW±SE | n | YW±SE |
|----------|---|-------|---|-------|---|-------|
| Male (M) |   |       |   |       |   |       |
| AA       | 13| 12.9±0.6 | 13| 74.3±4.9 | 13| 122.3±8.4 |
| AG       | 13| 13.0±0.6 | 13| 73.4±4.9 | 13| 120.6±8.4 |
| GG       | 0 | 0     | 0 | 0     | 0 | 0     |
| Total    | 26| 13.0±0.3 | 26| 73.9±3.3 | 26| 121.4±5.9 |
| Female (F) |   |       |   |       |   |       |
| AA       | 10| 12.8±0.7 | 10| 76.5±5.5 | 10| 126.2±9.6 |
| AG       | 14| 14.1±0.6 | 14| 70.1±4.7 | 14| 115.4±8.1 |
| GG       | 1 | 13.0±2.0 | 1 | 89.9±17.5 | 1 | 150.7±30.3 |
| Total    | 25| 13.5±0.5 | 25| 73.5±3.5 | 25| 121.1±5.9 |
| M + F    |   |       |   |       |   |       |
| AA       | 23| 12.9±0.2 | 23| 75.3±3.8 | 23| 124.0±6.8 |
| AG       | 27| 13.6±0.5 | 27| 71.7±3.1 | 27| 117.9±5.1 |
| GG       | 1 | 13.00   | 1 | 89.9     | 1 | 150.7   |
| Total    | 51| 13.3±0.3 | 51| 73.7±2.4 | 51| 121.3±4.2 |

n = number of samples; SE = standard error; BW = birth weight; WW = weaning weight; YW = yearling weight; M = male; F = female

Based on table 4, it can be seen that the genotype of the GHR|AluI gene is not significantly associated with birth weight, weaning weight and yearling weight in Grati-Bali cattle. This result is different from the study reported by Maskur et al (2014) that the GHR gene with restriction enzyme HpyCH4III was significantly associated with weaning weight and daily gain but not significant on birth weight of Mataram-Bali cattle [40]. This difference is probably due to differences in the number of samples used so that the diversity is also different. The body weight data used in the association analysis in this study were only 51 individuals so that the diversity was low. Table 4 showed that GG genotype in the female population tends to have a weaning and yearling weight higher than AA and AG genotypes, this is in line with the results reported by Maskur et al (2014) that GG genotype had the highest weaning weight and daily gain compared to AA and AG (82.1±10.31 kg and 0.47±0.08 kg) [40]. Polymorphisms and associations of GHR|AluI genes in Bali cattle have not been widely reported, however, GHR|AluI gene polymorphisms in several other local cattle have been reported by Said et al (2016) that the GHR gene was significantly associated with weaning weight and daily gain in Pasundan cattle and Putra et al GHR gene has an association with body length, withers height and body weight in Pasundan cattle [41,42].
4. Conclusion
The polymorphism detection of GH and Pit1 genes in Grati-Bali cattle population is monomorphic, while GHR gene is polymorphic and it can be used as growth gene candidates for Grati-Bali cattle. The analysis of GHR gene association on body weight of Grati-Bali cattle was found to be insignificant. Detection of GHR gene polymorphisms in Grati-Bali cattle population needs to be continued to the validation stage in a larger number of samples, so that it has a potential as marker assisted selection (MAS).

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