Polymorphisms of the Myostatin gene (MSTN) and its association with growth traits in Bali cattle

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ABSTRACT
This research aims to develop a method for selection based on myostatin gene markers. The experiment was conducted in Bali cattle. Blood samples were collected from 100 cattle irrespective of age. PCR-RFLP was applied with primers for amplifying the 1346bp of the Myostatin gene were (F) 5' -CCCTACAGAGGCCACTTCAA-3' and (R) 5' -CTCGCTGTTCTCA TTCAGA TC-3', and HaeIII restriction enzymes. PCR-RFLP results showed that not all genotypes present in the population, despite populations that were observed quite polymorphic (PIC = 49.5%) with the conditions of gene frequencies. The results showed that almost all variables influenced by myostatin genotype where the genotype BB is better, except the chest girth showed a better result in genotype AB. It suggested that MSTN is strong candidate gene that influenced growth traits. Moreover, it is also expected that this MSTN could be used in marker-assisted selection.

Key words: Bali cattle, Growth traits, Myostatin gene, Polymorphism, PCR-RFLP.

INTRODUCTION
The uncertain direction of beef cattle breeding program led to the failure in fulfilling meat demand in Indonesia. National cattle breeding still depends on conventional methods that were conducted by the local ranch. It is also caused by crossbreeding between local cattle and imported cattle (Limousin, Simmental, Angus etc) which resulted in weight increase compare to local beef cattle. It encourages the farmer to do crossbreeding because it has more benefits such as heavier weight, faster growth, easier adaptation to environment and simpler animal feed. Increasing weight and size, and exotic appearance will have better selling price. Meanwhile, the previous study showed that crossbreeding adversely affects reproduction performance, such as decreases conception Rate (CR) and increases services per conception (S/C) (Putro, 2009; Busono and Nugroho (2012). It is supported by another study that demonstrated similar result in Bali cattle performance and its breeds with Simmental (Ashari et al., 2012). The reproduction performance of crossbred cattle will cause loss of benefits because of the threat of extinction of indigenous cattle.

The Bali breed is among one of the four existing indigenous cattle breeds (Aceh, Pesisir, Madura and Bali) in Indonesia. Some studies suggested that there is a loss of quality of Bali cattle genes shown by the low average adult weight. The average weight of male Bali cattle in 1990 was 350 kg and fell off into 278.3 kg in 2007, there was 4.5 kg/cattle/year weight loss or 1.28% from adult weight (Arman et al., 2007). As an indigenous cattle breed, Bali cattle is more readily adapted to harsh environments compared to imported ones. It was proved by its high fertility and birth rate. Thus, Bali cattle ability to produce meat should be optimized to give a contribution towards beef stock in national beef cattle demand.

Based on the actual situation, the performance of local cattle breed is not suitable for its body size. It means that there is a loss in the quality of local cattle breeds that needs continuous breeding improvement in breeds sources. The same thing happened in Indonesia, Bali cattle actually has good potential to meet beef demand but it seems rather difficult to refer to the current Bali cattle performance. Programs that may be applied to improve Bali cattle performance are: (1) production and reproduction recording program, (2) selection program.

Recently, there is an accurate and fast selection based on the genetic marker. Previous studies showed that polymorphism of GH gene is associated with dairy cattle weight. In one study, the allele frequency of a normal allele (Msp') is 34% and mutated allele (Msp1') is 66%, respectively, with 22% in degree of polymorphism. It also demonstrated that genotype Msp1' Msp1' and Msp1' Msp1' gave positive effects on cattle weight compared with genotype Msp1' Msp1 (Maylinda, 2011) Other study stated that GH1 polymorphism associated with the ability to digest low-quality animal feed in Ongole breed cattle and Holstein Friesian breed cattle and the interaction between ordo and genotype affect dry matter digestibility.
In response to the above, a method is developed to select Bali cattle breed using a genetic marker with superior performance in specific desirable traits. Our approach is based on marker-assisted selection using myostatin gene and growth hormone (GH) gene.

**MATERIALS AND METHODS**

**Animals and data collection:** A total of ninety cattle was divided into two groups based on their age (pre-weaning and post-weaning). The cattle were obtained from village breeding center (Pulukan, Jembrana, Bali). The research was conducted in two steps. The first step involved collecting phenotype data or cattle performance from a field experiment. Growth traits measured in the value-based care (VBC) population were: sex, age, birth weight, weaning weight chest girth, cattle height, wither height. The second step involved isolation of DNA to separate it from the nucleus and PCR-restriction fragment length polymorphism (RFLP)-based analysis.

**Samples and DNA preparation:** Genomic DNA samples were obtained from ninety Bali cattle and divided into two groups based on their age (pre-weaning and post-weaning). Genomic DNA was extracted from all blood samples and was isolated for the further protocol. A total of 5 ml jugular venous blood samples from each cattle were collected using venoject and then preserved in vacutainer tubes which have been given EDTA. DNA isolation was done based on the standard protocol of isolation from a blood sample.

**PCR amplification:** According to the NCBI database of the myostatin gene, one primer pair forward (5’-CCCTACAGAGGCCACTTCAA-3’) and reverse (5’-CTCGCTGTCTCTCAGATC-3’) were designed to amplify 1.346-bp fragments from myostatin gene (Zhang et al., 2007). PCR amplification was performed in 25 μl reactions containing 100 ng of genomic DNA, 1.5 mM MgCl₂, 200 μM each of the four dNTP, 5 pmol of each primer and 1 U of Taq DNA polymerase under the following conditions: one cycle 94°C 3 min; 39 three-step cycles 94°C 3 s, 63°C annealing temperature for 30 s; and 72°C for 1 min followed by last extension for 10 min at 72°C.

**PCR-RFLPs analysis:** RFLP was carried out to recognize wherever fragment mutation happens using HaeIII restriction enzyme (Zhang et al., 2007). HaeIII enzyme is a restriction enzyme with recognition site sequence:

\[ \text{HaeIII} \quad \text{GG} \uparrow \text{CC} \quad \text{(theLabRat.com, 2005)} \]

The PCR product was stored at 4°C and was detected by 2% agarose gel electrophoresis.

**Statistical analysis:** Based on DNA marker pattern, degree of polymorphism was tested using formula as follow:

\[ \text{PiCi} = 1 - \Sigma p_{2ij} \quad \text{(Budak et al., 2003)}; \]

where PiCi is polymorphic information content for ith locus and pij is the frequency of the jth allele for ith locus. Chi-square test was used to determine whether the alleles are in Hardy-Weinberg equilibrium or not. After getting the result of allele frequency and genotype, data were analyzed using Minitab Release 13.2 Software.

**RESULTS AND DISCUSSION**

Bali cattle performances or growth traits were shown by birth weight average, weaning weight, chest girth, length, and height. Meanwhile, genetic diversity in population was shown by a polymorphism in the myostatin gene. The result from PCR-RFLP revealed that not all genotypes found in the population are polymorphic.

The average value of different growth performance traits of Bali cattle is shown in Table 1. It indicates that the effect of sex was strongly significant in all variables except birth weight. Essien and Adesope (2003) reported that sex has a great effect on the size of N’Dama calf in Southwest Nigeria. Growth performance was affected by gender (males vs females) (Daza et al., 2014). There was a significant relationship between sex and body weight of Ankole and Friesian calves (Lagu et al., 2012). The sex of the animal had an obvious effect on Fatty Acid composition in the intramuscular fat from Qinchuan cattle (Zhang, 2010).

**Polymorphism of myostatin genes:** The result of PCR-RFLP showed myostatin gene fragment from several samples. We found the polymorphism of myostatin gene (MSTN) (figure 1, Table 2).

The genotype frequencies of MSTN in Bali population agreed with Hardy-Weinberg equilibrium. The result from polymorphism analysis showed that myostatin gene is quite polymorphic (Table 3). Meanwhile, Chi-Square test showed that the result was not statistically significant. It meant that the population was still in balance condition according to Hardy-Weinberg equilibrium. In contrary, it was different with the research conducted by Jakarta and Noor (2011). Their result showed that the growth hormone gene in Bali Cattle BPTU only LL which get all genotypes while most of Bali Cattle in Lombok island get genotype LV. It is assumed that the population used in the study were calves that probably bred from previously observed cows.

### Table 1. Average of birth weight, weaning weight, chest girth, length, and height

<table>
<thead>
<tr>
<th>Sex</th>
<th>N</th>
<th>Birth weight (kg)</th>
<th>Weaning weight (kg)</th>
<th>Chest girth (cm)</th>
<th>Body length (cm)</th>
<th>Height (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>45</td>
<td>18.11 ± 1.51</td>
<td>93.22 ± 19.11 ²</td>
<td>102.80 ± 13.42 ³</td>
<td>85.17 ± 5.17 ²</td>
<td>96.67 ± 11.44 ³</td>
</tr>
<tr>
<td>F</td>
<td>45</td>
<td>17.96 ± 1.13</td>
<td>81.35 ± 17.58 ³</td>
<td>96.67 ± 11.44 ³</td>
<td>89.0 ± 5.34 ²</td>
<td>91.4 ± 4.42 ³</td>
</tr>
</tbody>
</table>

Note: N: amount of the cattle, different superscript in same column indicated highly significant (P < 0.01)
Table 2: Allele and genotype frequencies at MSTN genes Bali cattle breed

<table>
<thead>
<tr>
<th>Total genotype</th>
<th>Genotype frequency</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB = 80; BB = 9</td>
<td>Freq. (AA) = 0, freq. (AB) = 89.9 %, freq. BB = 10.11 %</td>
<td>Frequency (A) = 0.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Frequency (B) = 0.45</td>
</tr>
</tbody>
</table>

The present study revealed that the population is still quite polymorphic for myostatin (MSTN) gene although genotypes incomplete. Similarly, the balance of gene frequency according to Hardy-Weinberg equilibrium is adequately met. Myostatin gene that has a stronger effect is the mutant allele (recessive), thus, homozygous recessive (BB) genotype has a better effect. However, the heterozygous genotype in weaning chest girth has more influence. It is suggested that this MSTN gene could be used for marker-assisted selection in beef cattle breeding. These studies will be of practical importance for the improvement of local cattle and the breeding of indigenous beef cattle in Indonesia.

REFERENCES


