Identification of Growth Hormone Gene of Bali Cattle with Qualitative Superior in Bali Province

Ni Ketut Suwiti[1*], I Wayan Suardana[1], Ni Luh Watiniasih[2], I Nengah Kerta Besung[1], Chandra Yowani[2]

[1]The Faculty of Veterinary Medicine, Udayana University
[2]The Faculty of Mathematics and Basic Science, Udayana University
*Corresponding author: nk_suwiti@unud.ac.id

Abstract. A research has conducted, in order to identify the genetic markers of Bali cattle that can be used as an indicator of their qualitative superior. The genetic marker identified was the growth hormone gen. As many as 50 whole blood samples which were collected from 50 individual cattle which were certificated as good breed or had qualitative superior, were used in this study. The DNA fragments were identified by using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method with primers, GH5 (5'-CCC ACG GGC AAG AAT GAG GC-3' and GH6 (5'TGA GGA ACT GCA GGG GCC CA-3), respectively. Furthermore, the PCR products were restricted with MspI endonucleic restriction enzyme, before electrophoresis in 2% agarose The result showed that the growth hormone gene of bali cattle with qualitative superior has polymorphism which characterized by the formation of two fragments i.e. 230 and 329 bp that categorized as: allele +/+ and allele -/-.

Keywords: **Bali cattle, growth hormone gene, PCR-RFLP, qualitative superior**

I. INTRODUCTION

Bali cattle (Bos javanicus) are indigenous of Indonesia, and have a significant economic importance to Indonesia due to their high fertility, adaptability to various environmental conditions and suitable to smallholder management systems [1][2]. Bali cattle are one of important local beef cattle breeds that contribute to the development of livestock and devote meat production in Indonesia [3][4]. recently, amount of bali cattle with their qualitative superior has been certified as a good breed. According to Milfa et al. (2015), one of the genetic factors that influence the qualitative superior is the variation in growth hormone gene [5].

The bovine growth hormone gene (bGH) can play a significant role in the stimulation of growth and milk production [6]. The bovine growth hormone gene is located in q22 of bovine chromosome 19 and consists of five exons and four introns with 217 amino acids [7].

Growth hormone is secreted in somatotropic or acidophilic cells of the anterior pituitary gland of mammals [6]. The secretions of its are influenced by growth hormone (GH) gene which was known potential encoding morphological characteristics related to growth, such as body weight, shoulder height, body length, and chest circum ference [8]. Growth hormone regulates expression of many genes including insulin-like growth factor I (IGF-I), that influences the growth rate, body composition, health, and milk production [9].

The bovine growth hormone (bGH) gene has been intensively studied in livestock because of its effects on wide physiological activities. Its functions including the regulation of growth, the development of lactation and mammary gland, gluconeogenesis, the activation of lipolysis, and the enhancement of amino acid incorporation into muscle protein [10]. There is also evidence that growth hormone may be involved in the pubertal development and testicular function [8].

Genetic variations may occur as a result of the differences in genes coding regions, but not due to mutations. All of natural genetic variations are caused by the changes in the nucleotide sequence of the DNA [11]. According to the background above, it is necessary to identify genetic marker of the growth hormone genes in bali cattle with qualitative superior. There are refer to the theory of Korkmazagaoglu and Akyuz, (2013) who investigated

the polymorphism of growth hormone gene will be infect to the production trait in farm animals and GH gene has been suggested as a putative candidate for variability [12].

II. RESEARCH METHOD

Sample preparation

Three milliliter of blood samples were collected from jugular vein into vacuum test tubes, which contained EDTA as an anticoagulant. Whole blood samples were collected from 50 individual cattle which have been certificated as good breed, and there were managed by farmers with traditional ways.

DNA extraction

DNA was extracted from blood samples using according to the method described Putra et al. (2016) [13]. The 200 µL blood sample was mixed with 800 µL buffer A solution in an Eppendorf tube for 1.5mL, then centrifuged at10,000 rpm for five minutes. After removal of the supernatant, the pellet was resuspended with 300 µL buffer A solution, then centrifuged again at 10,000 rpm for five minutes. This step was repeated until the pellet color is be complate. The pellet was added with 270 µL of buffer B and further added with 30 µL buffer C, then the mixture were incubated at 50 oC for overnight. The next day, the mixture was added with 71 µL of 5 M NaCl solution, shacked vigorously for 15 second, and then was centrifuged at 10,000 rpm for 10 minutes. The 300 µL to player was transferred in to a new 1.5 mL Eppendorf tube, added with 600 µL of 96% Ethanol, and mixed slowly. After emergence of DNA, then the tube was centrifuged at 12.000 rpm for 10 minutes. The supernatant was carefully deposed, and the DNA pellet was washed by addition of 100 µL of 70% ethanol and subsequent centrifugation at 12.000 rpm for 5 minutes. The supernatant was discarded and the DNA pellet was air-dried until it became semi-transparent. The dried DNA was added with 100 µL of TE (Tris-EDTA) solution (pH 7.4) then left for overnight to dissolve DNA repeated for 30 cycles and the reaction ended with a final extension at 72oC for 10 minutes. The PCR products were separated on a 0.8% agarose gel, stained with ethidium bromide, and visualized under UV light in UV transilluminator.

Restriction Fragment Length Polymorphism (PCR-RFLP)

Growth hormone gene variants were identified by a PCR-RFLP method. Two primers, namely GH5, (5'-CCC ACG GGC AAG AAT GAG GC-3') and GH6 (5'TGA GGA ACT GCA GGG GCC CA-3), respectively. The positive results were characterized by PCR product 390 bp. Furthermore, the products were restricted with endonucleic restriction enzyme MspI. The total volume of PCR-RFLP mixture was 15 µL that contain 5 µL of PCR product, 0.5 µL of MspI restriction enzyme 1.5 µL of 10x buffer tango, and 8 µL aquabidest. The mixture then incubated at 37ºC for 3 hours in multy heater. PCR-restriction fragments were separated by electrophoresis on 2% agarose gel in 1x TBE buffer at 50 V for 2 hours, and visualized under UV light after staining with ethidium bromide.

III. RESULTS AND ANALYSIS

Growth hormone gene has been amplified that were characterized by PCR product 390 bp (Fig. 1). After restricted with MspI restriction enzyme, produced the patterns consisting of two fragments, with a base length of 230 bp and 329 bp (Fig. 2). Both fragments were identified as allele +/+ and allele -/-..

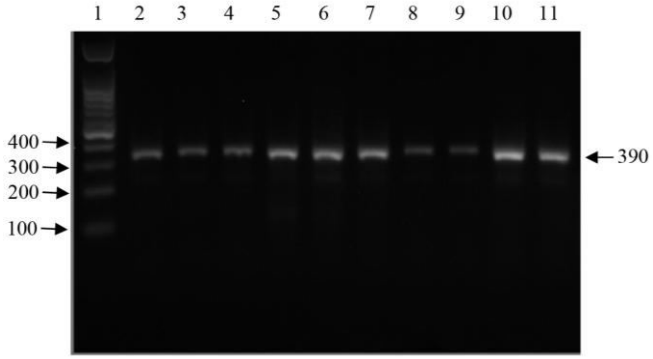

Fig 1. Amplification of growth hormone gene of bali cattle in 2 % agarose gel (1; marker 100 bp, 2-11 : samples).

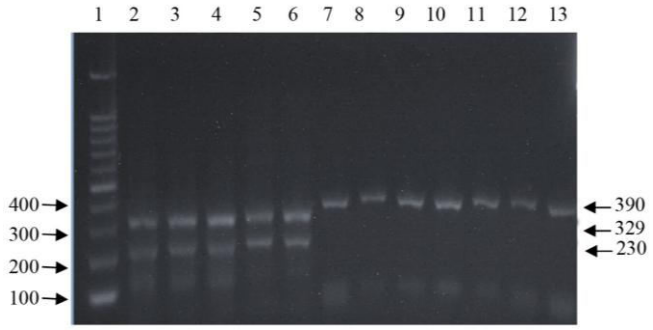

Fig 2. Visualization of growth hormone gene after restriction with MspI restriction enzyme in agarose gel 2 % (1; marker 100 bp, 2-13; GH gene amplification, R1 - R4; MspI restriction GH gene)

The genotype of growth hormone gene which was splitted with restriction enzyme showed polymorphism. The results indicated, the gene of growth hormone in Bali cattle with qualitatively superior was variously. The site of restriction enzyme by using MSpI was located in 1161 bp, which split growth hormone gene to be two fragments in position 230 and 329 bp, respectively. According to the previously study, there was known, the polymorphism was occurred as a result of the change in restriction sites of MSpI enzyme in position 1161 bp. The change of its can be produce new patterns of restriction sites [14][15]. The polymorphism of growth hormone gen in bali cattle produced by transition C to be T of nucleotide sequence in position +837 [16]. Polymorphism had been found in dairy cattle [17][18], Bavarian Simmental [19] India cattle [20] and Hereford [8].

Polymorphism of growth hormone gene was known affected by several factors i.e. mating, mutation, and natural or artificial selection [21]. Mutation at the DNA can be derived from deletion, insertion, and recombination. These changes will be affecting to the phenotype expression of organism. [19].

In this study, Bali cattle with qualitative superior was found splitted their locus of growth hormone gene into two allele i.e. +/+ and -/- These facts are predicted as a results of mutations in their growth hormone gene which effected for their expression. This hypothesis was supported by the study of several researchers who found the genotype +/+ and -/- have positive impact for the increasing of body weight and the quality of meat [9]. The other study found the frequency of gene was influenced by selection, mixing of population with different gene frequency, inbreeding, out breeding and genetic drift [22][11][23]. According to the several reasons above, researchers concluded the polymorphism of growth hormone which was showed by different allele can be used as a molecular marker for bali cattle with qualitative superior.

IV. CONCLUSION

Growth hormone genes of Bali cattle with qualitative superior are polymorphism which was characterized by the formation of two fragments with the length of 230 bp, 329 bp. Both fragments are identified as allele:+/+ and allele -/-.

ACKNOWLEDGMENT

The authors would like to thank Prof. Dr. Supar, MS for his kindness to provide control isolates of E. coli O157:H7 ATCC 43894, and also wish to thank Udayana University Institute for Research and Community Service for supporting this project through Basic Research Grant contract No: 0161/023-04.2/XX/2010 date on December, 31st 2009.

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