Mapping of Growth Hormone Gene of Body Weight of Krui Cattle in Pesisir Barat Regency, Lampung, Indonesia

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ABSTRACT

The research objective was to map the growth hormone (GH) gene of body weight of Krui cattle in Pesisir Barat Regency. This research used 30 blood samples of 30 Krui cattle. The method used was by taking quantitative data and blood samples from adult Krui cattle in Pesisir Barat Regency, then the blood samples were analyzed by DNA isolation method. PCR amplification used was a pair of GH-Forward primers: 5 'ATC CAC ACC CCC TCC ACA CAGT 3' and GH- reverse: 5 'CAT TTT CCA CCC TCC CCT ACA G 3', as well as digestion using the RLFP method at the UPT Integrated Laboratory and Innovation Center, University of Lampung. Association between genotype and body weight was analyzed descriptively. The results showed that Krui cattle had polymorphic genes with three genotypes found, namely: CC, CT, and TT. Cattle with CT genotype has the largest average body weight or meat production compared to those with other genotypes. These results indicated that the GH gene identifier has strong evidence that it can be used as a selection tool with the help of genotypes of body weight traits of Krui meat production in Pesisir Barat Regency. Krui cattle with CT genotype can be developed further because it has high economic value with a high average body weight and meat production.

Keywords: Gene mapping, Growth hormone, body weight, Krui cattle.

INTRODUCTION

Krui cattle is a local cattle in Pesisir Barat Regency, Lampung Province. Currently this Krui cattle is being proposed to Ministry of Agriculture of Republic Indonesia to be a local breed which is only available in Pesisir Barat Regency. Krui cattle have a relatively small body size compared to other breeds of cattle in Indonesia such as Bali cattle and Madura cattle, and it has characteristics which are different from the other local cattle in Indonesia. Krui cattle are able to survive in high ambient temperatures and low quality of feed (Prawira, 2015). The population of Krui cattle in Pesisir Barat region reaches 10,090 heads (Central Statistics Agency, 2019). The cattle are raised semi-intensively, during the day they are grazed until the afternoon, then the cattle are kept at night.

Krui cattle need to be preserved as germplasm originated from the Pesisir Barat Regency, because the longer the existence of Krui cattle will be increasingly rare if not

paid attention. The current belief is that the performance of Krui cattle always decreases from year to year due to the decreasing quality of the environment for the cattle and the possibility of ongoing inbreeding. This is due to the absence of a clear breeding program. In order to improve the performance of Krui cattle production, it is necessary to apply careful and continuous selection. Selection based on individual livestock records through a quantitative genetic approach has been shown to be able to improve the genetic quality of livestock and their productivity. However, this method requires a very long time and accuracy in recording. In addition, this conventional selection is less accurate and does not cover the genotypic aspects of livestock that reflect their potential genetic advantage.

Growth hormone (GH) gene plays a role in meat growth, and has been shown to increase the growth rate and carcass composition after being given growth hormone (Etherton and Bauman 1998). In relation to this function, this gene is used as a strong candidate for genetic markers for meat growth traits (Silveira et al., 2008). This trait is the most concerned aspect in improving the genetic quality of beef cattle. The mapping between the GH gene and growth traits in beef cattle can be seen by linking the mapping of GH genes to the growth traits of beef cattle.

Growth hormone (GH) plays a role in physiological processes in livestock, such as growth, carcass composition and milk production. Allele variations in the GH gene have been reported to be associated with variations in milk production (Sabour et al., 1997), carcass characteristics such as carcass weight gain, meat value, live weight and others (Grochowska et al., 2001).

Factors that influence meat growth are genetic, physiological and environmental factors. In terms of genetic factors, in general the differences between breeds of cattle are caused by genetic differences that regulate a trait. Variations in the characteristics of production in each individual cattle in the same breed are due to heredity. Differences in production capability between breeds of cattle reflect genetic differences in terms of differences in the frequency of genes that regulate the quantity and quality of production (Soeharsono, 2008).

Yip et al. (1999) described that gene mapping is the variation in the frequency of alleles at a locus within a gene. Much research has been done in trying to determine the relationship of gene mapping to production traits. If the relationship can be found and the relationship is close enough and is characteristic of the entire population, then it can be

used for selection as an indicator of productivity. One way to detect polymorphisms is through the PCR-RLFP method (Sulandari and Zein, 2003).

Selection based on genetic markers has been believed to increase the efficiency and acceleration of selection implementation so that genetic progress can be increased (Ge et al., 2001). This study aimed to determine the association or relationship between the genetic markers of growth hormone genes and body weight of Krui cattle in the Pesisir Barat Regency.

MATERIALS AND METHODS

Data collection and blood sampling

Data collection was carried out from thirty Krui cows aged 3-4 years reared by farmers in the Pesisir Barat Regency. The data collected were body weight, body length, shoulder height, chest girth, and blood sample. About 5 ml of blood from each cow was collected and then put into a 15 ml propylene tube which contains the anti-coagulant EDTA. Blood samples were analyzed at the UPT Integrated Laboratory and Innovation Center, University of Lampung.

Molecular DNA Analysis

DNA isolation from cow blood was using the SDS-PK extraction method, which is a modification of the method of Sambrook et al. (1989). The results of the isolation were then carried out DNA electrophoresis and the results were seen visually using Ultraviolet (UV) light. Amplification of specific DNA fragments was using the PCR (Polymerase Chain Reaction) method. The results were seen visually using UV light. Polymerase Chain Reaction-Restriction Fragment Length Polimorphism (PCR-RLFP) was carried out on the GH gene target. The results were seen visually using a UV trans-illuminator. PCR products that are recognized by this enzyme will have three possible results of digestion, namely: two bands showing the CC genotype, three bands showing the CT genotype, and one band showing the TT genotype (Sun et al., 2003; Maucilla et al., 1997; Choudhary et al., 2004).

The allele and genotype frequencies are calculated by the following formula (Warwick et al., 1983):

Allele frequency A = ∑ locus A / ∑ (locus A + locus B)
Allele frequency B = ∑ locus B / ∑ (locus A + locus B)
Genotype frequency AA = (∑ locus AA / ∑ individual in population) x 100%
Genotype frequency AB = (∑ locus AB / ∑ individual in population) x 100%
Genotype frequency BB = ∑ locus BB / ∑ individual in population) x 100%

If the calculation of the most allele frequency of GH genes found in the studied population did not exceed 0.99, then the GH gene was categorized as polymorphic

(Harris, 1994). The mapping of the GH gene on the growth traits of meat was analyzed using descriptive methods.

RESULTS AND DISCUSSION

The mean, standard deviation and variation coefficient of body measurements of Krui cattle in the Pesisir Barat Regency are presented in Table 1.

Table 2. Mean of body weight and body measurements of Krui cattle in the observation area

Variabel kuantitatif *(Quantitative Variable)*	Population Kabupaten Pesisir Barat *(Pesisir Barat Regency)(n=30)*
Body weight (kg)	163,87±38,00
Body length (cm)	100,93±5,93
Shoulder height (cm)	100,03±15,03
Hip height (cm)	103,52±13,52
Chest width (cm)	30,62±7,62
Chest girth (cm)	125,97±11,97
Dalam Dada (cm)	54,83±13,83
Hip circumference (cm)	137,31±18,31
Head width (cm)	42,52±2,52
Head length (cm)	18,10±2,1

Body Weight

The average body weight of Krui cattle in Pesisir Barat Regency is 163.87 ± 38.00 kg. Roviki et al. (2014) stated that the factor affecting body weight and cattle production is nutritional adequacy, if livestock are deficient in vitamins and minerals, it will affect the metabolic process which results in inhibition of productivity and growth. According to Basuki (2002), there are two factors that influence growth in beef cattle, namely internal factors (breed, age, genetics, gender and hormones) and external factors (feed, environmental temperature, disease, environmental stress and work / training).

Body Length

The results showed that the average body length of Krui Cattle in Pesisir Barat Regency was 100.93 ± 5.93 cm. Body length is influenced by feed and maintenance management in cattle. If the livestock ration during growth is deficient in nutrients, the bone formation will be less than perfect. Body length also has a relationship with body weight. According to Niam et al (2012), body length shows a spinal growth that includes the spine and lumbar. The direction of livestock growth begins with upward growth then extends and then circles on the chest.

Shoulder height

The results showed that the average shoulder height of Krui cows in Pesisir Barat Regency was 100.03 ± 15.03 cm. Shoulder height is one of the body measurements that can be used as supporting data in determining livestock performance. The relationship between shoulder height and weight will get tighter over time (Niam et al., 2012). Body measurements such as chest girth, body length and shoulder height can provide indications of body weight with sufficient accuracy. The correlation between chest

circumference, body length, and shoulder height with live weight is very high compared to other body measurements (Suryadi, 2003).

Chest girth

The results showed that the mean chest circumference of Krui cattle in Pesisir Barat Regency was 125.97 ± 11.97 cm. The average Krui cattle chest girth is thought to be influenced by environmental factors, including maintenance management and geographical conditions. According to Niam et al. (2012) that chest girth has the closest relationship with body weight, the relationship between body weight and chest girth lasts more until older age than the close relationship between body weight and other body measurements. Chest girth is the best parameter for estimating body weight at all age levels. The increase in body weight will be followed by an increase in chest girth (Basbeth et al., 2015).

DNA Analysis

DNA Isolation

DNA isolation from cow blood using the SDS-PK extraction method, which is a modification of the method of Sambrook et al. (1989). The results of electrophoresis of genome DNA from cow blood samples were visualized using 0.8% agarose gel with ethahunidium bromide dye which fluoresces in ultraviolet light. The DNA results look like in Figure 1.

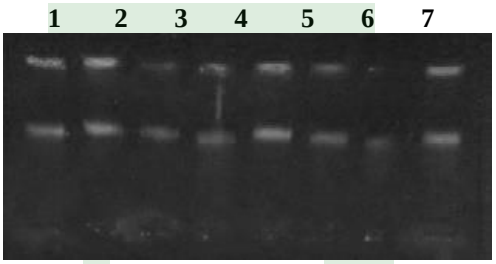

Figure 1. Results of visualization of DNA isolation products.

The results of electrophoresis showed a thick and bright visible band. The DNA image that appears on the electrophoresis results is an illustration of the success rate of the extracted DNA. The bright band indicates that DNA has been isolated, where the thicker the band, the more DNA is obtained. According to Sulandari and Zein (2003), DNA extraction from eukaryote organisms (humans, animals and plants) is carried out through the process of destroying cell walls, removing proteins and ribonucleic acid (RNA), DNA deposition and harvesting.

***Polymerase Chain Reaction* (PCR)**

Amplification of specific DNA fragments (growth genes) using the PCR (Polymerase Chain Reaction) method is an in vitro DNA synthesis technique that is able to rapidly multiply DNA fragments of a certain size enzymatically through the mechanism of temperature change (Sulandari and Zein, 2003). The primers used in this study are according to Reis et al. (2001) used a pair of primers, GH-forward and GH-reverse. GH-Forward: 5 'ATC CAC ACC CCC TCC ACA CAG T 3' and GH-Reverse: 5 'CAT TTT CCA CCC TCC CCT ACA G 3'. The PCR program includes predenaturation:

97oC 1 minute 30 seconds, denaturation: 94oC 1 minute, 65oC 1 minute annealing, 1 minute 72oC extension, and 72oC 1 minute final extension, 34 times.

Mapping detection of Growth Hormone (GH) genes in cattle in this study was carried out using PCR techniques, namely amplification of specific DNA fragments along 891 bp. The results of electrophoresis of PCR products using 3% agarose gel are shown in Figure 2.

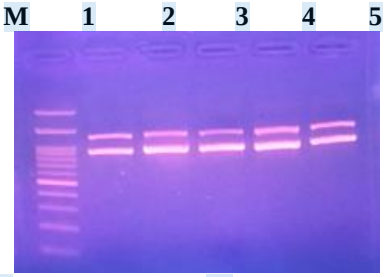

Figure 2. Visualization of gene PCR products

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RLFP)

The detection of Growth Hormone (GH) gene polymorphisms in Krui cattle in this study was carried out using the PCR-RLFP technique, namely the amplification of specific DNA fragments along 891 bp which was then followed by cutting (digestion) at a certain point using the Msp1 enzyme.

Digestion electrophoresis used 10% polyacrylamide gel because 10% polyacrylamide gel has a higher resolution capacity than agarose gel, and can separate DNA in a narrow size range, so that 52 bp fragments can be seen. The results of electrophoresis of PCR-RLFP products using 10% polyacrylamide gel are shown in Figure 3.

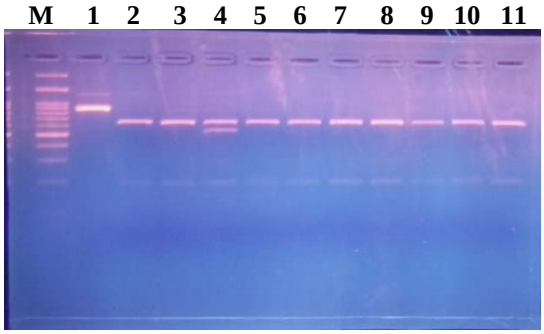

Figure 3. Visualization of GH 891bp gene PCR-RFLP products with Msp1 enzyme

Digestion of PCR products along 891 bp with restriction enzyme Msp1 in this study produced two alleles, namely C and T alleles. The CC genotype was characterized by cutting DNA at the 194 bp, 63 bp, 110 bp and 524 bp positions. The CT allele was characterized by cutting DNA at the 194 bp, 63 bp, 110 bp, 524 bp and 634 bp positions. The TT allele was characterized by cutting DNA at the following positions: 194 bp, 63 bp and 634 bp. The PCR-RLFP results in this study showed that there were CC, CT and TT genotypes in Krui cattle.

Allele and Genotype Frequency

Allele frequencies and genotypes of GH genes in this research of Krui cattle are presented in Table 2. Krui cattle population was polymorphic because the frequency of the common allele (T allele) is not more than 0.99. The frequency of the T allele of the GH gene in Krui cattle was 0.93 and the C allele was 0.067. The results of allele frequency calculations show polymorphism, which is stated by Harris (1994) that if the common allele frequency of the GH gene found is not more than 0.99, the cow population studied is polymorphic.

Table 3. Allele genotype frequencies of GH genes in Krui cattle

N	Genotype frequency			Allele frequency	
	TT	CT	CC	T	C
30	0,90	0,07	0,03	0,93	0,07

Table 2. Genotype and allele frequency of C/T in GH-MSp1 gene in cattle

Breed of cattle	N	Genotype			Alel		Sources
		CC	CT	TT	C	T	
Bali Pesisir	47	0,00	0,00	1,00	0,00	1,00	Jakaria, *et al.*, 2009
Aceh	133	0,05	0,30	0,65	0,20	0,80	Jakaria *et al.*, 2007
Limousine	41	0,00	0,00	1,00	0,00	1,00	Putra, *et al* 2013
Simmental	22	0,41	0,45	0,14	0,64	0,36	Jakaria, *et al.*, 2009
Grati	18	0,77	0,23	0,00	0,89	0,11	Jakaria, *et al.*, 2009
Ongole	43	0,16	0,35	0,49	0,34	0,66	Maylinda, 2011
Madura	114	0,43	0,50	0,07	0,74	0,26	Hartatik, *et al.*, 2010
	49	0,23	0,22	0,55	0,44	0,56	Hartatik *et al.*, 2010

Genotype Association with Body Weight or Krui Beef Production

The association between GH gene genotype and the average body weight of the research cattle is presented in Table 3.

Table 4. Relationship of GH gene genotypes to average body weight in research cattle

Krui cttle	N	Genotype		
		TT	CT	CC
Body weight (kg)	30	182,92	241,50	160,00

The results showed that Krui cattle with CT genotype had the greatest average body weight or meat production compared to those with other genotypes. Thus these results showed that the GH MspI gene identifier has strong evidence that it can be used as a selection tool with the help of genotypes on body weight or Krui cattle production in Pesisir Barat Regency. Several other studies related to the polymorphism of the GH MspI

gene reported that the heterozygous genotype (CT) had a body weight greater than the homozygous genotype (CC / TT) in Angus and Brangus cattle (Garcia et al., 2003).

The results of this study are in line with the results of research by Sutarno et al. (2005) on the mapping of the GH gene in the analysis of the relationship between allele types and growth traits in Ongole Grade cattle (PO cattle), it was found that the CT genotype of GH gene had a significant effect on meat growth, where cattle with CT genotype were individuals who were superior in meat growth. Thus, Krui cattle with CT genotype can be developed further because they have high economic value with high average body weight and meat production.

Conclusion

The results showed that in Krui cattle there were three kinds of genotypes, namely, CC, CT, and TT. The CT genotype has the largest average body weight or meat production compared to cows with other genotypes. Thus these results show that the GH gene identifier has strong evidence that it can be used as a selection tool with the help of genotypes on body weight traits or Krui beef production in Pesisir Barat Regency. Thus, Krui cattle with CT genotype can be developed further because they have high economic value with high average body weight and meat production.

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