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Identification of Locus GH/AluI Polymorphisms of Kuantan and Pesisir Cattle

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ABSTRACT

Kuantan cattle is a local beef cattle of Riau Province that adaptive and breed well along the Kuantan river flow, found in Indragiri Hulu Regency and Kuantan Singingi Regency, have smaller morphometric performance compared to other local beef cattle and are relatively the same as Pesisir cattle. One of the genetic markers of growth determinant in beef cattle is the GH/AluI gene locus. Identification of the diversity of the GH/Alu I gene was carried out using the PCR-RFLP. Fifty four samples of Kuantan cattle consisting of 25 samples from Indragiri Hulu Regency and 29 samples from Kuantan Singingi Regency, as well as 25 samples of Pesisir cattle from BPTU-HPT Padang Mengatas Payakumbuh, West Sumatra, were used in this study. The oligonucleotida forward F-5'GCTGCTCCTGAGGGCCCTTC-3' and reverse R-5'CATGACCCTCAGGTACGTCTCCG-3' used in this study along 211 bp of GH Gene. The PCR mixture consisted of DNA templates 20 - 25 ng, 0.4 μL primer forward dan primer reverse (10 ng primer forward and primer reverse), 25 μL dream tag green master mix and ddH_2O up to a 50 μL. PCR machine conditions consisted of 94^0C pradenaturation for 5 minutes, 94^0C denaturation for 30 seconds, annealing 65^0C for 1 minute, 72^0C extension for 50 seconds and 72^0C final extension for 5 minutes. Identification of GH gene diversity using AluI at an incubation temperature of 37^0C for 3 hours. The results in this study were found monomorphic of GH/AluI genes in Kuantan and Pesisir cattle. The monomorphic in Kuantan cattle can be used as an indicator to assess the purity of Kuantan cattle in this areas.

Keywords**:** AluI, GH Gene, Kuantan cattle, Monomorphic, Pesisir cattle

Introduction

Kuantan cattles is one of Indonesia's local beef cattle based on Decree of the Minister of Agriculture Number: 1052/Kpts/SR.120/10/ 2014, were lived and adapted well in areas along the Kuantan river flow from Indragiri Hulu Regency to Kuantan Singingi Regency. The highest population of Kuantan cattle (Figure 1) was found in Indragiri Hulu Regency with a total of 5,959 heads and in Kuantan Singingi Regency was 2,386 heads (Department of Animal Husbandry and Animal Health Riau Province, 2011). At present, no official information is found on the number of Kuantan cattle populations in this area, and it is predicted that the population has decreased from year to year.

Qualitatively and morphometrically, the cattles have a variety of coat color, horn shape, and morphometrics. The results of Zulfikar (2018) showed that the coat color variations of male and female adult cattle high significantly to differences the size of chest circumference, body length, shoulder height, hip height, hip width and circumference of the scrotum (male), where cows with light coat color have morphometrics higher than the dark coat color of Kuantan cattle. Kuantan cattle have relatively small body size and bodyweight compared to Bali cattle breeds and are relatively the same as Pesisir cattle (Decree of The Minister of Agricultural Number: 2908/Kpts/OT.140/6/2011). The morphometric of the bull i.e height of shoulder is 99.28 cm, the body length is 103.78 cm and the chest circumference is 126.22 cm with a bodyweight of only about 138 kg while for the cows i.e the height of shoulder is 99.19 cm, the body length is 102.35 cm and the chest circumference is 123. 27 cm with the weight the body is only around 132.18 kg (Decree of The Minister of Agricultural Number, 1052/Kpts/SR.120/10/2014).

Growth is a process of deposition, transfer of the substance of cells and an increase in the size and number of cells at different levels and points in a certain time that is influenced by growth genes (Lawrence and Fowler, 2002). Growth in cattles is controlled by a complex system that is through the presence of somatotropic hormones (growth hormones). The existence of somatic hormones is regulated

Figure 1. Kuantan Cattle in Indragiri Hulu Regency.

through the presence of genes that encode the hormones responsible for growth. Gene that have a relationship with postnatal growth is Growth Hormone (GH) which has an impact on bone and muscle growth after birth, anabolism such as bone growth and protein synthesis. GH is also needed for tissue growth, fat metabolism, normal growth (Etherton and Bauman, 1998), superovulation response, ovulation rate, fertility rate and embryo quality (Sumantri *et al.*, 2011). GH consists of 191 amino acid sequences that are synthesized and secreted by the anterior pituitary gland under hypothalamic control, the two hormones it produces are GH RH (GH Releasing Hormone) and SRIF (Somatotrophin Releasing Inhibiting Factor) (Silveira *et al.*, 2008). GH is encoded by the GH gene consisting of 5 exons and 4 introns found at 19q26qter on the bovine chromosome (Hediger *et al.*, 1990).

Polymorphisms of GH/AluI genes as growth markers in cattle has been reported by several researchers, namely in Sahiwal cattle (Biswas *et al.*, 2003), Pesisir cattle (Jakaria *et al.*, 2007), Bali cattle, PO cattle, SIMPO cattle, LIMPO cattle (Mu'in, 2008), FH cattle (Muin and Zurahmah, 2009), Limousin cattle (Jakaria *et al.*, 2009), Limura cattle (Volkandari *et al.*, 2013), FH cattle (Volkandari *et al.*, 2013), and there have been no reports for Kuantan cattle. The AluI mutation point is at position 2141 (C>G) of the bovine GH gene, converts the amino acid Leucine to Valine on the 127th sequence of proteins (Volkandari *et al.*, 2013).

Increasing the productivity of Kuantan cattle could be done through selection and crossbreeding. Conventional selection requires high costs, a long time and a large population. Application of marker assisted selection through identification of GH/AluI gene polymorphisms, is one solution that could be done. Identification of gene diversity at the DNA level allows early detection of genetic potential in the early growth phase (Curi *et al.*, 2005; Dekkers, 2004). Identification of locus polymorphisms of DNA can also be used as genetic markers to increase livestock production if polymorphic is found, and can also be used to assist conservation efforts if found monomorphic.

Materials and Methods

Sampling and DNA extraction

Fifty-four samples of Kuantan cattle bloods consist of 25 samples from Indragiri Hulu Regency (Figure 1) and 29 samples from Kuantan Singingi Regency and 25 Pesisir cattle from BPTU-HPT Padang Mengatas West Sumatra were used in this study. Blood collection using a 5 mL syringe on vena jugularis vein, 4-5 mL blood was collected in the EDTA vacuntainer tube and carried in a cool box for DNA isolation. The process of DNA isolation was carried out at the Animal Molecular Genetic Laboratory of Faculty of Animal Science, IPB Bogor using Phenol Chloroform Method (Sambrook and Russel, 2001). The isolated DNA was then tested using agarose gel electrophoresis 1.5% and a random sampling using a spectrophotometer to calculate the concentration and purity of DNA.

GH gene amplification using the polymerase chain reaction method

DNA amplification was carried out at a total volume of 50 µL consisting of 4 µL (25 -50 ng) template DNA, each 0.4 µL oligonucleotida forward and reverse (10 ng), 25 µL dream tag green master mix and ddH2O up to 50 µL volume. The condition of the PCR thermocycler was 94^{0}C pradenaturation for 5 minutes, 94^{0}C denaturation for 30 seconds, annealing 65^{0}C for 1 minute, 72^{0}C extension for 50 seconds and 72^{0}C final extension for 5 minutes. Stages 2-5 of the PCR process were repeated 34 times. The success of PCR was tested agarose gel electrophoresis with a gel concentration of 2% in 1 x TAE shown by appearing a single band at the position of 211 bp (PCR product), using Bio Rad Documentation Gel. The DNA marker used is 50 bp. PCR amplification and AluI restriction process were done in Reproduction and Breeding Laboratory of UIN Suska Riau, Pekanbaru.

Identification of polymorphisms GH gene used Restriction Fragment Length Polymorhisms technique

The PCR product which was successfully amplified, then incubated the AluI enzyme (AG^CT) at 37^{0}C for 3 hours. RFLP mixture consist of 6 µL PCR products, 0.4 µL AluI enzymes (4 units) added, 2.4 µL 10 x buffer and 15.2 µL ddH$_2$O. Diversity visualization was performed on agarose gel 3% in 1 x TAE using Bio Rad Documentation Gel. The cutting site interpretation refer to Reis *et al.* (2001), LL genotype (two bands at 52 bp and 159 bp), LV genotype (three bands at 52 bp, 159 bp, and 211 bp) and VV genotype (one band 211 bp).

Data analysis

Analysis of genotype and allele frequency of GH/AluI locus in Kuantan and Pesisir cattle according to Nei (1987) and Hardy-Weinberg equilibrium (Guo and Thomson, 1992).

Results and Discussion

Concentration and purity of DNA isolation

The success of DNA isolation was carried out agarose gel of 1.5% in 1 x TAE qualitatively, determined by the appearance of a single band that was bright and clear and was above the marker (Figure 2). The average concentration and purity of isolated DNA were presented in Table 1. The concentration of DNA produced in this study ranged from 4.85 - 15.90 ng/µL indicated a relatively low range. Hidayati *et al.* (2016) reported that the concentration of DNA of Kuantan cattle in the same isolation method ranged from 27.45 - 121.45 ng/µL. Nsubaga *et al.* (2004) states that damage to the sample as a source of DNA material could affected the concentration of DNA produced. Factors causing damage to the source of DNA material are a long storage time, high environmental temperature so that it can damage the DNA hydrogen bonds so that it becomes irreversible and damaged purine and pyrimidine bonds from DNA, and the presence or number of cells (Hidayati and Aulawi, 2016). The lower the DNA concentration, the more volume is needed in the PCR process. DNA concentrations commonly used for the PCR process are in the range of 25-50 ng/µL (Hidayati *et al.*, 2016).

Absorbance ratio values of 260/280 and 260/230 are used to assess the level of purity of DNA from the isolation. The value of DNA purity range (260/280) ranges between 1.49-2.97 and the value of DNA purity (260/230) is 0.07 - 0.20 (Table 1). The results showed that based on the ratio of absorbance values of 260/280, 32% (n = 8) samples had a good purity because they had a purity value between 1.8-2.0, 4% (n = 1) had a purity below 1, 8 and the remaining 64% (n = 16) have above 2.0 (Figure 3). Maximum absorbance of DNA occurs at a wavelength of 260 nm and protein at a wavelength of 280 nm (Boyer, 2005). Purity values (260/280) indicate the purity of DNA from protein contaminants (Widiarthi *et al.*, 2014; Qomar *et al.*, 2017). The variation in absorbance ratio values of 260/280 was influenced by DNA pH, wavelength accuracy, composition of nucleotide bases (A: T: G: C). Efforts can be made to increase the purity of DNA from protein contaminants, according to Qomar *et al.* (2017), with an increase in Proteinase K (Prot K) and Washing Blood Cell repeatedly.

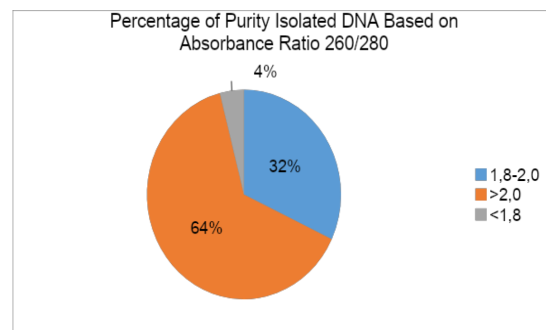

Figure 3. Percentage of Purity Isolated DNA Based on Absorbance Ratio 260/280.

The absorbance ratio of 260/230 was used to determine DNA purity from organic contaminants (Qomar *et al.*, 2017), with good purity values in the range of 2.0-2.2. DNA purity based on absorbance values of 260/230 in this study was very low with an average value of 0.13 ± 0.04 with a range of 0.07 - 0.20 (Table 1). Carbohydrates, phenols and Guanida HCL are materials that may be able to contaminate the DNA of the isolated results that can be absorbed at a wavelength of 230. The low value of DNA purity based on the absorbance ratio value of 260/230 is presumably due to the remaining

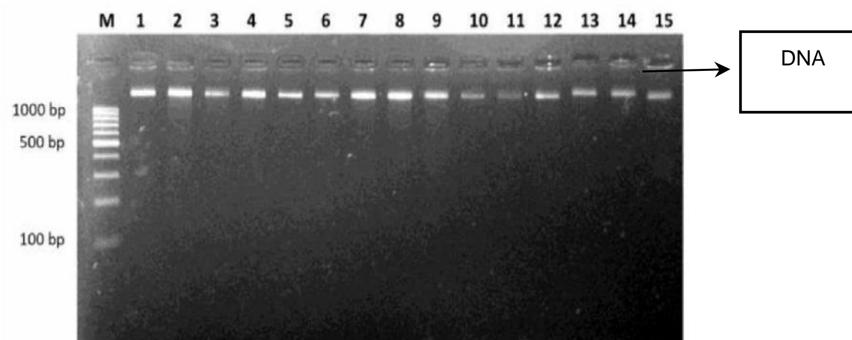

Figure 2. Qualitative Test of DNA Isolation, 1-15 = Sample Code, M = DNA Leader 100 bp.

Table 1. Average value of DNA concentration and purity of isolated DNA

Variables	Average ± Stdev	Maximum	Minimum
Concentration (ng/µL)	9.76 ± 2.87	15.90	4.85
Purity			
260/280	2.15 ± 0.33	2.97	1.49
260/230	0.13 ± 0.04	0.20	0.07

phenol contaminants added to the DNA isolation process in the form of compounds phenol chloroform.

Amplification GH gene and restricted fragment length polymorphisms with AluI enzyme on Kuantan and Pesisir cattle

Amplification of the GH gene of Kuantan and Pesisir cattle samples were carried out at annealing temperature of 65^{0}C for one minute. The results showed that the PCR product along 211 bp flanking intron 4 and exon 5, and the results of the RFLP by AluI enzyme were presented in Figure 4. Alu I enzyme cutting sites on PCR products at 52 bp and 159 bp were formed the LL genotype (Leucine-Leucine). LL genotype of the GH/Alu I locus was a common genotype that appears in cattle with small body sizes or light beef type such as Kuantan cattle and Pesisir cattle. The LL genotype of Kuantan cattle can be used to assist conservation efforts in the future. Jakaria *et al.* (2007) the same reported in Pesisir cattle from the Regency of Pesisir Selatan and Padang Pariaman, West Sumatra Province were LL genotypes. LL genotype was the dominant genotype in Pesisir cattle with LL, LV and VV genotypes were 0.985, 0.015 and 0,000 respectively. GH/AluI loci of Madura cattles were monomorphic with LL genotype and polymorphic in Limura cattles with LL, LV and VV genotypes frequency were 0.83, 017 and 0.00 respectively (Volkandari *et al.*, 2013). Kuantan and Pesisir cattle are beef cattle breeds included Bos indicus (Hidayati *et al.*, 2016). Bos indicus was group of cattle that have a dominant LL genotype compared to LV and VV genotypes (Biswas *et al.*, 2003; Jakaria *et al.*, 2007; Mu'in, 2008; Volkandari *et al.*, 2013), as well as Bos sundaicus (Bali cattle) (Mu'in, 2008) and buffaloes (Biswas *et al.*, 2003). Bos taurus such as Friesien Holstein (Biswas *et al.*, 2003; Muin and Zurahmah, 2009; Hartatik *et al.*, 2015), Karan Fries (Aruna *et al.*, 2004), Brazilian Canchim (Silveira *et al.*, 2008), Podolian cattle (Dario *et al.*, 2005), and Limousin (Jakaria *et al.*, 2009) were polymorphic, the frequency of L allele was not more than 0.99. Crosses Bos taurus X Bos indicus have produced polymorphic cattles at GH/AluI gene locus such as in Limousin X Madura (Volkandari *et al.*, 2013), Simmental x PO (Mu'in, 2008) and Limousin x PO (Mu'in, 2008). The LV genotype has better growth compared to LL genotype on SIMPO calf (Mu'in, 2008). Reis *et al.* (2001) also reported that LV genotypes from local Portuguese beef cattle i.e Alentejana, Marinhoa and Preta breeds have greater bodyweight than other breeds (Arouquesa, Barossã, Maronesa, Mertolenga and Mirandesa).

Genotype frequency and allele frequency of GH/AluI gene locus in Kuantan and Peisisir cattle

Genotype and allele frequency of Kuantan and Pesisir cattle of GH/AluI gene locus are presented in Table 2. The results showed that the GH/AluI gene locus in Kuantan and Pesisir cattle showed monomorphic (LL genotype). The GH/AluI

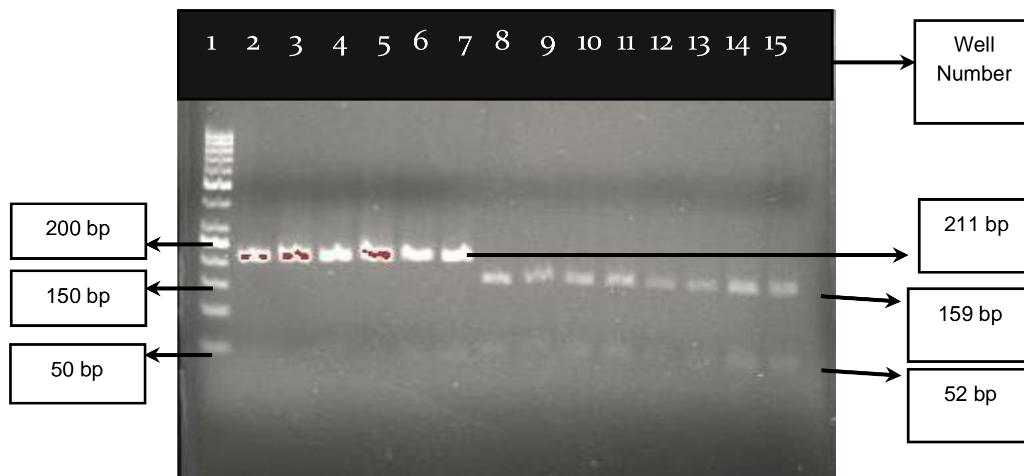

Figure 4 : 1th Well = 50 bp DNA Leader, 2nd – 5th Well (PCR Product of Kuantan Cattle sample code : 10, 24, 30, 51), 6th – 7th Well (PCR Product of Pesisir cattle, sample code: 4863, 4856), 8th – 15th Well (RFLP of Kuantan cattle, sample code 10, 24, 30, 51 and Pesisir cattle, sample code 4863, 4856, 4858, 4819), were formed LL genotype.

Table 2. Genotype and Alleles frequency of GH/AluI gene locus in Kuantan and Pesisir cattle

Breed	Genotype Frequency			Allele Frequency	
	LL	LV	VV	L	V
Kuantan Cattle					
a. Indragiri Hulu Regency (n= 25)	1.00	0.00	0.00	1.00	0.00
b. Kuantan Singingi Regency (n=29)	1.00	0.00	0.00	1.00	0.00
Total	1.00	0.00	0.00	1.00	0.00
Pesisir Cattle BPTU-HPT Padang Mengatas (n=25)	1.00	0.00	0.00	1.00	0.00

gene locus could not be used as one genetic markers of body growth genes in Kuantan cattle, but can be used to their conservation effort. A monomorphic allele if the allele frequency is equal to or less than 0.01 (Nei, 1987). In this study the frequency of L allele in Kuantan and Pesisir cattle was equal to 1.00, so that the Kuantan and Pesisir cattle don't mutated at the GH/AluI gene locus. One effort to bring up variations of the GH/AluI gene locus in Kuantan and Pesisir cattle through crossing with Bos taurus (Simmental, Limousin and FH) that have a VV or LV genotype. The results of Hardy Weinberg's equilibrium analysis were the population of Kuantan and Pesisir cattle wasn't in equilibrium, the value of chi square >1. This can be caused by the relatively small size of sample used in this study. Research using a larger sample size is recommended for further research.

Conclusions

The locus of the GH/AluI gene in Kuantan (n = 54) and Pesisir cattle (n = 25) were monomorphic with the LL genotype (1.00) so that the GH/AluI gene locus could not be used as genetic markers of growth body but used to conservation effort in the future.

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