

# Carcass Traits Association with GH/AluI Gene Polymorphism in Indonesian Aceh Cattle

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## Abstract

*This study was conducted in order to identify polymorphism of growth hormone gene in the exon five and to determine the association of GH/AluI polymorphism with carcass quality in Aceh cattle. A total of 42 DNA genome samples were extracted from two Aceh cattle population, i.e., Banda Aceh (12), Aceh Besar (30), and the PCR-RFLP was used to amplify 404 bp of GH gene. The result showed that the LL genotype was the only genotype found in Aceh cattle population, and the alele frequency of alel L is 1. This finding indicated that there was not evidence of polymorphism of GH/AluI in Aceh cattle, and there was not correlation of GH/AluI gene with carcass quality of Aceh cattle. It could be affected by small number of sampling size. However, this study suggests that GH gene could be possible used as genetic marker.*

*Keywords: Aceh cattle, GH gene, PCR-RFLP, Polymorphism*

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## Introduction

The enhancement of beef cattle's productivity in Indonesia will be more appropriate if it is done through a selection which is not only based on the phenotype, but also combined with direct selection on the level of DNA which codes the phenotype in which the quality needs to be improved. The bovine genome map made based on markers on genome DNA uses molecular technique such as RFLP, microsatellite, minisatellite, PCR-RFLP, and PCR-SSCP make it possible to identify gene locus which are responsible for trait variations having economic values.

GH gene as a genetic marker is frequently used in researches because growth hormone gene (GH) is one of the genes which influence growth (Di Stasio *et al.*

2005). Casas *et al.* (2004) reported that QTL for growth traits, carcass composition and beef quality was spread on chromosome 1, 2, 3, 16, 17, 19, 20, 21 and 26. GH gene has a great role in beef cattle's performance (Breier. 1999), hence it is very interesting to identify GH gene polymorphism on Aceh cattle.

## Materials and Methods

This research included field and laboratory activities. Field activity was conducted in Banda Aceh and Aceh Besar slaughterhouses. The DNA extraction and characterization of GH/Alu I gene diversity was conducted in Animal Molecular Genetics Laboratory, Faculty of Animal Science, Bogor Agriculture University, whereas the examination of carcass and meat quality was carried out in Ruminansia Besar Laboratory, Department of Animal Production and Technology. This activity was done in October 2009 - November 2010.

### *Blood Sample of Aceh Cattle*

This research used Aceh cattle's meat (muscle) in which this cattle is local ones originating from Aceh province. Twelve (12) samples were taken in RPH Banda Aceh, and thirty (30) samples from RPH Antassalam, Aceh Besar (Table 1). The sample used was *longissimus dorsi* muscle on the 12<sup>th</sup> – 13<sup>th</sup> rib. The cattle slaughtered were bred traditionally, in which during daytime, they were herded, and put in stalls at nights. The slaughter was done traditionally.

Table 1 The number of meat sample used for GH/AluI gene analysis

Population	Number
RPH Banda Aceh	12
RPH Aceh Besar	30
Total	42

### *Genome DNA Extraction (Meat)*

DNA extraction was done from the meat. The extraction procedure followed phenol-chloroform method (Sambrook *et al.* 1989).

### *GH Gene Amplification*

The amplification of GH gene fragment was done using PCR (*polymerase chain reaction*) method. The PCR machine used was *thermal cycler (Ependorf 5332)*. The arrangement of primers used can be seen in Table 2.

Table 2 Primers GH/AluI (Gen Bank M57764.1, Gordon *et al.*, 1983)

Locus	Temperature annealing	Product PCR	Sequences of Primers
GH/AluI	59 °C	404 bp	F:5'-TAGGGGAGGGTGGAAAATGGA-3' R:5'-GACACCTACTCAGACAATGCG-3'

### Genotype Determination Using PCR-RFLP

The genotype determination of each individual was done using *restriction fragment length polymorphism* (RFLP) which was visualized on agarose gel 1.5% with the buffer of 0,5x TBE (tris borat EDTA), functioned on 100 V for 40 minutes, and colored with ethidium bromide on *UV trans illuminator*. *AluI* was utilized as a cutter enzyme for the target gene fragment.

### Data Analysis

Gene Frequency. The genotype frequency was the ratio of the number of a certain genotype towards population number. Allele frequency was the ratio of a certain allele towards the whole allele in a locus in a certain population. The frequency of each allele in each locus was counted based on Nei dan Kumar (2000) formula:

$$X_i = (2n_{ii} + \sum n_{ij}) / (2N)$$

## Results and Discussions

### GH Gene Amplification Results

The amplification of growth hormone (GH) gene fragment done on Aceh cattle showed primers forward on the position of intron 4 and primers reverse on the position of flanking region 3 (Figure 1). The GH gene fragment amplification was conducted using thermal cycler (*Ependorf 5332*) machine on the temperature of annealing 63 °C.

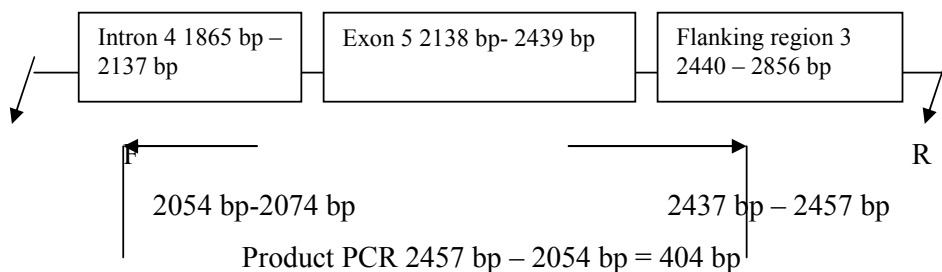


Figure 1. Primers forward position, primers reverse and PCR GH gene product

The result of gene fragment amplification visualized on agarose gel 1.5% is presented on Figure 2. The length of GH gene fragment amplification is 404 bp.

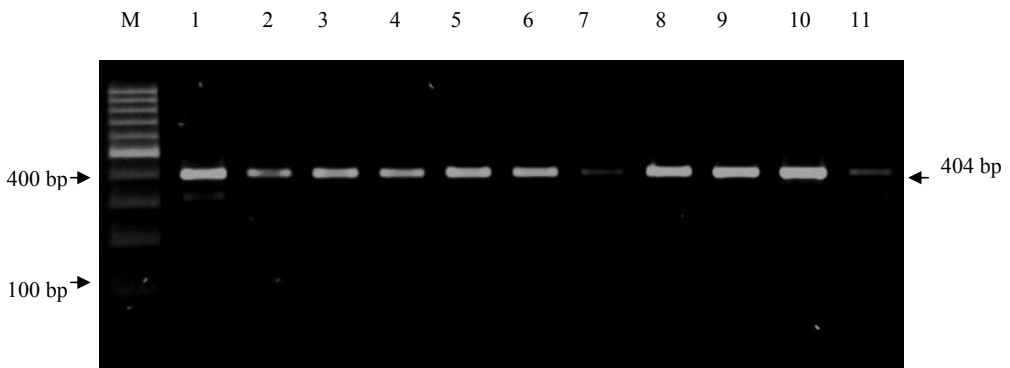


Figure 2. Visualization of GH gene fragment amplification result on agarose gel 1.5% (M: marker 100 bp, 1 -11: research sample)

The amplification results conducted by Gordon *et al.* (1983), Yao *et al.* (1996), Ge *et al.* (2003) and Zakizadeh *et al.* (2006) with the same primers showed that annealing primers GH/AluI gene fragment on 59 °C for 80 seconds, 65 °C for 30 second, and 57 °C for 60 seconds resulted in good PCR product. The annealing temperature used in this research was 63 °C for 45 second to obtain optimal PCR product so that it can be read clearly.

#### *Identification of GH gene variants using PCR-RFLP*

The determination of GH gene genotype in this research was carried out using PCR-RFLP with AluI as the cutter enzyme. AluI enzyme recognized AG|CT cutting site. Based on the sequence of GH gene DNA fragment being amplified, two AluI cutting sites were obtained; they are fragments with the length of 87, 132, and 185 which are knows as leucyne allele (L) (Picture 3).

The cutting using AluI enzyme on AluI GH gene fragment as much as 404 bp only resulted in one kind of fragment: a fragment which can be cut (two bars) known as LL genotype, whereas the fragment which cannot be cut (one bar) known as VV genotype and combined fragment (three bars) known as LV genotype cannot be found in this research (Figure 3).

The visualization result using agarose 1.5% shows that GH/AluI locus on the Aceh cattle sample population being observed is uniform. The genotype found on Aceh cattle in this research is LL genotype. Based on the analysis, the LL genotype frequency was one (1). This made the LV and VV genotype frequency zero (0). Based on Nei dan Kumar (2000) equition, allele L frequency is 1 and V allele frequency is 0. This result is likely caused by the very limited research samples.

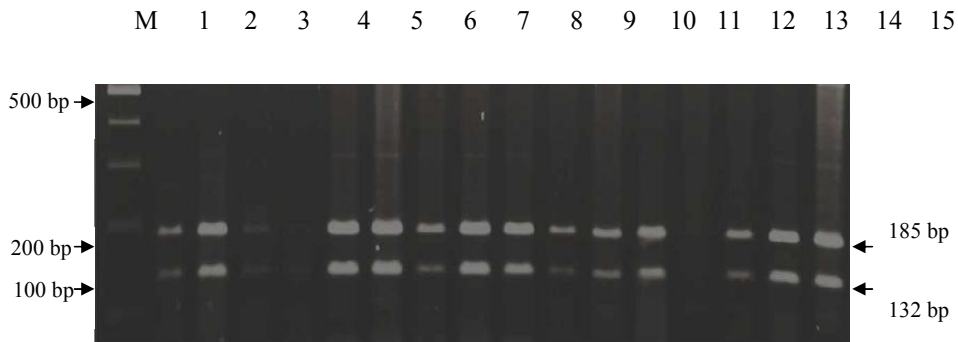


Figure 3. Visualization of GH|*AluI* gene fragment PCR-RFLP on aragose gel 1.5% (M: Marker 100 bp, 1-16: research sample)

### *Analysis Results on Aceh Cattle' Carcass and Meat Quality*

The value of parameter average of Aceh cattle carcass and meat quality can be seen on Table 3. The pH value of the meat in this research has the average of 5.46, which shows the pH range of normal meat 5.4 – 5.8. The meat color is in category I (score 1 – 5) according to SNI (Indonesian National Standard, 2008). The degree of tenderness 4 -5 is considered moderate, not too tender and not too tough.

Table 3 The results of Aceh cattle's meat and carcass quality

Parameter	n	Average ± standard deviation	Indonesian National Standard
Weight (kg)	42	301.88 ± 125.59	-
Eye muscle area (cm <sup>2</sup> )	42	34.19 ± 3.51	-
pH	42	5.46 ± 0.39	5.4-5.8
Tenderness (kg/cm <sup>2</sup> )	42	4.79 ± 1.74	4-5
Cooking loss (%)	42	35.38 ± 6.24	-
DMA (%)	42	29.94 ± 4.55	-
Meat color	42	3.8 ± 1.81	1-5

The research results identify that Aceh cattle's carcass/meat has finer meat fiber and its color is red. The quality and meat structure greatly depend on types of meat and location. Marbling is also very influenced by the breeding system and food given.

### Conclusions

Based on this research results, it can be identified that the use of GH/*AluI* only resulted in LL genotype and monomorphic, so that it cannot be used as a marker to

associate with the carcass quality on Aceh cattle. This phenomenon is likely due to the limited number of samples and the existence of natural selection towards LV and VV genotype as the consequence of Aceh cattle adaptation's to the local environment. Thus, a further research is still necessary, by using more samples and if diversity is found, sequencing needs to be done so that it results in more accurate research results.

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