CORRELATION OF POLYMORPHISM OF GROWTH HORMONE TO THE PRE-WEANING SABURAI GOAT GROWTH

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ABSTRACT

Polymorphisms of growth hormone gene were analyzed for association with pre-weaning growth of female Saburai goat. Analysis of PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) was conducted to determine polymorphism in two single nucleotide polymorphisms (SNPs) that A781G (Ser / Gly35) and A1575G (Leu147). The frequency of allele A, B, C, and D of 0.68, 0.32, 0.50, and 0.50. The results of analysis using SNPs A781G, 22 female saburai goat are heterozigot AB and 12 homozygous AA. Growth performance at birth with genotype AB was higher than AA (weight 3.04 ± 0.20 kg vs 2.94±0.05 kg, height 12.79±1.02 cm vs 11.12 ± 0.19 cm, body length 15.03 ± 0.55 cm vs 12.83 ± 0.76 cm, chest circumference 15.18 ± .55 cm vs 14.46 ± 0.21 cm), also growth performance at weaning with genotype AB was higher than AA (weight 29.92 ± 1.01 kg vs 29.56 ± 0.67 kg, height 51.34 ± 0.9 cm vs 50.90 ± 1.01 cm, body length 50.40 ±1.13 cm vs 49.44± 0.85 cm, chest circumference 60.93±1.92 cm vs 59.44±0.81 cm, ADG pre weaning 0.299 ± 0.010 kg vs 0.296 ± 0.007 kg). It was concluded that the growth hormone gene in exon 2 to exon 3 (SNPs A781G) was polymorphic and has correlation to growth performance pre weaning female Saburai goat.

Keywords : Saburai goat, growth hormon polymorphisms

INTRODUCTION

The Saburai goat is a local genetic resource in Lampung province and it was provisioned based on the Decree of Minister of Agriculture of Republic of Indonesia number 359/Kpts/PK.040/6/2015. This goat is a type raised for meat so that its growth is economical in its kind (Sulastri & Sukur, 2015).

The Saburai goat growth varies. It is suspected to be caused by the genotype diversity which control the goat growth. This genotype diversity is used as a basic for Saburai goat selection with a high growth performance to produce breed with higher performance that their parents. The growth performance selection can be done with identification of genetic polymorphism of growth hormone. The objective of this research was to find out the correlation between genetic polymorphism of growth hormone and the pre-weaning Saburai goat growth in Lampung province.

The goat growth genetic hormone has 17 chromosomes, it is composed from 2544 alkali nitrogen, and it has 5 exons and 4 nitrons (Kioka *et al*., 1989; Hua *et al*., 2009). Hua *et al*. (2009) studied GH gene polymorphism in the male Boer goat as a candidate for genetic markers of growth characteristics. Two SNPs, the A781G (Ser/Gly35) and A1575G (Leu147), were identified through the

analysis of *fragmenting* and *polymerase chain reaction-restriction fragment length polymorphism* (PCR-RFLP). The analysis results showed that in the Boer goat genetic hormone of growth, there were genotypes AA, AB, CC and CD. Based on these phenotypes, the goat could be grouped based on the body weights and body measures.

Sun *et al.* (2010) observed 254 of individual goats (43 Boer goats, 111 with *Xuhuai* goats, 100 *Chinese Haimen* goats) with PCR-SSCP and *fragmening* DNA methods. The observation results showed that only exon II from FIT2 (*fat-inducing transcript*) gene that was amplified with primary P5 from GIT gene which was proven to be polymorphic.

Irine (2011) reported his research results through *polymerase chain reaction-single strand conformation polymophism* (PCR-SSCP) method that the exon II growth hormonal gene in the PE goat, and the cross breed between PE and Saanen goats, was polymorphic. The close relationship between polymorphism of growth hormonal gene and the growth characteristics in some type of goats showed that this polymorphism could be used as markers in selecting goat or as the *marker asisted-selection* (MAS).

MATERIALS AND METHODS

This research was conducted to 34 pre-weaning female Saburai lambs, born as twins, and breed result of first parity product. Growth performance data including bodyweights and body measures at birth and weaning (2-3 years old) were obtained from recording goats owned by members of Pelita Karya III livestock farmer group in Dadapan village of Sumberejo sub district, in Tanggamus district. The measures included body length, chest circumference, chest depth, chest widt, waist height, ear length, and ear width.

3 ml of blood sample was taken from each of lamb individuals through jugular venous by using 22 ½ G syringe and contained in BD Vacutainer K2 EDTA (K2) tube. Blood sample was analyzed in the biochemistry laboratory of Faculty of Medicine in Gadjah Mada University. DNA isolation was conducted by using *Wizard® Genomic DNA Purification Kit* (Promega, USA). Analyses were done by using PCR KIT and GH gene primers (GHF-1 and GHR-1 primers; GHF-2 and GHR-2 primers) shown in Table 1 and restriction enzyme *Hae*III.

Table 1. Primer name and primer sequences GH gene, size of the PCR product and amplification

Primer name	Primer sequences	Size of the PCR product	Amplification
GHF-1	5'-CTCTGCCTGCCCTGGACT-3'	422 bp	*Exon* 2 dan 3
GHR-1	5'-GGAGAAGCAGAAGGCAACC-3'		
GHF-2	5'-TCAGCAGAGTCTTCACCAAC-3'	116 bp	*Exon* 4
GHR-2	5'-CAACAACGCCATCCTCAC-3'		

The Gene GH Amplification by Using *Polymerase Chain Reaction* (PCR) Method

The gene GH multiplication was conducted by using *Polymerase Chain Reaction* (PCR). Primers to use and design were based on the sequences of DNA gGH (*Gene Bank Accesion No. D00476*). These primers (Table 1) referred to the

research by Hua *et al.* (2009) in testing the correlation of GH gene polymorphism in the male Boer goat.

The 422 bp and 116 bp DNA fragments were amplified by using GH primer gene. The total of volume for PCR reaction in this research was 25 µl which contained of 20 µl PCR Master Mix; 2 µl for each of forward primer and reverse primer ddH2O and 1 µl DNA template. The PCR operational program was conducted according to recommendation of Hua *et al.* (2009) shown in Table 2.

Table 2. Operational program of PCR

Cycle	Temperature (°C)	Time
Predenaturasi	94	5 second
Denaturasi	94	30 second
Annealing	52	30 second
Elongasi	72	20 second
Post Elongasi	72	5 second
Siklus	25	

Source : Hua *et al.* (2009)

Detection of GH Gene Polymorphism with RFLP Method

422 bp and 116 bp PCR products were cut by using *Hae*III (GG^CC) restriction enzyme. Appearing alleles in the PCR 422 product (exon 2 and 3 regions) were allele A (366 bp and 56 bp) and allele B (422 bp). The genotypes having chance to appear were genotype AA (366 bp and 56 bp), AB (422 bp, 366 bp and 56 bp), and BB (422 bp). The *Hae*III enzyme cutting sites are shown in Figure 1.

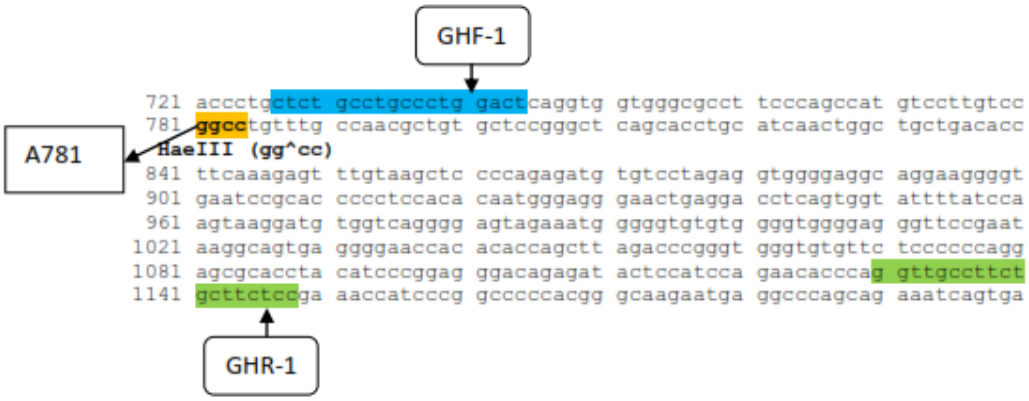


Figure 1. The layout of the enzyme HaeIII cuts in areas exon 2 dan3

The alleles having chance to appear in PCR 116 product (exon 4 region) were allele C (88 bp and 28 bp) and allele D (116 bp). Genotypes having chance to appear were genotype CC (88 bp and 28 bp), CD (116 bp, 88 bp and 28 bp), and DD (116 bp). The enzyme cutting sites are shown in Figure 2.

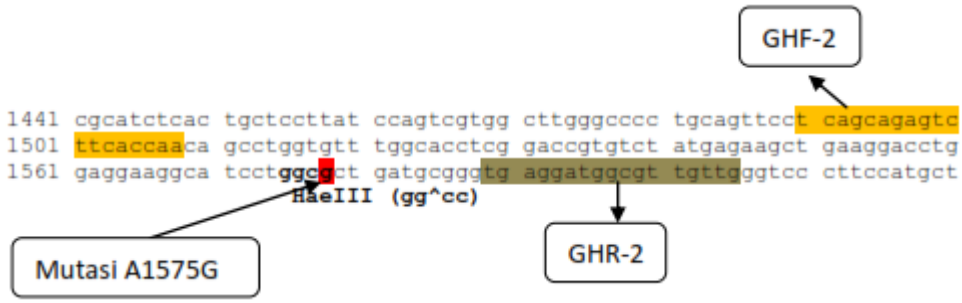


Figure 2. The layout of the enzyme HaeIII cuts in areas exon 4

Data Analysis

The frequencies of genotypes and alleles of GH genes in exon 2, 3, and 4 for each type of goat were estimated by using a formula recommended by Hardjosubroto (1998):

$$*) \text{Frequency of AA genotype} = \frac{\text{number of AA genotype}}{\text{number of individual in population}}$$

*) this formula applies for BB, AB, CC, CD, and DD genotypes

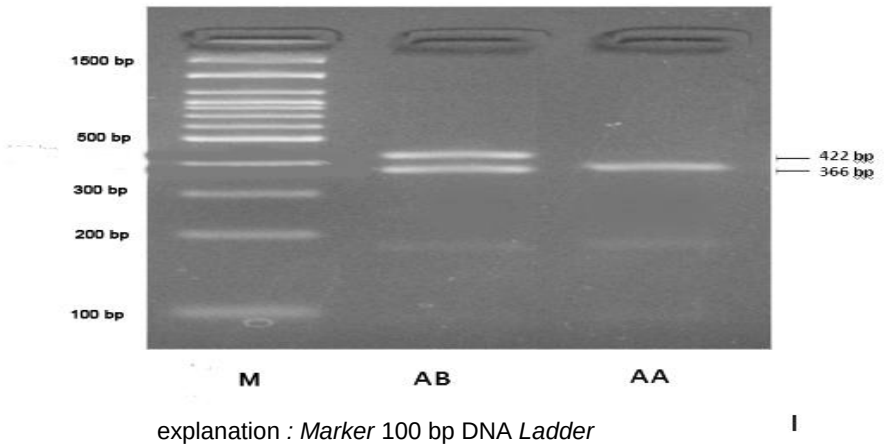
$$**) \text{Frequency of A allele} = \frac{\text{number of locus A}}{\text{sum of (locus A + locus B)}}$$

**) this formula also applies for frequencies of B, C, and D alleles

RESULTS AND DISCUSSION

Genotype in the Saburai Goat Growth Hormone Gene

Single nucleotide polymorphisms (SNP) A781G located in exon 2 to exon 3 were used to identify genotypes through PCR-RFLP procedures. Two fragment in PCR 22 base pair (bp) product digested by using *Hae*III restriction enzyme were identified successfully. AA genotype was identified as fragments with size of 366 and 56 bp. Three fragments were identified as AB genotype with sizes of 422, 366 and 56 bp (Figure 3).



explanation *: Marker* 100 bp DNA *Ladder*

Figure 3. PCR-RFLP pattern of SNPs A781G GH gene were digested with HaeIII

The research results showed that by using SNPs A781G, 22 Saburai lambs were identified as individuals with AB heterozygotes and 12 lambs as homozygote individuals with AA genotype. However, no individual had BB genotype.

Single nucleotide polymorphisms (SNPs) A1575G which was located in exon 4 could also be used to identify CD genotype with PCR-RFLP procedure. *Hae*III restriction enzyme was also used to digest PCR product with size of 116 bp. Frements with sizes of 116 bp, 88 bp, and 28 bp could be identified in individuals with CD genotype (Figure 4). Letter M in Figure 4 and 5 is a marker as it is recommended by Hua *et al.* (2009). All Saburai lambs identified by using SNPs A1575G were found to have CD heterozygote genotype.

GH gene polymorphism in 34 Saburai lambs showed that BB, CC, and DD genotypes were not found in all observed individuals. Individuals with BB genotype were also not found in the Boer goat population (Hua *et al.*, 2009).

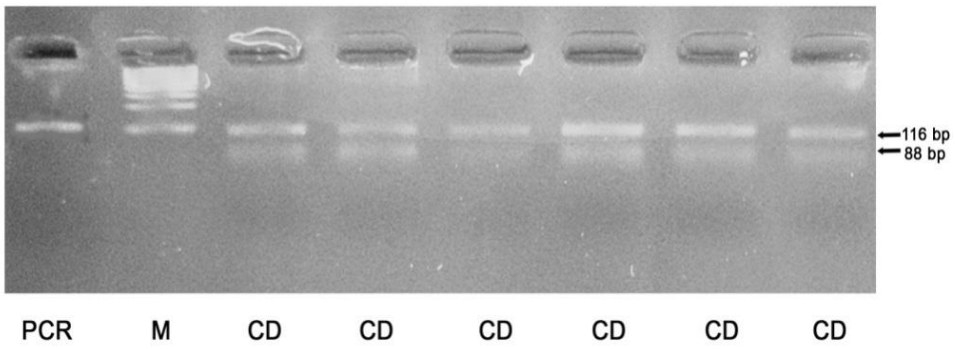


Figure 4. PCR-RFLP pattern of SNPs A1575G GH gene were digested with HaeIII

Frequencies of Allele and Polymorphism

Frequencies of allele A, B, C, and D in Saburai lamb GH gene were respectively 0,68, 0,32, 0,50, and 0,50. Saburai lamb GH gene was polymorphic because the frequencies of these alleles were respectively lower than 0.99. Gene is determined to be polymorphic when alleles in the gene are not more that 0.99 (Harris, 1994).

The allele A frequency (0.68) was higher than allele B (0.32), and this indicated that the change of allele A appearance in the Saburai lamb was higher than allele B. the reason was that allele A appeared in 22 lambs with AB genotypes and 12 lambs with AA genotype, while allele B appeared only in 12 AB genotype lamb. In addition, there was no Sabuari lamb having BB genotype, where this increased the chance of allele A to appear in the observed Saburai lambs.

The research result showed that there was no BB genotype found in SNPs A781G, and there were no CC and DD genotypes were found in SNPs A1575G. It was suspected because of the particular breeding setting. This breeding setting made lower chance of appearance of genotype which regulated characteristics not expected by the livestock farmers.

Unfound particular genotypes were also reported in other researchers. In the male Boer goat population, there was AA genotype found in 25 lambs and AB genotype in 129 lambs, but there was no BB genotype found. Allele A frequency (0.68) in pre-weaning female Saburai lamb in this research was higher than allele

B frequency (0.32). It was also similar to the research result of male Boer goat population which showed that allele A frequency (0.5812) was higher than allele B (0.4188) as it was reported by Hua *et al*. (2009).

The frequencies of allele C and D in this research was respectively 0.50 because the observed female Sabuari lambs were all having CD heterozygote genotype (CD genotype frequency of 100%).

The Correlation of GH Gen Polymorphism with Growth Performance

The research results showed that pre-weaning female Sabuari lambs with AB genotype had higher growth performance than those with AA genotype (Table 3). Higher growth performance of the Saburai lambs with AB genotype showed that GH gene polymorphism correlated to the pre-weaning female Saburai lamb performance. The gene polymorphism is determined to have correlation with a particular performance if this polymorphism is able to group performances in the different groups strictly (Hua *et al*., 2009).

Table 3. Frequency of alleles and genotypes at GH gene female Saburai goat

SNP	Genotype	Number of animals (head)	Genotype frequency	Allele frequency
1	AA	12	0.35	A (0.68)
	AB	22	0.65	B (0.32)
2	CD	34	1.00	C (0.50)
				D (0.50)

Table 4. Performance at birth and weaning Saburai goat kids on genotypes AA, AB and CD

No	Performance	SNPs A781G (*exon* 2 - 3)		T-test	SNPs A1575G
		AA	AB		CD
A.	**Birth**				
1	Birth weight (kg)	2.94 ± 0.05	3.04 ± 0.20	*P*<0.05	2.98 ±0.28
2	Body lenght (cm)	12.83 ±0.76	15.03 ± 0.55	*P*>0.05	14.25±1.23
3	Chest size (cm)	14.46 ± 0.21	15.18 ± 0.55	*P*<0.05	14.95±0.85
4	Chest lenght cm)	6.00 ± 0.16	6.78 ± 0.54	*P*<0.05	6.59±0.79
5	Chest width (cm)	2.18 ± 0.16	4.15 ± 1.00	*P*<0.05	4.47±0.96
6	Ears lenght (cm)	4.22 ± 0.15	5.24 ± 0.63	*P*<0.05	5.46±0.66
7	Ears width (cm)	2.26 ± 0.15	3.15 ± 0.61	*P*<0.05	3.40±0.66
8	Height (cm)	11.12 ± 0.19	12.79 ± 1.02	*P*<0.05	12.83±0.99
B.	**Weaning**				
1	Weaning weight (kg)	29.56 ± 0.67	29.92 ± 1.01	*P*<0.05	28.14±4.98
2	Body lenght (cm)	49.44 ± 0.85	50.40 ± 1.13	*P*<0.05	48.31±6.42
3	Chest size (cm)	59.44 ± 0.81	60.93 ± 1.92	*P*<0.05	58.51±7.92
4	Chest lenght cm)	25.28 ± 0.25	25.46 ± 0.48	*P*<0.05	23.88±3.50
5	Chest width (cm)	16.26 ± 0.18	16.36 ± 0.51	*P*<0.05	15.39±2.25
6	Ears lenght (cm)	14.04 ± 0.73	13.81 ± 0.53	*P*<0.05	12.98±1.67
7	Ears width (cm)	6.86 ± 0.43	7.07 ± 0.45	*P*<0.05	6.69±0.75
8	Height (cm)	50.90 ± 1.01	51.34 ± 0.96	*P*<0.05	49.16±6.65
9	ADG pre weaning (kg)	0.296 ± 0.007	0.299 ± 0.010	*P*<0.05	0.21±0.04

The inborn growth characteristics in individuals with AB genotype was higher than AA genotype except body length which did not show differences statistically (P>0.05). The growth performance at weaning from individuals with AB genotype was also higher than those with AA genotype (Table 3).

Higher growth performance of individuals with AB genotype than those with AA genotype indicated that genotype AB controlled higher growth in the observed Saburai lambs.

The explanation above indicates that female Saburai lamb GH gene polymorphism in exon 2 which is detected with SNPs A781G has a correlation to pre-weaning growth performance. Therefore, GH gene polymorphism is suspected to be able to use for a marker in selecting pre-weaning Saburai goat growth performance. Other researches also showed the correlation between polymorphism of GH gene detected with SNPs A781G in *exon* 2 to pre-weaning and post-weaning male Boer goats (Hua *et al*., 2009).

All of the Saburai goats in this research had CD genotype which showed weaning weigth of 28.14 kg, body length of 48.31 cm, and body height of 49.16 cm. Saburai goat body length and height in this research result are not significantly different to the research result of Hua *et al.* (2009). The body weight differences are suspected to be caused by genetic and environment differences. Observed Saburai goats in this research have PE genetics of local goat, so that cross breeding goat is easier and faster to adapt with local environment conditions. Boer goat is east to have adaptation with varying environment conditions so that it is often to be cross bred with other types of goats. Characteristics to consider when selecting type of goat for goat feed lot business are its adaptation ability, reproduction characteristic, and growth performance. Goat with high adaptation ability will be able to have maximum reproduction where ever it is raised (Casey & Van Niekerk, 1988).

CONCLUSION

It was concluded that the growth hormone gene in exon 2 to exon 3 (SNPs A781G) was polymorphic and has correlation to growth performance pre weaning female Saburai goat.

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