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Polymorphism of Growth Hormone Gene in Selecting Etawah Crossbred (PE) Goats

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ABSTRACT

Although Etawah Crossbred (PE) goat is considered to be dual purpose (meat and milk) goat, it is mainly raised for meat production. Since early 1990, there has been a growing interest of the farmer in some places to raise PE goat for milk production without sacrificing its role to produce kids for meat. Although milk yield of PE goat was not as high as milk yield of some other dairy goats, the ability of PE goat to cope with harsh local environment, particularly climate and feed conditions, was an advantage. Therefore, raising PE goat would still be an important part of farmer activities in the rural areas in Indonesia. Identification of the genes underlying livestock production traits leads to more efficient breeding programs and it is a promising way to improve production traits of farm animals. Growth hormone is a polypeptide hormone which is the major regulator of the metabolic procedures of growth and development and it is encoded by GH gene. The objective of this study was to detect the genetic polymorphism of GH gene in major Etawah Crossbred (PE) goat using PCR-RFLP. The PCR amplified fragment were digested with *Hae*III endonuclease and the result showed the presence of two genotype CC and CD. The total frequency were 47.0% and 53.0% for CC and CD genotype respectively in 94 tested goats. Statistical analysis showed that in the fragment amplified by the pair of primer, CD genotype had significant higher birth weight and weight of 100 days old (weaning weight) than CC genotype ($P<0.01$). In conclusion that GH gene may be a mayor gene or linked to the mayor gene to affect the weight traits and the polymorphic site could be used to select the goat weight in marker-assisted selection program.

Keywords: goat; birth weight; weaning weight; growth hormone (GH); PCR-RFLP.

INTRODUCTION

Etawah Crossbred (PE) goats, widely seen in the East Java Indonesia, are noted for their high milk production and meat production quality. They represent a unique genetic resources by virtue of their adaptability, resistance to many infectious diseases and prolificacy in the tropics of Indonesia. Animal exhibiting high genetic merit in growth and body measurements receive high priority in breeding programmers for meat purpose. Although, lot of progress has been achieved in animal improvement using conventional breeding methods, environmental influences limits accuracy of such methods for improving polygenic traits like body measurements. However, the genetic improvement of such traits can be enhanced by marker assisted selection, which is highly accurate in estimating breeding value of animals (Dekkers, 2004).

In view of the pivotal role of growth hormone in animal growth and development, GH gene may be used as a candidate gene for studying its polymorphism and association in relation to

growth. Growth Hormone (GH) plays a very important role in the growth of livestock as well as other biological processes such as metabolism, lactation and reproduction (Suparkon et al., 2007), and the pattern of which plays an important role in postnatal longitudinal growth and development, tissue growth, lactation, reproduction as well as protein, lipid, and carbohydrate metabolism (Akers, 2006). The growth hormone (GH) secreted by the pituitary gland exerts a key control in nutrient use, mammary development and growth and also modulates intermediary metabolism and other physiological process. Therefore, the GH gene is a promising candidate gene worth studying for its effects on milk performance, growth related traits and reproduction traits (Franco et al., 2005).

GH gene with its functional and positional has been widely used for marker in several livestock; including the dairy cattle (Maylinda, 2011), buffalos, goats (Chitra and Aravindhakshan, 2004; Hua et al., 2009; Wickramaratne et al., 2010; Marini et al., 2015 and, Singh et al., 2015) and GH gene can be used as a candidate gene for studying its polymorphism and association in relation to growth.

Recently, genetic polymorphism at candidate genes affecting economic traits have stimulated research interest because it is considered as an aid to genetic selection and to mark evolutionary relationship in different livestock breeds (Sodhi et al., 2007). Genetic polymorphism can be identified by several techniques. The most common method is polymerase chain reaction- restriction length polymorphism (PCR-RFLP). Hua et al. (2009) reported that there are two alleles at the GH gene locus in Boer goat bucks and had association with growth traits. The objectives of this study were to determine polymorphism of GH gene and its association with birth weight and weaning weight in Etawah crossbred goats.

MATERIALS AND METHODS

Blood Collection and DNA Extraction.

A total of 94 blood samples were collected from kids PE goat in keep by small farmer in Ampelgading East Java. Blood samples were placed into an EDTA tubes for DNA isolation. Genomic DNA was isolated using Genomic DNA Isolation kit (NORGEN) following the manufacturer's protocol. The quality of DNA was checked on 1.0% agarose gels and stained with ethidium bromide.

Amplification of GH Gene by PCR.

The polymorphism was performed by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) as proposed Hua et al. (2009). The sequences of the forward and reverse primers for the amplification of the GH gene (Accession: D00476) were:

Forward:
F5'-TCA GCA GAG TCT TCA CCA AC-3'.
Reverse:
5'-CAA CAA CGC CAT CCT CAC-3'.

PCR for the GH gene was performed PCR Master mix Solution, (intron) : in a 5 µl reaction mixture containing 2 µl ddH2O; 1 µl Primer F 1 µl Primer R; 5 µl master mix and 1µl of genomic DNA template. Thermal cycling conditions included: an initial denaturation step at 94°C for 5 min followed by 35 cycles of 94°C for 30s, 52°C for 30s, 72°C for 30s and a final extension at 72°C for 10 min. The PCR products were digested with 0.2 µl *HaeIII* restriction endonuclease (Fermentas) at 37°C for at least 60 min. PCR products and restriction fragments were electrophoresed on 2% agarose gels

respectively and stained with ethidium bromide.

Statistical Analysis.

Direct counting was used to estimate genotype and allele frequencies of GH gene genetic variants. Chi-square statistic ($\chi 2$) was used to check whether the populations were Hardy-Weinberg equilibrium.

RESULTS AND DISCUSSIONS

Amplification of GH Gene.

Recently, genetic polymorphisms at candidate genes affecting economic traits have stimulated research interest because it is considered as an aid to genetic selection and to mark evolutionary relationships in different livestock breeds (Sodhi et al., 2007).

GH gene fragments were successfully amplified is 116 bp in length (Figure 1). As a result of amplification product with *Hae*III digestion, two alleles, C and D, were observed. Restriction digestion of 116bp (Figure 2) an d PCR products with *Hae*III enzymes revealed two genotypes of CC (116 and 88 bp), CD (116, 88 and 28 bp) (Figure 2).

The pattern variation obtained from PCR-RFLP results using *HaeIII* enzyme shows the polymorphism of the GH gene in the PE goat, the potential factors of polymorphism are selection, migration, and mutation.

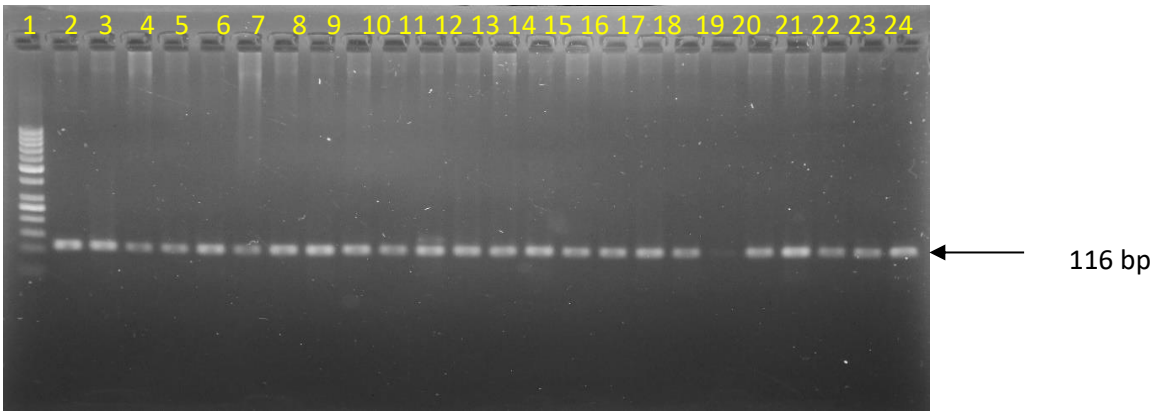

Figure 1. Visualization of GH Gene Amplification on 1.5% agarose gel. Lane M=1000 bp DNA Marker, Lanes 1-25= PE goat samples.

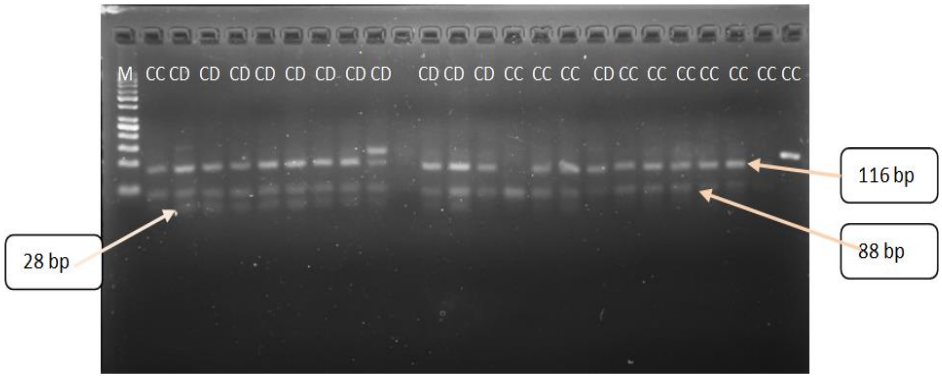

Figure 2. Electrophoresis profile for the GH gene in Etawah crossbred goats after digestion by *Hae*III. CC homozygous genotype with two digested fragment at 116 and 88 bp; CD heterozygous genotype with three digested fragment at 116, 88, and 28 bp.

Genotype and Alleles Frequency.

Genotype frequency is the proportion of individuals of each genotype in which the description of gene frequencies involves identifying alleles at each locus were analyzed and calculated the proportion of different types of alleles (Zein et al., 2012). Allele frequency is the relative frequency of an allele in a population or an allele of the total number of alleles present in a population (Nei and Kumar, 2000).

The frequencies of CC and CD genotype were 47.0 and 53.0% and the frequencies of allele C and D were 73.0 snd 27.0%. The results of Chi-square statistic reflected that breeds were in Hardy-Weinberg equilibrium. The present result agrees the previous results obtained by Hua et al. (2009) and Zhang et al. (2011), where presence of two genotype homozygous and the other heterozygous.

Association of Genotypes with Growth Traits.

In the present study, we assessed the association between different GH genotype and growth traits including birth weight and weaning weight (100 days old). The genotype CD is associated with high and best growth parameters in Table 1. The genotype CD was associated with significantly high ($P<0.01$) weaning weight and Thomas et al. (2007) reported that heterozygous genotypes for two GH polymorphisms appeared advantageous for traits of muscularity and adiposity in the cooperating breeding program. This genotype CD is associated with high and best growth traits parameters like birth weight, weaning weight (100 days old) and average daily gain (ADG). Hua et al. (2009) analyzed the polymorphism of GH gene as a genetic marker candidate for growth traits in Boer goat bucks. Two single nucleotide polymorphisms A781G and A1575G were identified by GH gene sequencing and PCR-RFLP analysis. AA genotype resulted in a significant decrease in birth chest girth ($p>0.03$) and weaning weight ($p>0.014$) comparing to AB genotype while CC genotype contributed to weaning height ($p>0.04$) greater than CD genotype.

It may be due to the presence of both serine and glycine amino acid in the heterozygous animals and this amino acids are interconvertible; when the body needs any of them, it does not obtained it from the digestion of food, it uses glycine to produce serine and vice versa. According to Othman et al. (2015) serine involved in the metabolic processes that burn glucose and fatty acids for energy and the body uses serine to make creatine which combines with water to “pump up” muscle mass.

Table 1. Effect Of The GH Gene Genotypes on Birth Weight and Weaning Weight (100 Days Old) of Etawah Crossbred Goats.

Growth Trait	Genotype CC (n= 44)	Genotype CD (n = 50)
Birth weight (kg)	3.64±0.79	3.78±0.66
Weaning weight (100 days old) (kg)	18.82±2.98	22.89±3.97
Average daily gain (ADG) (kg/day)	0.15±0.02	0.19±0.03

CONCLUSION

In conclusion, production improvement can be achieved by using new genetic technology for better selection of heritable traits through marker-assisted selection. GH gene may be a major gene or linked to the major gene to affect the weight traits and the polymorphic site could be used to select the goat weight in marker-assisted selection program. Due to the reported association between genotype possess CD genotype

with different growth trait parameter in Etawah crossbred (PE) goats.

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