The 6th International Seminar on Tropical Animal Production
Integrated Approach in Developing Sustainable Tropical Animal Production
October 20-22, 2015, Yogyakarta, Indonesia

Genotypic Profile of Ettawa Grade Goat with Different Head and Neck Color Based on MC1R Gene

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ABSTRACT: The exterior characteristic is the main reason for the most farmers in Indonesia to select and keep Ettawa grade goat. The farmers prefer to keep the goats with black head color instead of brown or mixed colors due to the price of black head color is more expensive. In order to indicate the genetic molecular basis of different head and neck color of Ettawa grade goats, a comparative analysis of *MC1R* gene polymorphism was conducted. The *Melanocortin-1-receptor* (MC1R) gene is known as an important candidate gene for the coat color trait. Total thirty Ettawa garde goats were divided in three group: CP (brown head and neck color with white body color), RP (brown or black head and neck color with various body color), HP (black head and neck color with white body color) were used in this study. The blood samples from all groups were collected for DNA isolation. The single nucleotide polymorphisms (SNP 676A<G) in exon 1 which located at 676 bp in MC1R gene were obtained by PCR-RFLP methods for genotyping of the goats by using *ear*I restriction enzyme. The results showed all of the goats in CP and RP groups were heterozygote (AG Genotype) which indicated 1.00 for their genotype frequencies. In HP group only had one goat with homozygote animal (GG genotype). Interestingly, none AA genotype found in this study. The A and G allele frequencies were similar 0.5 in both CP and RP group. However, the A allele frequency (0.55) was slightly higher than G allele (0.50) in HP group. These results indicated the spread of both alleles were equal in all groups and seems less genetic variability in the goat population study. In conclusion, the SNP 676A<G of MC1R gene may be regulated genetically in Ettawa grade goat with different head color. Further study need to be conducted in detecting the association of the gene that may affect production traits.

Key words: Genotypic, Phenotypic, Ettawa grade goat, Head and neck color

INTRODUCTION

Ettawa Grade goats are one of potential animals in Indonesia which are widely raised in small farmers. To increase the income, the farmers prefer keep the goat with black head and neck color due to the high price compare to other goats color. Those specific goats are also kept by farmers for goat competition purpose. However, the farmer's perception believed that color differences will not have impact on the productivity of goats. Initially, this perception needs to be confirmed based on genetic molecular basis which can describe genetic variability of the goat with different head color. In order to detect the genetic variability of goats, a comparative analysis of MC1R gene polymorphism was conducted.

The *Melanocortin-1-receptor* (MC1R) gene plays a central role in regulation of animal coat color formation. The gene has been widely used to identify the coat color in various ruminants

such as in sheep, cattle, goat and rabbit. The haplotype AATGT in MC1R was uniquely associated with black coat color in Minxian Black-fur breed (Yang *et al.*, 2013). The coat color *extension gene* (E) which encoded the transmembrane domain MC1R have been indicated affecting the coat color in French cattle breed (Rouzaud *et al.*, 2000). In goat, the p.267W missence mutation located in coding region of MC1R was present in all Murciano-Granadina black goats, whereas it was never identified in the brown ones (Fontanesi *et al.*, 2009). The c.[124A;125_130del6] was suggested responsible for a MC1R variant determining eumelanin production in the black area of the rabbit (Fontanesi *et al*., 2010). Moreover, the mutation at the position 676 bp of MC1R gene had been detected in Boer Goat with red head and neck color (Wu *et al.*, 2006). Therefore, the objective of this study was to identify the genetic profile of Ettawa grade goat with different head color based on MC1R gene.

MATERIALS AND METHODS

Animal and sampling. Thirty Ettawa grade goats which divided in three groups: CP (brown head and neck color with white body color), RP (brown or black head and neck color with various body color), HP (black head and neck color with white body color) were reared in the field laboratory in Faculty of Animal Science, Universitas Gadjah Mada (FAS UGM) with the same environments condition. The blood samples were collected for genomic DNA isolation using SDS/ ProteinaseK modified method (Sambrook *et al.*, 1989).

Polymerase Chain Reaction (PCR). The primer sequences (according to Wu *et al*., 2006), annealing temperature for PCR amplification and the restriction enzyme for PCR-RFLP are shown in Table 1.

Table 1. Primers for PCR amplification and restriction enzyme information for genotyping of MC1R gene

GenBank Acc. No	Primer	PCR product size	Restriction enzyme
Y13958	E1-F : 5' gtggaccgctacatctccat 3' E1-R : 5' ttgaagatgcagccacagg 3'	416 bp	EarI

Amplifications were performed at 10 min at 94ºC, 35 cycles of 30 s at 94ºC, 30 s at the annealing temperature (64ºC), and 30 s at 72ºC, and a final extension of 10 min at 72ºC. The PCR products were visualized in 1.5% standard agarose gels stained with ethidium bromide.

PCR-RFLP and Genotyping Determination. PCR products were sequenced using the same primers for PCR by PT Genetics Science Indonesia. The DNA sequences were analyzed with the BioEdit program ver. 7.00 (Tom Hall, Ibis Therapeutics, California, USA) and the SNP676A>G was confirmed based on the electrophoregram results. The SNP G.676A>G was genotyped by the PCR-restriction fragment length polymorphism (PCR-RFLP) method. The restriction enzyme digestion was performed in 20 µl reaction volumes with approximately 5 µl of PCR products and 2 units of each restriction enzyme. The digested products were run on 3% agarose gels.

Statistical Analysis. A chi-square test was performed to test the allele and genotype frequencies for Hardy Weinberg equilibrium. The following mathematical model was:

$$X^2 = \sum_{i=1}^{n} \frac{(Oi - Ei)}{Ei}$$

Where, X2 is Chi-square value, Oi is observed frequency, Ei is expected frequency, n is the number of possible outcomes of each event.

RESULTS AND DISCUSSION

SNP g.676A>G in MC1R gene was initially identified by direct sequencing using PCR product pool. The SNP was confirmed by BioEdit program and used for genotyping the goats (Fig.1a). Homozygote AA and GG were defined when the fragments size being recognized at 162 and 254 bp, and 416 bp, respectively. The heterozygote AG existed by PCR-RFLP method at the same position of the homologous chromosome with 162, 254 and 416 bp of fragments size (Fig.1b). As the results, most of animals in three groups have heterozygote (AG) genotype. Only one animal have GG genotype in HP group and no AA genotype detected in the study. The allele and genotype frequencies are shown at Table 2.

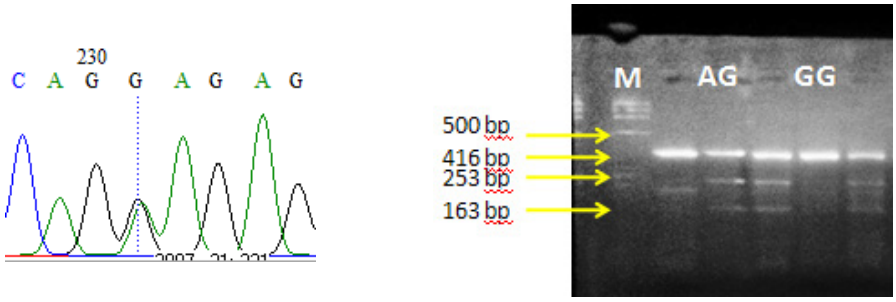

Figure 1 (a). Electrophoregram result for the identified SNP g.676A>G in MC1R gene with G & A peaks. (b) PCR-RFLP patterns of SNP g.676A>G (digested with EarI restriction enzyme).

Table 2. Frequencies allele and genotype of three groups Ettawa Grade Goats based on PCR-RFLP results using SNP g.676A>G

Group	Allele Frequency		Genotype Frequency		
	A	G	AA	AG	GG
CP	0.50	0.50	0.00	1.00	0.00
RP	0.50	0.50	0.00	1.00	0.00
HP	0.55	0.45	0.00	0.90	0.10

Based on Table 2, the results indicated the spread of both alleles A and G were equal in all groups. The A and G allele frequencies were similar 0.5 in both CP and RP group. However, the A allele frequency (0.55) was slightly higher than G allele (0.50) in HP group. The AG genotype frequencies were similar 1.00 in both CP and RP group. However, no AA genotype was identified in the study.. The results of Pearson 's Chi-square test indicated that the genotypes of the goats were deviated from the Hardy-Weinberg equilibrium (HWE). The deviation may due to variation of causes. Mutation, gene flow, non-random mating (assortative mating), genetic drift and selection

may lead to deviate from HWE (Falcorner and Mackay, 1996). In case of this study, non-random mating and small sample size may due to the deviation.

CONCLUSIONS

In conclusion, the Ettawa Grade goats with different head and neck color have same AG genotypes based on MC1R gene. Our results suggest that the SNP 676A<G of MC1R gene may associate with hair color and can be used to determine the genotype profile of Ettawa Grade goats

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