TRANSFERRIN POLYMORPHISM IN FOUR LOCAL BREEDS OF GOAT IN CENTRAL JAVA, INDONESIA

E. Kurnianto, Sutopo, E.T. Setiatin, D. Samsudewa, E. Purbowati, D.R. Dewanti and G.D. Brata
Faculty of Animal Science and Agriculture, Diponegoro University, Tembalang Campus, Semarang 50275 - Indonesia
Corresponding E-mail: kurniantoedy17@gmail.com

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ABSTRAK

Tujuan penelitian ini adalah menentukan frekuensi gen dan heterosigositas individual transferin pada empat bangsa kambing lokal di Jawa Tengah-Indonesia. Sampel darah diambil dari 96 ekor kambing, masing masing sebanyak 24 ekor kambing Kejobong (Kabupaten Purbalingga), Peranakan Etawah (Kabupaten Purworejo), Kacang (Kabupaten Grobogan) dan Jawarandu (Kabupaten Pemalang). *Polyacrilamide Gel Electrophoresis* digunakan untuk mengetahui tampilan pita-pita protein plasma darah. Frekuensi gen transferin dihitung dengan rumus umum genetika populasi. Heterosigositas yang diamati dan heterosigositas harapan duji dengan Chi-square untuk mengetahui status keseimbangan Hukum Hardy Weinberg untuk gen transferin (*Tf*). Hasil penelitian menunjukkan terdapat dua alele transferin, yaitu *Tf*A dan *Tf*B. Frekuensi gen *Tf*A lebih tinggi dibandingkan dengan *Tf*B. Heterosigositas transferin mengindikasikan tidak dalam keseimbangan Hukum Hardy Weinberg.

Kata kunci : frekuensi gen, transferin, heterosigositas, kambing

ABSTRACT

The objectives of this study were to determine the gene frequency and individual heterozygosity of transferrin in four local breeds of goat in Central Java-Indonesia. The number of blood samples were taken from 96 heads of goat, in which each of breeds were 24 samples, those were Kejobong (Purbalingga regency), Ettawa Grade (Purworejo regency), Kacang (Grobogan regency) and Jawarandu (Pemalang regency). Polyacrilamide Gel Electrophoresis was performed to detect the bands of blood plasm protein. Gen frequency was calculated using general formula of population genetics. Estimated heterozygosity and individual heterosizygosity were calculated to analysis the equilibrium condition of transferrin. Result showed there was two allele of transferrin, namely TfA and TfB. Gene frequency of TfA was higher than that of TfB. Transferrin gene and genotypes were in disequilibrium of Hardy-Weinberg Law.

Keywords : gene frequensi, transferrin, heterozigosity, local goat

INTRODUCTION

Eight breeds of Indonesia local goat have been identified by Research Institute for Animal Production (Pamungkas *et al.*, 2009; Prabowo, 2010), namely Marica, Samosir, Muara, Kosta, Gembrong, Kacang, Ettawa grade, and Benggala. In Central Java, there are four local breeds of goat, those are Kacang, Ettawa grade, Jawarandu and Kejobong. The first three breeds have been well-known because of its dissemination, but the last one has lack of information. Kejobong goat is just exists in Purbalingga Regency. Early study of phenotypic characteristics of Kejobong goat was conducted by Purbowati and Rianto (2010).

Study on goat breeding is very important to detect the genetic quality of that animal. The importance of goat quality is needed in order to select good performance of goat from a population (Kurnianto, 2009). Selection on a certain trait can be applied when base information on genetic variation of a population is available. According to Falconer and Mackay (1996), phenotypic variation is expression of genetic and environmental variation. Analysis of genetic variation in animal more indicates the genetic

condition of animal breed. Study on genetic variation through the blood protein polymorphism have been conducted in many countries, such as China, Argentine, and Japan (Nozawa *et al*., 1978; Katsumata *et al*., 1981; Chen *et al*., 2009; Deza *et al*., 2000). The blood plasm protein locus frequently observed are pre-albumin (*P-alb*), albumin (*Alb*), ceruloplasmin (*Cp*), transferrin (*Tf*), post transferrin (*P-tf*) and amylase-I (*Amy-I*). Information about the genetic status of local breed for breeding program is very important. Unfortunately, there is limitation of study on genetic variation in indigenous breed of Indonesia goat. On the basis of that reason, study on genetic variation in indigenous breed of goat was conducted. The objectives of this study was to evaluate the transferrin gene in Kejobong, Ettawa grade, Kacang and Jawarandu goats.

MATERIALS AND METHODS

Materials

Ninety six blood samples collected from the adult local breed of goat in Central Java were used as materials. Those samples were originated from Kejobong goat (Purbalingga Regency), Ettawa grade (Purworejo Regency), Kacang (Grobogan Regency) and Jawarandu (Pemalang Regency) as many as 24 samples, respectively. All of goats were raised by farmers under traditional system.

Sample Analysis

The blood sample was taken from jugular vein by syringe. The 5 ml blood was drawn into tube containing EDTA as anticoagulant. Blood plasm was separated from blood cell by centrifugation on 3500 rpm at room temperature during 10 minutes. For analysis process, blood plasm was drawn into labeled sample tube and stored in freezer until tested.

Polyacrilamide Gel Electrophoresis was performed according to Ogita and Markert (1979). Interpretation about gene structure was carried out to visualize the protein bands at six locus, those were pre-albumin (*P-alb*), albumin (*Alb*), ceruloplasmin (*Cp*), transferrin (*Tf*), post transferrin (*P-tf*) and amylase-I (*Amy-I*). Only transferrin gene was evaluated in this study.

Data Analysis

Gene frequency of each locus was determined by calculating a gene from all genes of interest (Warwick *et al*., 1990). Genetic variation at each breed of goat was calculated based on the performance of gene structure expressed as heterozigosity. Observed heterosigosity (Ho) and individual heterozygosity or expected heterozygosity (He) were calculated as described by Hartl (1988) and Nei (1973) below:

$$H_o = \sum \frac{n}{N} \quad \text{..... (1)}$$

Where H_o = observed heterozygosity; n = the number of individual having heterozygous transferrin genotype, N = the number of observed individual.

$$H_e = 1 - \sum_i q_i^2 \quad \text{..... (2)}$$

Where H_e= individual heterozigosity; q = i^{th} gene frequency at a transferrin locus. Hardy-Weinberg equilibrium for transferrin locus was tested between H_o and H_e by Chi-square (Steel and Torrie, 1980). Differences of body weight expressed by each of transferrin genotype were analyzed by SAS package (2004).

RESULTS AND DISCUSSION

Studies on genetic variation through the polymorphism are often conducted to detect genetic differences within population or between populations. Polymorphism was illustrated by protein plasm bands. Figure 1 presents one of slabs and diagrammatically scheme of blood protein locus. Transferin (*Tf*) moved slower than Pre-albumin (*Pre-alb*) and Albumin (*Alb*), but faster compared to Post-transferrin (*P-tf*) and Amylase-I (*Amy-I*). Moving of gene structure in electrophoretic tool relates to molecular weight (MW). Small MW showed slow motion. Meanwhile, the thickness of band was depend on protein content. The thick band indicated more content of protein. Transferrin has a molecular weight of around 80 KDa.

Transferrins are iron-binding blood plasma glygoprotein that control the level of free iron in biological fluids (Crichton and Charloteaux-Wauters, 1987). Transferrin imbalance can have serious health effects for those with low or high serum transferrin levels.

Observation to the transferrin band in four breeds of goat showed that two alleles were found in trasferrin locus, that was *Tf*A and *Tf*B. On the basis of gene expression theory on a trait, those

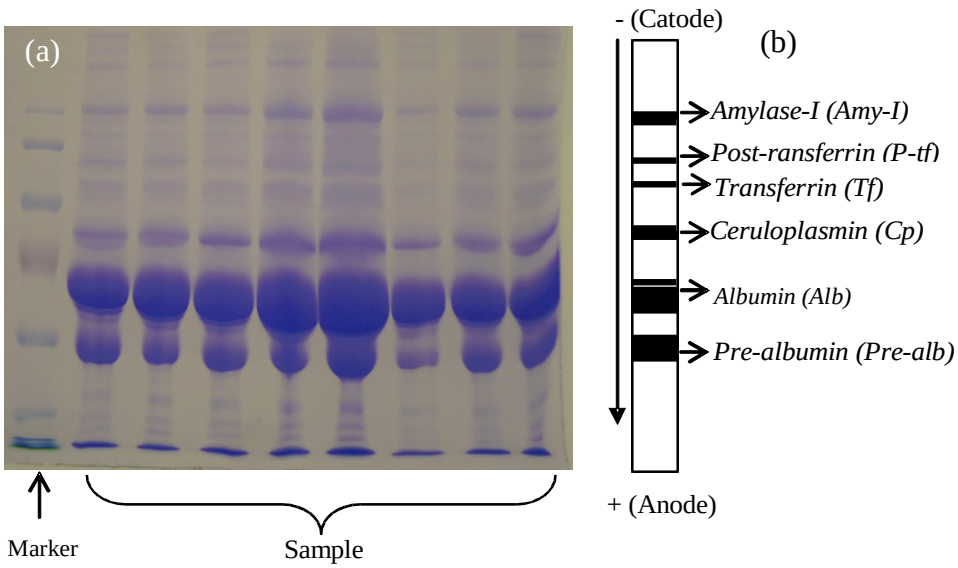

Figure 1. One of Slabs Illustrating Bands Observed from Electrophoretic (a) and Diagrammatically Scheme of Locus (b)

Table 1. Genotype and Gene Frequency at Transferin Locus in Four Local Goat

Breed	No. Sample	Genotype			Gene Frequency	
		AA	AB	BB	*TfA*	*TfB*
Kejobong	24	14	-	10	0.58	0.42
PE	24	15	1	8	0.65	0.35
Kacang	24	14	-	10	0.58	0.42
Jawarandu	24	8	-	16	0.33	0.67

two alleles controlled three genotype of transferrin (*Tf*AA, *Tf*AB and *Tf*BB) as autosomal codominant. In Ettawa grade, two allele formed codominant *Tf*AB as many as 1 sample. In other three breeds of goat, there was no codominant of transferrin allele. This result was in parallel to the results of Asher (1981) and Wang *et al.* (1991). Asher (1981) conducted the analysis of gene frequency in undomesticated goat (feral goat) di New Zealand, characterized gene structure by giving symbol *Tf* I-I, *Tf* I-II and *Tf* II-II. Wang *et al*. (1991) showed the results of study in five breeds of goat in USA and found the *Tf*A and *Tf*B. Before that, Watanabe and Suzuki (1973) found one allele of *Tf*C in goat population in Asia, in which TfC was the gene move slowly in running electrophoresis. Güney *et al.* (2003) conducted an experiment to detect haemoglobin and transferrin in Damascus goats by using a starch gel electrophoresis. It has been determine that nine transferrin genotypes, namely Tf AA, AC, AD, BB, BC, BD, CC, CD and DD exists, in which C and D are two common occurring allele at the b locus and A is the rare b-globulin variant. .

The number of allele of transferrin locus in sheep is more than that of in goat. Chudoba and Jablonska (1981) showed five allele of *Tf*A, *Tf*B, *Tf*C, *Tf*D and *Tf*E forming 13 genotypes.

Genotype and gene frequency in four breeds of goat in Central Java is presented in Table 1. Results showed the polymorphic character in transferrin locus. Gene frequency of *Tf*A was most frequent and larger than *Tf*B in Kejobong, Ettawa grade and Kacang goats (0.58-0.65 *vs* 0.35-0.42), whereas in Jawarandu the gene frequency of *Tf*A was smaller (0.33 *vs* 0.67). Chudoba and Jablonska (1981) and Yadav *et al*. (2010) who conducted study in sheep showed that *Tf*D was the

Table 2. Observed Heterozygosity (Ho) and Individual Heterozygosity (He) of Transferrin Locus

Breed	H_o	H_e	$X^2_{calculation}$
Kejobong	0.00	0.49	23.99
PE	0.04	0.46	19.84
Kacang	0.00	0.49	23.99
Jawarandu	0.00	0.44	24.02

Table 3. Transferrin Genotype and Body Weight in Four Local Goats

Genotype	Kejobong		Ettawa Grade		Kacang		Jawarandu	
	N	BW (kg)	N	BW (kg)	N	BW (kg)	N	BW (kg)
AA	14	34.6±11.1	15	44.2±14.3^{ab}	14	24.8±6.3	8	40.0±11.6
AB	0	-	1	62.2± 0.0^{a}		-		-
BB	14	31.7±12.0	8	32.6± 8.6^{b}	10	27.4±9.2	16	35.0±11.0

Different superscript at the same column indicate significantly different (P<0.05); N = number of sample; BW = body weight

highest gene frequency in transferrin locus.

Observed heterozygosity (Ho) and individual heterozigosity (He) are presented in Table 2. The high values of individual heterozigosity obtained in this study (0.44-0.49) indicated genetic variation in transferrin locus in four local goats. It means that there was possibility to conduct selection program to achieve good performance. Basically, the objective breeding program is to improve the genetic quality and population through selection on the basis of proper criteria. It was stated by select animal. Chi-square test showed highly significant in four breeds of goat, it means that each of goat population was in disequilibrium of Hardy-Weinberg Law. This result indicated that there is no guarantee for gene frequency and genotype frequency will be similar in the next generation.

Analysis of goat genotype and body weight is summarized in Table 3. In Ettawa grade, body weight was significantly affected by transferrin genotype, whereas in other three breeds there was no effect of genotype on body weight. Significantly different found in Ettawa grade was because of small number of AB heterozygous genotype expressing 62.2 kg compared to both of AA and BB. Transferrin gene and haemoglobin might give effect on performance, either in goat or sheep (Menrad *et al.*, 1994), but Güney *et al.* (2003) proved there was no relationship between transferrin and body measurements.

CONCLUSION

It was found two allele of *Tf*A and *Tf*B in four breeds of local goat in Central Java-Indonesia. Gene frequency of *Tf*A was higher than that of *Tf*B. Gene frequency and genotype frequency of transferrin were not in Hardy Weinberg equilibrium.

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