GENETIC VARIATION IN THREE BREEDS OF INDONESIAN LOCAL DUCKS BASED ON BLOOD AND EGG WHITE PROTEIN POLYMORPHISM

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ABSTRAK

Penelitian keragaman genetik pada 4 lokus protein darah dan 3 lokus putih telur dari 3 bangsa itik lokal Indonesia (itik Magelang = MAD, itik Tegal = TED dan itik Mojosari = MOD), dilakukan dengan poliakrilamid gel elektrophoresis (PAGE). Hasil penelitian menunjukkan bahwa 4 lokus darah (Pre-albumin, Albumin, Transferrin and Post-transferrin) and 3 lokus protein putih telur (Ovalbumin, Ovoglobulin and Conalbumin) ditemukan dalam bentuk polimorpik pada ketiga itik lokal. Tidak ada perbedaan yang nyata antara protein darah dan putih telur pada variasi genetik. Nilai rata-rata heterosigositas pada 3 jenis itik lokal mempunyai potensi genetik untuk menentukan kontrol sistem perkawinan dan seleksi dalam pemurnian tiap jenis itik. Hubungan kekerabatan yang paling dekat diantara ketiga jenis itik lokal terjadi antara MAD dan TED.

Kata Kunci: Itik lokal, polimorfisme protein, darah, putih telur, keragaman genetik.

ABSTRACT

A study of genetic variation of four blood protein loci and three egg white loci in three breeds of Indonesian local ducks (Magelang duck = MAD, Tegal duck = TED and Mojosari duck= MOD) was investigated by using polyacrilamide gel electrophoresis (PAGE). The result showed that four blood protein loci (Pre-albumin, Albumin, Transferrin and Post-transferrin) and three egg white loci (Ovalbumin, Ovoglobulin and Conalbumin) were found to be polymorphic in three breeds of local ducks. There was no difference on blood and egg white protein polyimorphism. The average value of heterozygosity on three breeds of local ducks had a genetic potential to determine the controlled mating system and selection to obtain pure line. The closest phylogenetic relationship among the three breeds of local ducks occurred between MAD and TED.

Keywords: Local duck, protein polymorphism, blood, egg white, genetic variation

INTRODUCTION

There are several local ducks in Indonesia having opportunity to be developed. Magelang duck, Tegal duck and Mojosari duck included the Indian runner duck breeds having high productivity. Local ducks are still maintained traditionally with uncontrolled mating system causing a genetic variation and lead to different productivity (Muzani *et al*., 2005; Setioko *et al*., 2005; Ismoyowati, 2008). The genetic variation is needed as a basis for the identification and genetic improvement in the term of conservation (Okabayashi *et al*., 2000). The genetic variation is also one of the bases to know the change of selection value in the population (Zhang *et al.*, 2002; Miguel *et al*., 2005; Johari *et al*., 2009). The genetic variation can show an alllele characters from the specific locus or body tissue like blood and egg white protein (Muzani *et al*., 2005; Prasetyo and Ketaren, 2005).

Blood and egg white protein polymorphisms have been reported, mainly in different locus of protein (Ismoyowati, 2008; Johari *et al*., 2009). The protein loci in blood often studied are pre-albumin, albumin, pre-trsnsferrin, trsnsferrin and haemoglobin (Warwick *et al*., 1990; Muzani *et al*., 2005; Johari *et al*., 2008), whereas protein loci from egg white are ovalbumin, conalbumin and lysosim (Ardiningsasi and Hashiguchi, 1998; Inafuku *et al*., 1998; Johari *et al*., 2009). It can be explained that blood and egg white have different

potential of protein content, but both of them can be used to study the genetic variation (Muzani *et al*., 2005; Setioko *et al*., 2005; Ismoyowati, 2008; Johari *et al*., 2009; Mahfudz *et al*., 2011). Consequently, it is very important to know the genetic variation of blood and egg white protein of three breeds of Indonesian local ducks.

This study was conducted to clarify the gene constitutions in three breeds of Indonesian local ducks and to analysis difference of polymorphism between blood and white protein in those breeds of duck.

MATERIALS AND METHODS

Samples

Blood and egg white were collected from 48 Indonesian local ducks of Magelang duck (MAD=16 birds), Tegal duck (TED=16 birds) and Mojosari duck (MOD=16 birds). The electrophoresis of blood protein of Pre-Albumin (Pa), Albumin (Alb), Trsnsferrin Tf) and Post-Trsnsferrin (P-tf) was performed by using PAGE-TLE (Johari *et al*., 2008). For egg white protein, electrophoretic separation of Ovalbumin (Ov), Ovoglobulin (G2) and Conalbumin (Cnb) was performed in 10-12% starch gel electrophoresis by the methods of Inafuku *et al*. (1998) and Kinoshita *et al*. (2001). The blood and egg white protein polymorphisms were used to analyses the genetic variation of local ducks.

Data Analysis

Genetic variability within the population was quantified by measuring the average heterozygosity (H). The average heterozygosity was estimated from the expected proportion of heterozygosity per locus by formula of Nei (1972):

$$H = \frac{1 - \sum_{i=1}^{m} q_i^2}{r}$$

Where q_i = the frequency of the allele at locus, m = the number of allele, and r = the number of locus. The genetic distance between population and average heterozygosity were estimated using the DISPAN (Johari *et al*., 2008).

The polymorphism between blood and egg white protein were tested by using t-test. The chi-square was applied to compare the heterozygosity values between observed and expected values:

$$x^2 = \sum_{i=1}^{k} \frac{(o_i - e_i)^2}{e_i}$$

Where o_i = observed value, and e_i = expected value

RESULTS AND DISCUSSION

Blood Protein Polymorphism

The gene frequencies of blood protein in the three breeds of local dukcs (4 loci) are presented in Table 1. All of 4 loci (P-Alb, Alb, Tf and P-Tf) were polymorphic as illustrated by the high vaule of its frequency gene. The gene frequency of pre albumin locus were varied for each breeds. In MAD, Pa^A gene frequency was relatively lower than that of the Pa^B gene, whereas the Pa^A gene frequency of TED was relatively higher than the Pa^B. The gene frequency of Pa^A similar to Pa^B in MOD. Variation in the frequency of pre albumin gene at this locus was reported in several studies. Okabayashi *et al*. (1999) stated that the overall gene frequency of Pa^A was higher than the frequency of Pa^B in MAD and TED. Pa^A gene frequencies was higher than the frequency of Pa^B, whereas the opposite result occured in MOD. Okabayashi *et al*. (2000) stated that the overall gene frequency of Pa^A was higher than Pa^B. Zhang *et al*. (2002) reported that in the chicken breeds, the gene frequency of Pa^A is generally lower than the frequency of Pa^B.

The results showed that gene frequency of Alb^A in MAD, TED and MOD were 0.719; 0.800 and 0.656, respectively, and gene frequency of Alb^A was generally higher than Alb^B (Table 1.). This is in line with the report of Azmi *et al.* (2006) in a study of "talang benih" ducks, in which the gene frequency of Alb^A was higher than the two other genes, namely Alb^B and Alb^C. The results obtained were in contrast to the results of research conducted by Ismoyowati (2008), in which the Alb^B gene frequency of TED was higher than Alb^A and Alb^C. It was stated by Johari *et al.* (2008) that in the Kedu chicken, Alb^Bgene frequency was higher than that of Alb^C. These differences were possibility due to the different population.

Ismoyowati (2008) explained that the homozygous AA had higher egg production than the heterozygous AB. This is because Alb^A was

Table 1. Gene Frequencies of the Blood Protein Loci and Average Heterozygosity of Three Breeds of Local Ducks

Locus	Allele	Gene Frequencies in Local Ducks		
		MAD (n=16)	TED (n=15)	MOD (n=16)
P-Albumin (*Pa*)	Pa^A	0.469	0.767	0.500
	Pa^B	0.531	0.233	0.500
Albumin (*Alb*)	Alb^A	0.719	0.800	0.656
	Pa^B	0.531	0.200	0.344
Trsnsferrin (*Tf*)	Tf^A	0.406	0.434	0.625
	Tf^B	0.594	0.566	0.375
Posttrsnsferrin (*P-tf*)	$P\text{-}tf^A$	0.312	0.466	0.750
	$P\text{-}tf^B$	0.688	0.534	0.250
Average heterozygosity		0.484	0.447	0.479

n = number of sample; MAD = Magelang duck; TED = Tegal duck; MOD = Mojosari duck

dominant to the Alb^B, in which it could potentially cause a drop in egg production. The results of this study found that the frequency of gene Alb^A was higher than Alb^B, so it can be expected that the three breeds of local ducks will have a high potential for egg production. Among the three breeds of local ducks, the TED has the higher potential to produce eggs compared to the MAD and MOD.

The results of transferrin loci in three breeds of local ducks also showed that there were two different types of alleles, those were the Tf^A and Tf^B. Zhang *et al*. (2002) and Ismoyowati (2008) stated that the transferrin locus allele had three variations, namely Tf^A, Tf^B and Tf^C. The statement was also expressed differently by Johari *et al*. (2008) that the kedu chicken transferrin locus is controlled by two alleles of the Tf^B and Tf^C.

Observations on transferrin loci showed that the frequency of the Tf^B gene in MAD and TED was relatively higher than the frequency of Tf^A gene. This is in line with those reported by Zhang *et al.* (2002), Azmi *et al.* (2006) and Ismoyowati (2008), that Tf^B gene frequency was higher than the frequency of other genes. Observations on the transferrin loci of MOD showed different results, in which the gene frequency of Tf^A was higher than that of Tf^B.

Gene frequency of $P\text{-}tf^A$ in MOD was higher than the gene frequency of $P\text{-}tf^B$ gene . This result was in contrast to the results found in MAD and TED.

Heterozygosity of Blood Protein

The higher average heterozygosity was found in MAD populations being about 0.484 followed by 0.479 for MOD and the smallest average heterozygosity was found about 0.447 for TED (Table 1). These values of heterozygosity were higher when compared to the average heterozygosity reported by Okabayashi *et al.* (1999) being 0.136, 0.118 and 0.135 for MAD, TED and MOD, respectively. Based on the results above, it can be concluded that there is a development of genetic diversity in populations of MAD, TED and MOD during the period of 11 years, which is possibly due to mating and migration. The average value of heterozygosity of this study was higher than the average heterozygosity of local ducks in Vietnam, that was 0.098 to 0.179 (Okabayashi *et al*., 1999) and in Laos, which was 0.118 to 0.132 (Okabayashi *et al*., 2000).

The matrix of genetic distances among the three breeds of local ducks are presented in Table 3. The closest genetic distance was between MAD and TED (0.0168). The fartest genetic distance

Table 2. Gene Frequencies of the Egg White Protein Loci and Average Heterozygosity of Three Breeds of Local Ducks

Locus	Allele	Gene Frequencies in Local Ducks		
		MAD (n=16)	TED (n=15)	MOD (n=16)
Ovalbumin (*Ov)*	*OvA*	0.812	0.750	0.562
	OvB	0.188	0.250	0.438
Ovoglobulin (G_2)	G_2^A	0.875	0.844	0.812
	G_2^B	0.125	0.156	0.188
Conalbumin (*Cnb*)	Cnb^A	0.562	0.594	0.625
	Cnb^B	0.438	0.406	0.375
Average heterozygosity		0.361	0.398	0.494

n = number of sample; MAD = Magelang duck; TED = Tegal duck; MOD = Mojosari duck

Table 3. Matrix of Genetic Distance among Three Breeds od Local Ducks Population

Population	MAD	TED	MOD
- Blood protein loci:			
MAD	0.0000		
TED	0.0168	0.0000	
MOD	0.0322	0.0289	0.0000
- Egg white protein loci:			
MAD	0.0000		
TED	0.0014	0.0000	
MOD	0.0221	0.0126	0.0000

MAD = Magelang duck; TED = Tegal duck; MOD = Mojosari duck

was between MAD with and MOD being about 0.0322. Dendogram of Figure 1 shows that the closest genetic distance was between MAD and TED. In fact, MAD and TED are on the same province, whereas MOD populations located in different provinces so that the MOD are rare to migrate to other area.

Egg White Protein Polymorpism

There were two different types of Albumin alleles, those were Ov^A and Ov^B (Table 2). It was stated by Grunder (1990) that there were two allele of ovalbumin in the chicken (A and B). Repot of Johari *et al.* (2008) showed that there were 3 genotypes in ovalbumin, those were AA, BB and AB. Ovalbumin genotypic characters can be recognized by the thickness of band in electrophoretic, in which ovalbumin with B allele move faster towards than the A allele.

The genetic frequency of ovalbumin loci on three breeds of local ducks showed that overall allele frequency was higher in allele than that of B, which is 0.812; 0.750 and 0.562 in MAD, TED, MOD, respectively. This is consistent with

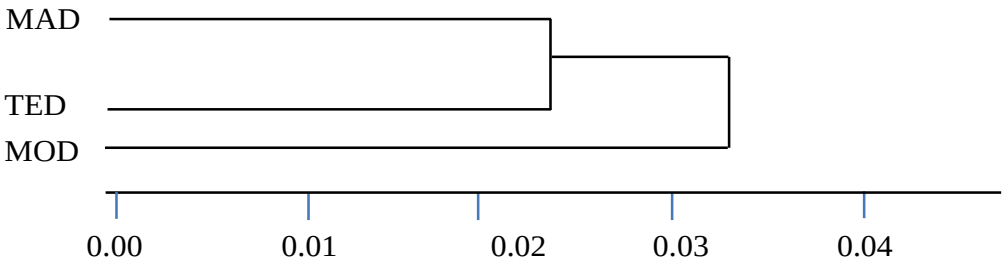

Figure 1. Dendogram of Local Ducks by Blood Protein Polymorphism

that reported by Inafuku *et al.* (1998), in which the ovalbumin was polymorphic loci with the highest gene frequency in allele A. Furthermore, Obeidah *et al.* (1977) found that there was a relationship among body weight, egg weight and ovalbumin, in which the AA genotype had a greater body weight and heavier eggs production than other genotypes. Based on the above, it can be expected that the MAD potential to have a greater body weight and produced heavier eggs than TED and MOD.

The results in the locus of ovoglobulin G_2 showed that overall G_2^A gene frequency was higher than that of G_2^B, that was 0.875, 0.844 and 0.688 in MAD, TED and MOD, respectively. The results were different from those suggested by Obeidah *et al*. (1977), in which the G_2^A gene frequencies were lower than the G_2^B and G_2 gene frequencies. Overall results suggested that three breeds of local ducks had a genetic potential to produce egg that heavier.

Obeidah *et al.* (1977) and an Mahfudz *et al.* (2011) found that no significant effect on egg production in G_2 locus. Furthermore, allele G_2^A had the potential to produce heavier eggs. The results showed that the overall gene frequency of G_2^A was higher, and more AA genotype appeared, so it possibly that the three breeds of duck eggs have the potential to produce heavy egg.

Observations on conalbumin locus showed that the overall gene frequency of Cnb^A was relatively higher than the gene frequency of Cnb^B , those were 0.562; 0.594 and 0.625 in MAD, TED and MOD, respectively. The observation was in contrast to report of Inafuku *et al*. (1998) and Ardiningsasi and Hashiguchi (1988) that the gene frequency of Cnb^B was higher than the frequency of Cnb^C. However, the results of Kinoshita *et al.* (2002) was corresponding with the results of this study, the gene frequency of Cnb^A was higher than Cnb^B.

Heterozygosity of Egg White Protein

There was difference in average heterozygosity in the three breeds of local ducks (Table 2). The highest average heterozygosity found in the population in ordered was MOD (0.494) followed by TED (0.398) and lowest one was found in MAD populations, being 0.361. The average value of heterozygosity was higher when compared to the average heterozygosity reported by Okabayashi *et al*. (1999), being 0.136, 0.118 and 0.135 in MAD, TED and MOD, respectively. The average value of heterozygosity of this study was higher than the local ducks in Vietnam, namely from 0.098 to 0.179 (Okabayashi *et al*., 1999) and the average value of the local ducks in Laos, which was 0.118 to 0.132 (Okabayashi *et al*., 2000).

The matrix of genetic distances between the three types of local ducks is presented in Table 3. It was known that the closest genetic distance was between MAD with TED (0.0014). Genetic distance between the farthest was found between MAD and MOD, being 0.0221.

Genetic distance of three breeds of local duck egg white protein polymorphisms by using the UPGMA program DISPAN by Nei *et al.* (1983) is presented in Figure 2. Dendogram shows that the closest genetic distance is between MAD with TED, when compared to MOD. The difference may be caused by MAD and TED [which] are on the same province, so the movement or migration of ducks may occur frequently. MOD population is located in different province, so rare or no migration to other MAD and TED area.

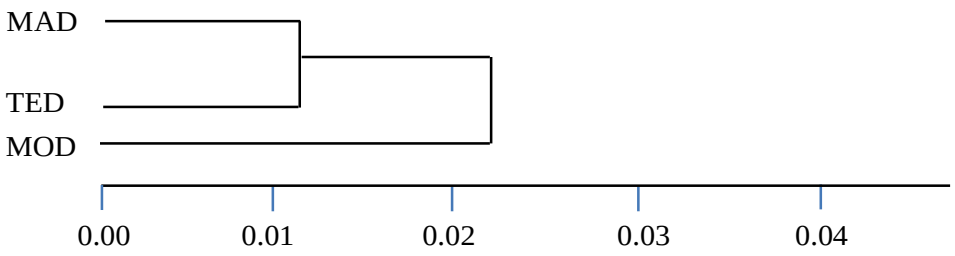

Figure 2. Dendogram of Local Ducks by Egg White Protein Polymorphism

Table 4. Heterozigosity in Blood and Egg White Protein

Population	Treatment		Average	Significance
	Blood	Egg White		
MAD	0.4837	0.3614	0.42255	ns
TED	0.4469	0.3984	0.42265	ns
MOD	0.4787	0.4944	0.48655	ns
Average	0.4698	0.4181		

ns= non-significant (P>0.05)

Heterozygosity Based on Blood and Egg White Protein

The average heterozygosity of three breeds of local ducks based on the blood and egg white protein are presented in Table 4. The highest heterozygosity of blood protein polymorphism was found in MAD followed by MOD and TED. Different things shown egg white protein, the highest average heterozygosity found in the MOD and the lowest was found in MAD. There was no significantly different between blood and egg white protein (P>0.05). This may imply that the blood and egg white protein polymorphism can be used to identify the genetic variation (Johari *et al.*, 2008; Mahfudz *et al.*, 2011). Heterozygosity values obtained in the study were not significantly different (P> 0.05) with the expected value, either the value of heterozygosity based on blood protein polymorphisms and egg whites.

CONCLUSION

There was no significantly differerent on genetic variance between blood and egg white polymorphism in three breeds of local ducks. The average value of the three offspring heterozygosity on all three breeds of local ducks have the potential to determine the genetic control of mating systems and selection for strains of each breeds of duck. The closest relationship among the three breeds of ducks was between the MAD and TED. Another thing is to do a migration between populations on the same breed of ducks, as well as maintaining the purity of these ducks.

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