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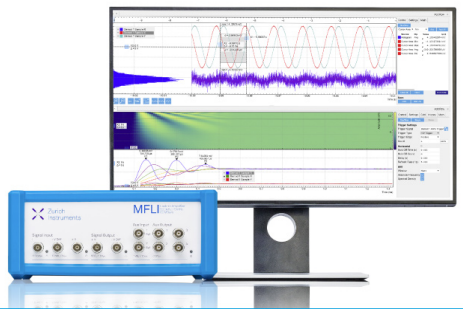

Genetic Characteristic of Swamp Buffalo (*Bubalus bubalis*) from Pampangan, South Sumatra Based on Blood Protein Profile

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Abstract. Swamp buffalo (*Bubalus bubalis*) is an endemic species and one of the genetic wealth of South Sumatra with a distribution area in the district of Pampangan (OganIlir and OganOganIlir). Suspected inbreeding causes decreased phenotypic properties. Inbreeding among various swamp buffalo is certainly not only lower the qualities but also genotypes and phenotypes. It is of interest to determine kinship variants swamp buffaloes from Pampangan through the analysis of a blood protein profile. Blood protein profile of four variants swamps buffalo was studied by using five electrophoresis system i.e. pre-albumin (Palb), albumin (Alb), ceruloplasmin (Cp), transferrin (Tf) and transferrin post (Ptf). In this paper, it is obtained that there was no significant differences among the four variants of the buffaloes were used as a sample. Prealbumin has two alleles (Palb1 and Palb2), albumin has three alleles (Alba, AlbB, AlbC), ceruloplasmin has one allele (BPA), post-transferrin has one allele (PTFA) with an allele frequency 1.0000 at any time transferrin has two alleles (TFA and TFB) with the allele frequency of 0.7500 and 1.0000. Characteristics prealbumin (Palb), albumin (Alb), ceruloplasmin (Cp), and post-transferrin (P-tf) is monomorphic, while transferrin is polymorphic average heterozygosity values all loci (H) 0.1286. Based on average heterozygosity, the swamp buffalo (*Bubalusbubalis*) from Pampangan has low genetic variation and closest genetic relationship.

INTRODUCTION

The habitat population of swamp buffalo *(Bubalus bubalis)* in the Pampangan area is mainly in the Southeast Asian region and named according to the name of the area or their location. Swamp buffalo (*Bubalus bubalis*) adapt to the monotonous marsh areas that are not cultivated. Swamp buffalo *(Bubalus bubalis)* in the Pampangan area endemic species and one of the genetic wealth of South Sumatra with deployment in District Pampangan (Ogan Ilir and Ogan Komering Ilir). These species are also found in Banyuasin area with four variants (black, red, striped and Lampung). The recent population now is only 3,623 individuals. High consumption of this swamp buffalo as meat supplies (± 10% per year), and low population increase (± 0.64% per year) causing the total population of swamp buffaloes declines every year (Windusari *et al.*, 2015).

Swamp buffalo *(Bubalus bubalis)* is a type of livestock that have high protein content. The quality of this livestock decreased due to population stocks. The pollution stock is also depending on the way marriage between individuals who have close family ties which are called inbreeding. Suspected inbreeding causes decreased phenotypic properties eg. body size, fertility, and endurance. If this conditions continuously happened, feared buffalo would be extinct. In the long term effect, the people of South Sumatra will suffer huge losses due to loss the germplasm (Murti, 2002).

Allendorf & Luikart (2007) and Frankham (2003) stated that conservation genetics or the application of genetics to the preservation of species has received increasing attention in recent years. The development of science and technology in this decade gave some effects to the conservation of germplasm through molecular genetic approaches. Molecular genetic approaches can be used to assess genetic variation swamp buffalo in South Sumatra.

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Diversity or genetic variation can be used as a starting point for improving the quality and quantity of swamp buffalo. Thus, information about the genetic crossbreeding and kinship in cattle including swamp buffalo is crucial in the preservation of germplasm (Windusari *et al*., 2016). Genetic variation of species is very important in maintaining the population. The sustainability of genetic diversity is the key factor for generation of the species (Hall & Bradley, 1995).

Genetic diversity became the important information in evaluating the genetic potential for the development, utilization, and conservation of species. Determination of genetic diversity at the gene level can be tested using fractions of blood proteins through protein polymorphism by electrophoresis method, which a chemical analysis is way based on the movement of protein molecules charged in an electric field. The pattern of proteins showed genotypes of individuals and variation will produce differences in allele frequency in a population.

The research on the blood protein profile of swamp buffalo (*Bubalus bubalis*) is still rare. Therefore, it is important to carry out research for conservation of swamp buffalo *(Bubalus bubalis)* from Pampangan as one of germplasm endemic in South Sumatra. This research aims to determine the blood protein profile and the pattern of kinship some kind of swamp buffalo in the area Pampangan, Banyuasin, South Sumatra.

MATERIALS AND METHOD

Sample

Blood samples were collected from four variants of swamp buffalo from Pampangan (red buffalo, black buffalo, striped buffalo and lampung buffalo). All the individuals fit the corresponding phenotypic breed type.

Laboratory Analyses

A Sample of blood was collected from the jugular vein in tubes containing EDTA then stored at 4◦C. A blood sample was centrifuged at 400 rpm for 5 min to separate plasma and erythrocytes. Both plasma and erythrocytes were stored at -20◦C before analysis. prealbumin, albumin, ceruloplasmin, transferrin, and post transferrin were performed using the polyacrylamide gel electrophoresis (PAGE) as described by Gahne *et al*. (1977).

Data Analyses

The frequency of alleles and genotypes of each protein locus is calculated according to the equation from Warwick et al., (1995).

$$xi = \frac{2xii + xij}{2n} \quad (1)$$

xi = i^{th} allele frequencies.
xii = allele homozygotes.
xij = allele heterozygotes.
n = number of samples observed.

Genetic diversity is determined by the value of heterozygosity per individual (H) according to Nei (1987).

$$h = 2n[1 - \sum_{i=1}^{m} xi.xi] \div (2n - 1) \quad (2)$$

h = heterozygosity per locus.
xi = i^{th} allele frequencies.
N = number of samples observed.

Zymogram protein created by the migration distance of the formula Y = ax + b through linear regression line equation. Dendogram created using NTSys 2.1 (Crnokrak & Roff, 1999).

RESULT AND DISCUSSION

Protein Profiles

Protein is a macromolecular compound composed of amino acids linked together by peptide bonds. Protein is one of the results of the expression of genes that are not affected by environmental changes. The structure of a protein consisting of the amino acid sequences will describe the sequence of bases in the DNA of each organism.

Based on the direction and speed movement of the molecular weight of each electric charge, then in Figure 1 is shown that there are five different zones. The first zone is pre-albumin with a relatively thin ribbon, then albumin zone which has thicker characters with the movement towards the anode (positive pole) quickly, then the ceruloplasmin zone, transferrin zone, and post transferrin zone.

Results of analysis using polyacrylamide gel electrophoresis showed there are no significant differences among the four variants of the buffalo were used as a sample. Blood plasma proteins in the swamp buffalo Pampangan which includes streamers pre-albumin *(Palb),* albumin *(Alb)*, transferring *(Tf)*, ceruloplasmin (Cp) and post transferring *(PTF)* that are in the same molecular weight. The results of the analysis of a blood protein profile buffalo presented in Fig. 1.

Differences in the velocity of each locus are affected by the molecular weight of the protein. The higher the molecular weight will cause the movement of molecules of the protein is getting slower and closer to the negative pole. After electrified within 2-3 hours and *destaning* it will form the locus of several zones according to speed the movement of protein.

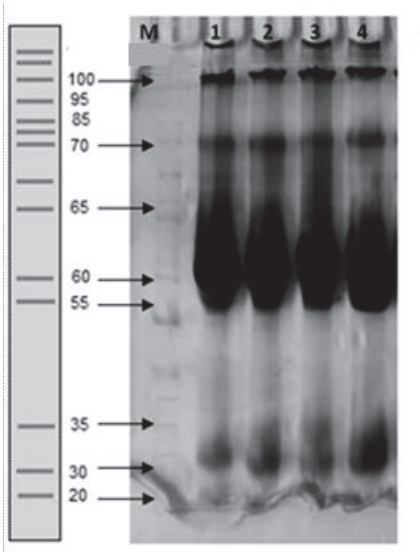

FIGURE 1. Blood protein profile of swamp buffaloes (M= Marker, 1= Black Buffalo, 2= Striped Buffalo, 3= Red Buffalo, 4= Lampung Buffalo)

Prealbumin (Palb)

Locus prealbumin (Lab) located at the top of the locus of albumin (Alb) and has two typeonomorphic. PA1 has a characteristic move faster towards the positive pole (anode) whis of alleles that PA1 and PA2 which have the same allele frequency of 1.0000 because it is mle PA2 moves slower than the PA1. Both of these alleles form the shape of thin ribbons. This locus is the molecular weight of 100-150 kDa.

Albumin (Alb)

The Data (Figure 1) showed that all the alleles at the locus of the blood protein albumin (Alb) displayed by every variation of samples analyzed. There are three bands shown between each individual of all the samples. Types A *(Alb^A)*, Type B *(Alb^B)* and type C *(Alb^C)*. Each of these alleles has allele frequencies of 1.0000 as it shows the same

tape forms one with the other. Locus albumin appears more clearly than the other locus. It can be seen on the tape that is thicker than the other locus. The molecular weight of albumin in the range of 60-70 kDa.

Ceruplasmin (Cp)

Ceruloplasmin only had one allele band formed. Ceruloplasmin has a molecular weight of 50 kDa and monomorphic. Allele found at this locus is a Cp^A with allele frequency allele 1.0000. No difference band formed from each of these variants so that the buffalo allegedly proteins are produced by the same gene in every variant.

Transferin (Tf)

Transferrin locus in this study has the molecular weight of 30 kDa. There are two bands formed. Tf^A alleles that have allele frequencies 0.7500 and Tf^B alleles that have allele frequencies 1.0000 indicates the characteristic polymorphic allele. Tf^A allele was not found in the black buffalo, while the Tf^B alleles found at every variant. This locus moved rapidly toward the positive pole (anode) because it has a small molecular weight compared to another locus.

Post Transferin (Ptf)

Post transferrin locus in this study is at a molecular weight of 20 kDa. Band formed tend to be thin and to be monomorphic in each variant of the swamp buffalo. There is only one type of ribbon is formed with Ptf^A alleles that have allele frequencies of 1.0000 for the same at each locus. This locus moving toward the positive pole (anode) fastest when compared with the other four locus because it has the smallest molecular weight.

Based on analysis of the entire banding pattern variation swamp buffalo, the characteristics of polymorphic only found on transferrin, while four other locus showed monomorphic characteristics. The results above also show that in the fourth variation of the swamp buffalo has a low genetic variation. This can happen due to the mating system do farmers tend towards inbreeding. According to Warwick *et al*. (1990), inbreeding tend to increase gene homozygosity and mating with another family will increase the gene heterozygosity also show genetic variation. Allele frequencies are presented in Table 1.

TABLE 1. Allele frequencies of the locus of blood protein

Locus	Allele	Allele Frequency
Prealbumin	Pa^1	1.0000
	Pa^2	1.0000
Albumin	Alb^A	1.0000
	Alb^B	1.0000
	Alb^C	1.0000
Ceruloplasmin	Cp^A	1.0000
Transferin	Tf^A	0.7500
	Tf^B	1.0000
Post Transferin	Ptf^A	1.0000

Heterozygosity

Heterozygosity obtained from the calculation of the frequency of alleles at each locus. Declared by Nei (1987) that genetic diversity can be measured through individual heterozygosity and the average heterozygosity (h) at a polymorphic and monomorphic locus. Based on the results of this study can be found that all four locus are prealbumin, albumin, Transferrin Post Ceruloplasmin show the characteristics of monomorphic, whereas only the transferrin locus showing the characteristic polymorphic. Heterozygosity is presented in Table 2.

Based on Table 2, it can be seen that the swamp buffalo pampangan have low genetic variation. This means that the four variants of the swamp buffalo have a very close genetic relationship. The closer kinship indicates a high similarity to the blood protein locus were observed. The more distant kinship indicates a high diversity or variation in blood protein locus were observed. Crosses between livestock that have close kinship ties (inbreeding) can increase the genes that are homozygous (two genes have the same genotype).

TABLE 2. Heterozygosity value of locus

Locus	Heterozygosity (h)
Pre albumin	0,0000
Albumin	0,0000
Ceruloplasmin	0,0000
Transferin	0,6428
Post transferin	0,0000
Average of heterozygosity (h)	0,1286

Results of previous studies of the characteristics of swamp buffalo from Pampangan, South Sumatera, Indonesia has done Windusari *et al*. (2016) and is expressed in the form dendogram showed that the correlation coefficient of fourth variant range between 0.57 and 0.85. The indicates that the variance between the buffalo have a close genetic relationship and probably derived from the same parent, expecially for variant with index kindship close to 1. It is show in Fig. 2. According Hoda & Marsan (2012) that all the genetic distance measures employed to estimate inter-breed closeness showed low levels of distances between the breeds.

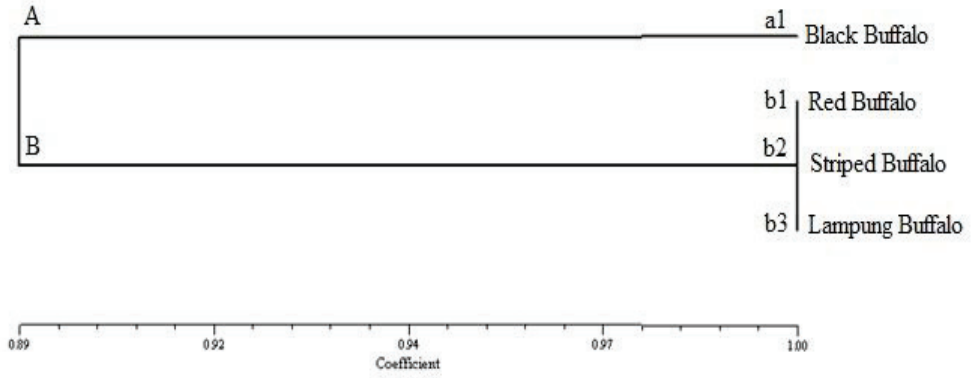

FIGURE 2. Dendogram of the kinship of swamp buffalo (Source: Windusari *et al.*, 2016)

In conservation genetics, knowledge of the relatedness between individuals is particularly important in captive breeding programs that seek to reduce incestuous matings in order to minimize inbreeding and the loss of genetic variation. It is well established that a decline in genetic variation reduces the ability of a population to adapt to environmental changes and therefore decreases its long term survival. The loss of genetic diversity also results in lower individual fitness and poor adaptability (Lande, 1988 in Arif & Khan, 2009). The fate of small populations is linked to genetic changes.

The fate of small populations is linked to genetic changes. The captive breeding of endangered wildlife animals is often necessary for their conservation; however, this strategy potentially increases the chances of inbreeding that, in turn, causes poor fitness of these populations (Crnokrak & Roff, 1999). According by Land & Lacy (2000), inbreeding is known to decrease genetic diversity and to reduce reproductive and survival rates leading to increased extinction risk. Therefore genetically impoverished endangered populations often fail to exhibit signs of recovery until crossed with individuals from other populations. Saccheri *et al*. (1998 in Arif & Khan, 2009) suggest that wildlife populations with lower genetic diversity are at greater risk of extinction.

CONCLUSIONS

Blood plasma protein profile of the fourth variant of swamp buffalo *(Bubalus bubalis)* from Pampangan based locus prealbumin, albumin, ceruloplasmin, and Post transferrin showed monomorphic characteristics and Transferrin showing the polymorphic characteristics. Swamp buffalo *(Bubalus bubalis)* from Pampangan have low genetic variation. Average of heterozygosity (h) is 0.1286 which has the meaning of the genetic relationship of buffalo variants closely. The mean allele number (allele diversity) provides a reasonable indicator of the levels of variability present.

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