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Short Communication:
Genetic diversity of Ongole Grade Cattle of Rembang District, Central Java, Indonesia, based on blood protein polymorphism

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Abstract. *Sutiyono, Sutopo, Ondho YS, Setiatin ET, Samsudewa D, Suryawijaya A, Lestari DA, Kurnianto E. 2018. Genetic diversity of Ongole Grade Cattle of Rembang District, Central Java, Indonesia, based on blood protein polymorphism. Biodiversitas 19: 1429-1433.* The objective of this study was to identify genetic diversity in Ongole Grade cattle of Rembang District, Central Java, Indonesia, based on blood-protein polymorphism. A total of 34 blood samples were collected from unrelated Ongole Grade cattle and the blood plasm was used to identify the blood proteins. Blood protein analysis was performed using Sodium Dodecyl Sulfate Polyacrilamide Gel Electrophoresis (SDS-PAGE). Observed bands were used to perform allele interpretation of four loci, namely Albumin (*Alb*), Post-albumin (*Po-Alb*), Ceruloplasmin (*Cp*) and Amylase-1 (*Am-1*). Results showed the presence of polymorphism in all of the four blood-protein loci, average heterozigosity value (*H*) being 0.506 and almost all of the blood-protein loci studied showed deviation from Hardy-Weimberg Equilibrium (HWE). In conclusion, the study indicated that population of Ongole Grade cattle in Rembang, Indonesia has high genetic diversity.

Keywords: Blood-Protein Locus, Electrophoresis, Genetic diversity, Ongole Grade, SDS PAGE

Abbreviation: *Alb*: Albumin, *Po-Alb*: Post-albumin, *Cp*: Ceruloplasmin, *Am-1*: Amylase-1, *HWE*: Hardy-Weimberg Equilibrium, *H*: Average Heterozogosity

INTRODUCTION

As one of the local cattle of Indonesia, Ongole Grade (Ind.: *Peranakan Ongole*) is the most breed farmed cattle in Java island, especially in Central Java (Rohyan et al. 2016). Based on the Decree of the Ministry of Agriculture No. 404/Kpts/PK.010/7/2017, Rembang district is designated as one of the Ongole Grade cattle germ locations in Central Java. Nevertheless, Rembang District becomes the second regency, after Kebumen District which was previously established, to be designated as Ongole Grade cattle germ location in Central Java. As a result, Rembang's Ongole Grade cattle have been certified free from zoonosis and deserve to be used as livestock germs. This, in turn, can play an important role in materialising the government's desire of increasing cattle population in Indonesia, especially in Central Java Province.

Ongole Grade cattle is the result of a cross between Sumba Ongole and Java cattle (Suyadi et al. 2014). Sutarno and Setyawan (2016) stated that Ongole Grade cattle have white-gray skin, long tail, black fur around the eyes, short head, short horns, long straight ears, and a rather large belly and they are the crossing result of Javanese cattle (*Bos javanicus*) and Ongole cattle (*Bos indicus*). An earlier study based on mtDNA Cyt b gene sequence has indicated that the Ongole Grade cattle in Indonesia, especially in Kebumen, have blood relation with Brahman cattle (Hartatik et al. 2018). SRY gene sequence study has also revealed close relationship with *Bos indicus* and Madura cattle (Hartatik et al. 2017). It has been known for their tame and strong body, Ongole Grade cattle are famous as working and beef cattle and they are also able to wtithstand and grow under limited environmental conditions (Sutarno and Setyawan 2016). Ongole Grade cattle also have lean meat (Ngadiyono et al. 2014) and good reproductive performance (Suyadi et al. 2014; Rohyan et al. 2016; Ngadiyono et al. 2017). As one of the local livestock genetic resources, Ongole Grade cattle needs to be maintained and preserved. It is necessary to know about the genetic diversity of Ongole Grade cattle population in Rembang in order to understand their genetic status. An insight into the pattern and extent of genetic diversity in a breed may assist in the development of more rational breeding programs and is also a prerequisite in the conservation of its genetic resources (Felius et al. 2014).

Recent studies on genetics and breeding of Ongole Grade cattle explores various aspects like genetic relationship (Hartatik et al. 2017; Hartatik et al. 2018), heritability of body size (Paputungan et al. 2015) and breeding value estimating of sire superiority (Sumadi et al. 2017). But, the genetic diversity of Ongole Grade cattle is rarely investigated. Besides through DNA (deoxyribo

nucleic acid), genetic diversity can be also measured through blood protein locus (Agaviezor et al. 2013; Johari et al. 2013; Egena and Alao 2014; Pal and Mummed 2014; Oguntunji and Ayorinde 2015; Demir and Mert 2015; Zaabza et al. 2015; Musa et al. 2016; Akintan et al. 2017; Windusari et al. 2017). Blood protein also can be used as livestock physiological characteristic data (Utomo et al. 2017), imunogenetic marker of animals (Hrinca 2015; Tothova et al. 2016) and identification of phylogenetical relationships (Nigussie et al. 2016).

Furthermore, identification of protein polymorphism can be used to study gene sequences that have impact on physiological pathway and closely related to phenotypic traits. Previous researchers have also explored the possibility of using protein polymorphism as genetic markers for production and reproduction traits (Ismoyowati 2008; Yadav et al. 2013; Yakubu et al. 2014; Aygun 2016; Yuwono et al. 2017; Sutiyono et al. 2018). Discovery of such genetic markers has the potential to substantially enhance the accuracy of selection, especially in economical traits such as body weight. The body weight is the main parameter of the production performance and it determine the sale value of a livestock.

Polymorphism of blood-protein can be detected by polyacrylamide gel electrophoresis model (Gahne et al. 1977). Individual genotypic variation would be described by polymorphism of blood-protein and it can lead to variation of gene frequency in population (Sari et al. 2011). Thus, this study was aimed to identify genetic diversity in Ongole Grade population in Rembang District, Indonesia, based on blood-protein polymorphism.

MATERIALS AND METHODS

Sample collection

A total of 34 blood samples were collected from unrelated Ongole Grade cattle in Rembang District, Central Java, Indonesia. Blood was taken by using 5 cc disposable syringe through *Jugular venous* that was cleaned with alcohol before. Later it was transferred to vacutainer tubes with an anticoagulant (EDTA), stored in a cool box containing ice gel and transported to the laboratory for analysis. The blood was centrifuged to separate blood plasm and blood cell. The separated blood plasm was then used to identify blood protein. The chest circumference of the cattle was also measured to obtain estimated value of their body weight.

Electrophoresis

Blood plasm was diluted using aquadest (blood plasm: aquadest = 1 µL: 19 µL) and mixed with blue juice (blood plasm: blue juice = 4: 1). Sample was then heated to 100°C for 3 minutes and was cooled in ice water. Blood protein analysis was performed using Sodium Dodecyl Sulfate-Polyacrilamide Gel Electrophoresis (SDS-PAGE) (Gahne et al., 1977). 10 µL sample was loaded into gel and running time was 2 hours at 120 V. The gel was stained with staining solution (20 ml Glacial Acetic Acid, 80 ml Methanol, 100 ml Aquadest, 0.2 g Coomassie Blue) for 1 hour on a shaker and destained with destaining solution (same as staining solution but without Coomassie blue) 2 times for 45 minutes. Observed bands were used to perform allele interpretation of four locus consisting of Albumin (*Alb*), Post-albumin (*Po-Alb*), Ceruloplasmin (*Cp*) and Amylase-1 (*Am-1*).

Data analysis

Allele frequency was calculated using the following formula of Warwick et al. (1995):

$$F_{Ai} = \frac{\sum \text{Allele Ai}}{\sum \text{Allele Ai} + \sum \text{Allele Bi} + ... + \sum \text{Allele Ni}}$$

Where:
F_{Ai} = Allele frequency of allele A on locus i

Genetic diversity within Ongole Grade population was quantified by measuring average heterozigosity (*H*). *H* was estimated from expected propotion of locus heterozigosity using formula of Nei (1987), as follows:

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

Where:
qi = allele frequency in each protein locus
m = number of allele
r = number of locus

Expected genotype frequency value was calculated based on Hardy-Weinberg Equilibrium (HWE) theory (Falconer and Mackay 1996) by following equation:
$p^2 + 2pq + q^2 = 1$ (for 2 alleles), or
$p^2 + 2pq + 2pr + q^2 + 2qr + r^2 = 1$ (for 3 alleles)

Where:
p = allele frequency of first allele
q = allele frequency of second allele
r = allele frequency of third allele

HWE was calculated using Chi-square test that was applied to compare the heterozygosity value between expected and observed genotype value (Hartl and Clark 1997):

$$x2 = \sum_{i=1}^{k} \frac{(oi - ei)^2}{ei}$$

Where:
X^2 = Chi square value
o_i = observed genotype frequency value
e_i = expected genotype frequency value

X^2 table using 5% significance level for HWE test

Body weight data was calculated by using body weight estimation formula from Schoorl formula (Kusumo et al. 2014):

$$BW = \frac{(cc + 22)^2}{100}$$

Where:
BW = estimated body weight (kg)
cc = chest circumference (cm)

General Linier Model procedure and Duncan test were used to test the significance of differences between means of body weight to discover the impact of genotype differences in each locus.

RESULTS AND DISCUSSION

The blood protein plasma electrophoresis clearly distinguished 4 protein loci, which are Albumin (*Alb*), Post-albumin (*Po-Alb*), Ceruloplasmin (*Cp*) and Amylase-1 (*Am-1*) as presented in Figure 1. A total of 10 alleles for 4 blood protein locus were observed. Estimated allele frequency are presented in Table 1.

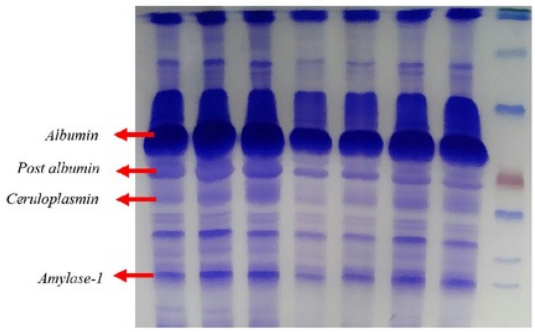

Figure 1. An example result of the blood protein plasma electrophoresis of Ongole Grade Cattle population in Rembang District, Central Java, Indonesia

Table 1. Allele frequency

Locus	Allele	Frequency
Albumin (*Alb*)	A	0.206
	B	0.500
	C	0.294
Post Albumin (*Po-Alb*)	A	0.353
	B	0.647
Ceruloplasmin (*Cp*)	F	0.412
	S	0.588
Amylase-1 (*Am-I*)	B	0.456
	C	0.544

Ongole Grade had three alleles distributed in Albumin loci, which were Alb^A, Alb^B and Alb^C. Alb^A allele moved faster to positive pole than Alb^B allele, and Alb^C allele was the slowest. Frequency of Alb^B allele was the highest compared to the other two alleles. Four genotypes were identified in Albumin loci consisting of homozygous BB genotype and heterozygous AB, BC and AC genotypes, while homozygous AA and CC genotypes were not found. This result was different from a previous study in which only two alleles (Alb^A and Alb^B) were discovered on Albumin loci in Ongole Grade dam (Yuwono et al. 2016). However, this result is parallel with that of Sutopo et al. (2001) which reported that Ongole Grade has three alleles in Albumin loci, namely Alb^A, Alb^B and Alb^C with allele frequency 0.233, 0.722 and 0.045, respectively. Further, findings of this study are also supported by other studies which have indicated that most of the Indonesian cattle such as Bali cattle, Madura cattle, Java cattle and Ongole Grade cattle have three alleles in their Albumin loci (Sutopo et al. 2001; Johari et al. 2007). Similar result was obtained with Hainan cattle and Wenshan cattle from China (Nie et al. 1999). Only two alleles for Albumin loci has been reported in some European cattle such as Slovak Spotted cattle (Zitny et al. 2007), Droughtmaster Cattle (Ashton et al. 1966), Brangus-Ibage cattle (Henkes et al. 2000) and Romanian Black Spotted cattle (Rebedea et al. 2005).

Post albumin loci in Ongole Grade has 2 alleles, namely $Po\text{-}Alb^A$ and $Po\text{-}Alb^B$. $Po\text{-}Alb^A$ allele moved faster than $Po\text{-}Alb^B$ allele toward positive pole. Only homozygous genotypes were observed in post albumin loci, namely AA and BB. It is noteworthy that Ongole Grade population in Rembang had no heterozygous genotypes for this loci. Post albumin loci was predominated by $Po\text{-}Alb^B$ allele and homozygous BB genotype with frequency of 0.647. This is similar to the research conducted by Gahne et al. (1977) in Swedish Friesien, Charolais, Simmental and Hereford cattle where in Post albumin loci was dominated by $Po\text{-}Alb^B$ alleles with allele frequency 0.68, 0.86, 0.75 and 0.98, respectively. On the contrary, Yuwono et al. (2016) reported that homozygous AA genotype and $Po\text{-}Alb^A$ allele frequency was the highest in Post Albumin loci of Ongole Grade. According to Nie et al. (1999), some Chinese cattle is monomorphic (100% allele frequency) in Post Albumin loci which had only B alleles, causing genetic fixation.

In this study, two alleles were identified in Ceruloplasmin loci. Cp^F alleles migrated more quickly to positive pole than Cp^S alleles. Both homozygous and heterozygous genotypes were observed in this study, namely FF, SS and FS with frequency 0.294, 0.471 and 0.235, respectively. The allele gene frequency of Cp^S (0.588) was higher than Cp^F (0.412). This result is in accordance with Yuwono et al. (2016), who reported that Ongole Grade has higher Cp^S allele gene frequency than Cp^F. This differs from the finding of Sutopo et al. (2001) that allele frequency in Ceruloplasmin was 0.750 and 0.250 for Cp^F *and* Cp^S, respectively. Ceruloplasmin plays a role in maintaining cell integrity through cytoprotective and various antioxidative activities and it also facilitates binding of Fe to Transferin protein (Demir and Mert 2015).

Two alleles *Am-1*^{B} and *Am-1*^{C}, were observed in Amylase-1 loci in Ongole Grade cattle. *Am-1*^{C} alleles (0.544) were commonly found compared to *Am-1*^{B} alleles (0.456) and three genotypes namely BB, CC and BC were obtained. *Am-1*^{C} allele migrated slower than *Am-1*^{B} to positive pole. However, there were alleles that are slightly lower in position (slower) than *Am-1*^{C} allele in 2 of the 34 samples investigated. It was named *Am-1*$^{C'}$. Further research is needed to confirm whether this allele is a novel discovery or not. According to previous reports, Ongole Grade as well as other Indonesian cattle has two alleles in Amylase-1 loci (Yuwono et al. 2015; Sutopo et al. 2001).

Genetic diversity of Ongole Grade population in Rembang was estimated by heterozygosity value and Chi-square test was performed to determine HWE (Table 2). Average heterozygosity (*H*) of Ongole Grade population was up to 50% and the highest loci heterozygosity (h) was observed in Albumin. Chi-square test for HWE showed all protein-blood loci were not in HWE. *H* value in this study was higher than previously reported values for other local cattle. Sutopo et al. (2001) reported that heterozygosity value for Bali cattle, Madura cattle, Java cattle and Ongole Grade cattle was 0.057, 0.210, 0.195 and 0.172, respectively. Sutarno et al. (2015), based on analysis of five microsatellite loci, reported that genetic diversity in Ongole Grade cattle was in moderate level. Nei and Kumar (2000) stated that heterozygosity value is the average percentage of individual heterozygous loci or the percentage of heterozygous individuals in a population. The *H* value of this study indicates that Ongole Grade population in Rembang has high diversity. It is advantageous to have high *H* value because of the fact that farther the genetic relationship, lesser is the possibility of inbreeding. High heterozygosity value shows that animals have various traits, either favorable or unfavorable (Warwick 1995). High genetic diversity indicates a relatively higher genetic inheritance potential in genes derived from each protein locus (Ismoyowati, 2008). According to Nei (1987), *H* value ranges from 0-1, in which higher the value, higher is the genetic diversity. Most of the blood protein loci in Ongole Grade showed deviations from HWE. It may be due to mutations, genetic drift, gene flow, non-random mating and natural selection (Jankowska et al. 2011).

The result of body weight analysis of Ongole Grade cattle in Rembang is presented in Table 3. The highest body weight means, in each locus, the genotype is homozygous. They were BB genotype in *Alb* loci, AA genotype in Po-*Alb* loci, FF genotype in Cp loci and BB genotype in Am-1 loci. However, within the same blood protein locus, no significant association between body weight and genotype was observed (P>0.05). Similar study in Pigs by Yanfang et al (2002) showed that AA genotype in Am-1-1 loci was significantly higher than other genotypes in birth weight and the AA genotype in Pa loci was significantly higher in daily gain of live-weight. Yakubu et al (2014), showed that AA genotype in HB loci was significantly higher than other genotypes in body weight of goat.

Table 2. Genotype frequency, HWE and heterozygosity

Locus	Genot.	Freq.	Obs.	Exp.	Test of HWE	h	*H*
Albumin (*Alb*)	AA	0	0	1.44	X^2 = 19.01*	0.621	0.515
	BB	0.324	11	8.50			
	CC	0	0	2.94			
	AB	0.088	3	7.00			
	BC	0.265	9	10.00			
	AC	0.324	11	4.12			
Post Albumin (*Po-Alb*)	AA	0.353	12	4.24	X^2 = 34*	0.457	
	BB	0.647	22	14.23			
	AB	0	0	15.53			
Cerulopasmin (*Cp*)	FF	0.294	10	5.76	X^2 = 8.52*	0.484	
	SS	0.471	16	11.77			
	FS	0.235	8	16.47			
Amylase-1 (*Am-1*)	BB	0.324	11	10.07	X^2 = 7.40*	0.500	
	CC	0.411	14	7.06			
	BC	0.265	9	16.87			

Note: h: individual heterozygosity, H: average heterozygosity

Table 3. The body weight of Ongole Grade cattle in Rembang District, Central Java, Indonesia

Locus	Genotype	Body weight
Albumin (*Alb*)	BB	372.32
	AB	346.13
	AC	372.08
	BC	357.43
Post Albumin (*Po-Alb*)	AA	373.26
	BB	362.03
Ceruloplasmin (*Cp*)	FF	370.42
	SS	364.36
	FS	363.71
Amylase-1 (*Am-1*)	BB	373.48
	CC	364.19
	BC	359.64

In conclusion, population of Ongole Grade cattle in Rembang has high genetic diversity as showed by polymorphism observed in their alleles and genotypes, and a high heterozygosity value. Most of the blood protein loci showed deviation from HWE. This high genetic diversity condition needs to be maintained for the purpose of conservation and also for avoiding inbreeding.

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