International Refereed Journal of Engineering and Science (IRJES)
ISSN (Online) 2319-183X, (Print) 2319-1821
Volume 3, Issue 3(March 2014), PP.01-14

Genotype Distribution of Local Chicken Crosbred in Poultry Breeding Centre Temanggung-Central Java

Abubakar[1], E. Suprijatna[2] and Sutopo[3]
Master of Animal Science Study Program Faculty of Animal and Agricultural Sciences Diponegoro University

ABSTRACT:- This study was aimed to conduct in genotype distribution of local chicken crosbred in Poultry Breeding Centre Temanggung-Central Java. Total amount of 41 blood sample from different offspring were used. The parameter observed are genotype distribution of pre albumin (Pab), albumin (Alb), transferrin (Tf), post transferrin (Ptf), ceruloplasmin (Cp), and amylase (Amy-1) loci through each of distribution of genotype and gene frequency. Genotype distribution calculated by sum of genotype revealed at each individual. Gene frequency counted by Warwick *et al*. (1990), genetic differentiation are determined by using heterozygosity (h) and average of heterozygosity (H) according to Nei (1987). Based on identification of gel electrophoresisi indicate that local chicken crosbred had two allele allele at each loci observed and could not obtain different allele. The result of this study showed that there were no significant ($P \geq 0,05$) genotype distributiom among local chicken crosbred in Poultry Breeding Center-Temanggung

Keywords:- Indonesia local chicken, Genotype Distribution *****
Seminar Paper of Animal Science Study Program
[1] Author
[2] Advisor
[3] Vice Advisor

I. INTRODUCTION

Background

Kampung and Kedu chicken are common Indonesian local chicken. The chicken looks very diverse, so are very broad due to the nature of the fenotype. Spreading of domestic poultry population (not race) is found in cities and villages. An exotic chicken in Central Java were found such as Arab chicken and Lingnans chicken. This exotic chicken usually came from crossbreed between Arabic chicken and Indonesian Local Chicken itself. Arab Chicken is one of the eminent start laying hens has been developed in Indonesia because it has a more attractive appearance than the usual free-range chicken, egg productivity of laying hens almost like race and has characteristics that resemble Kampung chicken (Natalia *et al*., 2005). Arab chicken is superior laying hens were classified into mild type chickens weighing age 52 weeks reached 2035.60 ± 115.7 g in males and 1324.70 ± 106.47 g in females (Nataamijaya *et al*., 2003). Arab chicken egg production is high, namely 190-250 eggs / year with a weight of 30-35 g eggs and almost no brood properties so time becomes longer spawn (Sulandari *et al*., 2007).

The Arab cock laying types is a local chicken from Egypt. Among people of Egypt, the chicken is better known by the name of fayoumi or bigawi chicken. Chicken has long been settled and developed since before Christ and are found along the River Nile. This chicken has characteristics-traits such as body posture slender and small, agile, like flying, and has a high adaptability. The advantages of this chicken are a fast sex mature and begin laying eggs at the age of four months. Qualitative properties of Chicken feathers are silvery white in color from the head to the neck and white plumage black spots on the body, shank green or blue tree, his DOC has a color with a blend of brown, black and white, and the head of brownish purple. The age and weight at sexual maturity of Fayoumi Chicken were 155.0 days and 1240g and 163.63 days and 1253±16.42g respectively (Khan et al, 2006).

In Indonesia, there are any kind of chicken which is come from crossbreed between arabic chicken and Indonesia local chicken. This kind of crossbreed can be lead to heterozigosity in the next filliation of chicken. The purpose of crossbreed between arabic chicken and Indonesian local chicken usually to improve the egg productivity or to improve the value of those chickens. In this study, we are going to evaluate the genetic diversity between the fillial of arabic chicken and Indonesian local chicken. This evaluation will be using a polymorphisms method to give us a wide overview about the genetic diversity and genetic distribution in chicken.

Polymorphism is genetically useful to help determine the origin, phylogenetic relationships compiled among species and or groups within the species. Most of the blood protein polymorphism was genetically regulated by pair of alleles or sequence of alleles without dominance (Warwick *et al*., 1990). Protein

polymorphisms have been used to determine the genetic relationship of livestock, as is done in ducks (Brahmantiyo *et al.*, 2003). Polimorphism itself is when two or more different phenotypes in the population of a species - or, in other words, the appearance of more than one form. To be referred to as polymorphism, these forms should be in the same habitat at the same time and belong to the random mating population.

According to Dobzhansky (1970), polymorphism many appear in nature and related to biodiversity, genetic variation, and adaptation. Those functions usually are to keep the variation in the population which is living in a variable environment. The most obvious example is sexual dimorphism in many organisms. Among the types of blood proteins that are known to be polymorphic is globulin (transferrin), albumin, and hemoglobin (Warwick *et al*., 1990). According to Wulandari (2008) on the analysis of chicken blood plasma protein by electrophoresis, Kedu chicken shows 4 loci that are polymorphic such as prealbumin (*Palb*), albumin (*Alb*), tansferin (*Tf*), and post-transferrin (*Ptf*). Whereas on native chicken found at four different loci were polymorphic protein, hemoglobin, albumin, post-albumin and transferrin (Johari, 1999).

Polymorphisms in this research can give us a broaded view about genetic distribution in Indonesian local chicken that were chickens crossbreed from female arabic chicken and male lingnan chicken. Genetic distributions of indonesian local chicken that came from crossbreed chicken rarely discuss in research. That was the main reason why result in this study can give us deeper knowledge about genetics code in Indonesian local chicken like never before.

Research Objectives

The purpose of this study was to evaluate genotype distribution of Indonesian local chicken crossbred in poultry breeding center Central Java.

Useful of the Research

The useful of this research hopefully could be known genotypic of Indonesian local chicken crossbred in poultry breeding center and to obtain the latest information on potential genotyping as be basic information in the effort to improve genetic of local chicken.

The Scientific Framework

Exotic chickens from abroad in Central Java namely are Arab chicken and Lingnans chicken. Arab Chicken is superior because the type chickens laying eggs high weight of 40 g. Eggshell color varies namely white, yellow and brown that sometimes a lot of people who do not know the difference between chicken eggs and Arabic which chicken eggs. Productivity of lingnans chicken approach Arab cock, hen day reached 50%. Arab chicken production ranges 150-180 eggs per year. This type of chickens is growing very fast and efficient in feed. Lingnans chicken weight can reach 1.1 to 1.3 kg with an average feed consumption of 2.5 kg per period. Growth performance of the lingnans chicken is faster than the growth of normal chicken. However, further genetic analyses concerning the successfully crossbreed between local chicken and result mating between Arab chicken (cock) and Lingnan chicken (hen) have been limited due to few data available in Poultry Breeding Center-Temanggung.

To our knowledge, expected from the results of this study can provide basic information about evaluation of genotype distribution. The useful of this research could be known as result of evaluation genotypic distribution as basic information in an effort to improve the quality of local chicken. The scientific framework was

Illustrated 1.

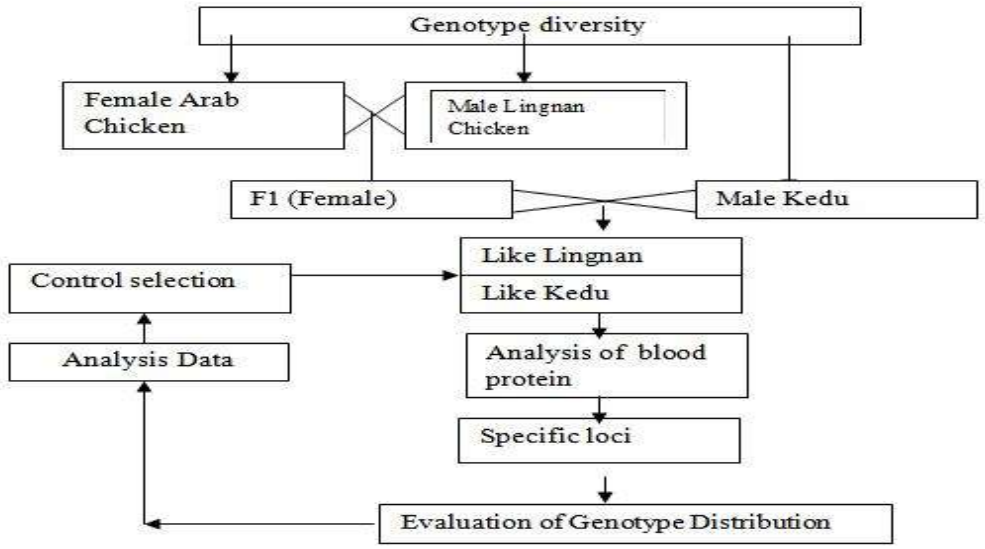

Illustration 1. Scientific Framework of Research

From the framework above, we know that Indonesian local chicken was not a pure filliation but crossbreed chicken. In poultry breeding centre, Temanggung, four kind of chicken was used in this research to figure out the genetic distribution among them. The framework above, can give us a little perspective that chicken in Poultry Breeding, Temanggung was not a pure breed but a crossbreed from female arabic chicken and male lingnan chicken. The filliation of this crossbreed has two specific characteristics which is like Kedu chicken and like lingnan chicken. As we analysis the blood of these chicken, we will know the genetic distribution of the chicken and we can conclude if the chicken was a pure breed or crossbreed like we assumed before. Evaluation of genotype distribution among these chickens can also give us deeper knowledge about the genetic coding of the chicken. As we know more about the genetic distribution of the chicken in Poultry Breeding Centre, Temanggung, we can use the information to improve productivity or to preserved a specific characteristics of the chicken that can added more value for the chicken itself.

Research Hypothesis

The hypothesis of the research supposed that there was genotype distribution between the F1 (resulting from Lingnan and Arabic chicken) and local chicken crossbred.

II. LITERATURE REVIEW

Arabic Chicken. There are two types of Arabic Chicken, namely Arab silver chicken (Brakel Kriel-silver) and the Arab golden chicken (Brakel Kriel-gold). According to the development information, the Arab silver chicken is more widely known and cultivated than the Arab golden chicken. Both types are distinguished by Arab cock in his fur color as the name suggests. Arab Chicken has silver hair color from head to neck silvery white and black spotted fur color white / black and white striated. The chicken has a typical Arab golden plumage on the head to the neck red and golden colors of red spotted golden fur loss (Natalia *et al*., 2005).

Arab Chicken is one of the eminent start laying hens that has been developed in Indonesia because it has a more attractive appearance than the usual free-range chicken, egg productivity high productivity of laying hens almost like race and has characteristics that resemble chicken eggs Kampung (Natalia *et al*., 2005). Chicken Arab is superior laying hens were classified into mild type chickens weighing age 52 weeks reached 2035.60 ± 115.7 g in males and 1324.70 ± 106.47 g in females (Nataamijaya *et al*., 2003). Arab chicken egg production is high, namely 190-250 eggs / year with a weight of 30-35 g eggs and almost no brood properties so time becomes longer spawn (Natalia *et al*., 2005; Sulandari *et al*., 2007). Chicken egg of Arabic were white because it has dominant genes derived from chicken imports, although in Indonesia has interbred with the local chickens. The weight of a chicken egg Arab namely 34.24 ± 1.38 g per item to the age of first spawning is 168.52 ± 3.20 days and egg production per 6-month period is 51.41 ± 4.61%. Natalia *et al*. (2005) states that Arabs chicken meat are look like thin and black skin so less favored consumers, in addition to weight of culling chicken are relatively low at only 1.1 to 1.2 kg.

Local Chicken is chicken which is native to Indonesia crosses with jungle fowl (*Gallus bankiva*), spread throughout the islands of Java and Nusa Tenggara (Gallus varius) and are not directed to a specific production purpose (Budipurwanto, 2001). Meanwhile, according to Blakly and Bade (1994), the ancestors of the local chicken are red jungle fowl (*Gallus gallus* or *Red jugle fowl*).

Local chicken types such as Kampung chicken (spread across all regions in Indonesia), chicken Pelung (Cianjur, West Java), Sentul (Kudat, West Java), wareng (Indramayu, West Java), Lamba (Garut, West Java) , Ciparege (Karawang, West Java), Rintit / Walik (spread in Indonesia, but in small amounts), Black Kedu (Kedu Village, Temanggung-Central Java), White Kedu (Kedu Village, Temanggung, Central Java), Cemani (Kedu village, Temanggung, Central Java), Olagan (Bali), Tukung, Ranged, and Cangehgar / Cukir / Alas (*Green Jungle fowl*) (Nataamidjaya, 2000). Local chicken has advantages those were has a good adaptation to the tropical climate in Indonesia. Local chickens are more resistant to disease, meat and eggs taste appreciated by the public and the production cost is relatively cheaper than chicken (Rasyaf, 1989).

Kedu Chicken was another kind of Indonesian Local Chicken that have a higher economic value compared to other kind of Indonesian Local Chicken. Kedu chicken can be beneficial from their meat and also their egg. Beside that, Kedu chicken frequently used in Indonesian traditional ritual. Kedu chicken also have a higher egg productivity compared to other chicken. Creswel and Gunawan (1982) stated that, produce of egg in Black Kedu chicken annualy reach the number of 215 when other kind of chicken just reached the number between 119-197.

Blood Protein Polymorphism, Polymorphism is a genetic variation that occurs at the level of DNA and proteins, and is often expressed in the form of different phenotypes in a population. Polymorphism can occur at three levels including at the level of chromosomes, genes, and the restriction fragments were polymorphic DNA (Stansfield & Elrod, 2002). Harris et al (1994) states that if a population whose members

have two or more phenotypes protein encoded by two or more alleles at a particular gene locus, then it are known as polymorphism. Further explained that the so-called polymorphic locus allele frequencies if not greater than 0.99. Polymorphism is the main result of the action of genes that are highly useful in basic biological research, especially to determine the origin of livestock, construct phylogenetic relationships between species and the people or groups in the species. In general, among the types of blood proteins are known to be polymorphic globulin (transferrin), albumin, blood enzymes and hemoglobin (Warwick *et al*., 1990). The results Wulandari (2008) on the analysis of chicken blood plasma protein using polyacrylamide gel on Kedu chicken shows 4 loci that are polymorphic include pre albumin (Palb), albumin (Alb), tansferin (Tf), and post-transferrin (PTF). At the local chicken found four polymorphic loci protein hemoglobin, albumin, post-albumin and transferrin (Johari, 1999).

Transferrin., Transferrin has a molecular weight range of 85,000 Dalton (Da). The results Johari *et al*. (2008) showed that in Kedu chickens, locus transferrin (Tf) is controlled by two alleles, the TFB and TFC. The banding which moving faster toward the positive pole, is called allele B, while the slower-moving bands called allele C. Both of these alleles can combine be the character of heterozygous BC. Ismoyowati (2008) reported the identification of phenotype or genotype transferrin locus in Tegal ducks acquired three alleles or gene combinations form four different genotypes, namely, Tf AA, Tf AB, Tf BB and Tf BC with each gene frequency is 0.25 of Tf A, Tf B gene frequency is 0.64 and the gene frequency of Tf C is 0.09. Tf AA homozygote genotype had the highest egg production potential compared with other genotypes. Genotype or allele heterozygotes are Tf AB with Tf A genes or gene alleles dominant to Tf B, so a combination of both causes decreased egg production potential. Tf BB homozygote genotype has the lowest potential for egg production. Heterosigot Tf BC with genotype or allele dominant to the allele gene of Tf C and Tf B, so the combination of both of them led to potential egg production higher than Tf BB genotype.

Albumin (*Alb*)., Albumin polymorphism was reported by Ashton (1964) which states that has three alleles; Alb A; Alb B and Alb C. At Alb B move faster than Alb C (Gahne *et al*., 1977). Characteristics of albumin in the native Indonesian goats showed characteristic C-type homozygote (Katsumata *et al*., 1981). Research Nozawa *et al*. (1981), Mongolian native goat blood plasma protein albumin are found in loci with no variation or homozygous CC. In sheep Cham people in Vietnam found albumin (Alb) homozygote with type C (Tsunoda *et al*., 1998). Research of Tsunoda *et al*. (1999) stated that the Central Mongolian sheep albumin locus that has two alleles is controlled by AlbC and Albx. Albumin molecules are smaller and have a greater payload showed the fastest migration rate (Simm, 2000).

Prealbumin (Palb)., Prealbumin is a plasma protein that began its structural life in scientific research in the laboratory of Dewitt Goodman where it was isolated and sequenced (Kanda, et al, 1974). It was named prealbumin because it ran ahead of albumin on serum protein electrophoresis gels (this is true of the human but not the bovine protein). Prealbumin plays important physiological roles as a transporter of thyroxine and retinol-binding protein. Prealbumin has a monomer molecular weight of approximately 14,000 Da (Hamilton & Benson, 2001). Prealbumin was found in goslings as early as the first day of their life, whereas no such equivalents were recorded in hens, turkeys, Japanese quails or ducks (Brodacki *et al*., 1986).

Brodacki and Smalec (2001) found ten phenotypes in geese, four phenotypes have a single, intensively stained band, and six phenotypes, each of which is represented by two bands. The distance between the bands C and D was twice as long as between the bands A and B or between B and C, which may suggest that there is an additional band migrating with intermediate speed in relation to the speed of C and D (Brodacki & Smalec, 2001). In the former study, Kuznetov (1994) presented five alleles, A, B, C, D and E, which encode the pre-albumin subregion proteins. In Brodacki and Smalec (2001), the band B was commonly found in all the geese, whereas the phenotype A was present only in the birds that originate d from *Anser anser*, and the bands C and D only in the geese that originated from *Anser cygnoides*.

Amylase-I (Am-I)., Amylase-I (Amy-I) is an enzyme protein in the blood that are useful in increasing the rate of metabolism and is used in the determination of gene loci through analysis amylase protein-I (Amy-I) has a molecular weight of 110000-120000 daltons with ribbon allele moving faster toward the anode is called allele B (Wyne *et al*., 1990). According Khana (1973), amylase-I found on the donkey that had genotype C and Amy-I Amy-I B and has a gene frequency of allele Amy-I C higher than Am-I B alleles and found also in the Hereford Cattle that have genotype Amy-I B, I C and Amy-I BC which has a gene frequency of allele B Am-I higher than Amy-I allele C.

Post-transferrin (P-tf)., Research by Namikawa *et al*. (1982) mentions that there are two alleles at loci post-transferrin, namely Ptf F and Ptf S on local Indonesian cattle. However, research results Sutopo *et al*. (2001) showed that the Bali cattle found no P-tf S, whereas in cattle Madura, Java and Peranakan Ongole, P-tf S is more common than the P-TFF. The results on the local goat and sheep of Indonesia and Vietnam by using

polyacrylamide gel electrophoresis method are not identified loci post-transferrin (P-tf) (Katsumata *et al*., 1981, Tsunoda *et al*., 1998).

Ceruloplasmin (Cp)., Ceruloplasmin is a ferroxidase essential enzyme that catalyzes the oxidation of iron, ceruloplasmin deficiency can cause a condition genetic, or aceruloplasminemia which is a disease that shows the important role of copper in iron distribution (Hubbard, 1999). According Wyne *et al*., (1990), the molecular weight of the protein ceruloplasmin were 70000-75000 daltons. Electrophoresis results in a cross between a sheep and a lamb Texel Sheep Suffolk Cp S allele was found higher than the Cp F.

Electrophoresis is a separation technique based on size cellular molecules using electric fields that are drawn on a medium containing the sample to be separated. This technique can be used with the existing electrical charge on macromolecules, such as DNA is negatively charged. If the negatively charged molecules passed through a medium, such as agarose gel, and then electrified from one pole to the opposite pole charge, the molecules will move from the negative to the positive pole. Motion of the molecules depends on the ratio (ratio) charge to its mass, and depending also on the shape of the molecule (Yuwono, 2005) stated that electrophoretic techniques can be used for analysis of DNA, RNA or protein. In general, protein electrophoresis technique sometimes called allozyme analysis (Feldhamer *et al*., 1999). Protein electrophoresis is basically done by similar principles as used in DNA electrophoresis, but gel used is polyacrylamide gel. Electrophoresed proteins can be analyzed by using coomassie blue staining. These compounds are typically added together with the samples. Painting proteins can also be done with a solution of silver nitrate is more sensitive than coomassie blue (Yuwono, 2005).

Genotype distribution is one way to obtain a complete picture of the genetic code in living organisms, which in this study is the distribution of the genetic code in chickens in Poultry Breeding Centre, Temanggung, Central Java. Understanding of the genotype distribution will help researcher to clearly map the genetic relatedness among species of chicken that is in Poultry Breeding Cenre, Temanggung, Central Java. This mapping can help researcher and farmers, particularly, to focus on some of the genes that can increase the productivity of the species of the chicken itself. This productivity can be associated with egg productivity, speed of growth of the chicken or chicken resistance to certain diseases that are expected to provide benefits to the poultry farmers in Poultry Breeding centre, Temanggung, Central Java. The genotype distribution can be obtained through the blood polymorphism chickens in Waterford, which focuses on six loci, namely albumin, pre-albumin, ceruloplasmin, transferrin, post-transferrin and amylase-1.

III. MATERIALS AND METHODS

This study was conducted at chicken breeding center (Temanggung district) from May to June 2013. The process of blood analysis performed at the Laboratory of Biochemistry Faculty of Veterinary Medicine of Gadjah Mada University and Laboratory of Genetics, Breeding and Reproduction Diponegoro University for data analysis.

Materials Research

Materials research on "Genotype Distribution of Local Chicken Crossbreed in Poultry Center, Temanggung, Central Java" through blood protein polymorphism analysis. The equipment used in study are vacutainer, syringe (5 ml) equipped with needles, test tube racks, ice flask, centrifuge, measuring pipette, micropipette (10µl capacity), glass beaker, measuring cups, stove power, sample bottles, plastic trays, analytical scales, magnetic stirrer, refrigerator, plastic ruler, plastic cylinder, plastic tray, electrophoresis kit (tank, tape, combs and spacers), power supply (DC power supply) for blood protein plasma analysis.

Chemicals used to test the blood plasma protein polymorphisms among others: Alcohol 70%, EDTA (as an anti-coagulant), 0.9% NaCl, distilled water, acrylamide, Bis-acrylamide, Tris, HCl, acetic acid, Glycine, Ammonium persulfate, glycerol, Methanol, N, N, N ', N'-Tetramethylenediamine (TEMED), trichloroacetic acid (TCA), Bromophenol blue (BPB), Dyes Amido Black 10 B, Ethanol, Sodium Dedochil Sulphate (SDS) 10%, 100 ml APS and Commassie Brilliant Blue.

Research Methods

Research on "Genotype Distribution of Local Chicken Crossbreed in Poultry Center, Temanggung, Central Java" through blood protein polymorphism analysis using the Indonesian local chicken bred. The design of the study is observational. The location was been chosen at chicken breeding center in Temanggung, Central Java.

Research Procedures

Blood samples were taken from 41 chickens (local chicken crossbred). Chicken blood samples were taken by using a syringe on the wing vein approximately 3 ml chicken, and then put in a 2.5 ml Eppendorf tube

filled EDTA as anti-coagulant and stored on ice thermos. Blood plasma is separated from red blood cells by means centrifuged at 8000 rpm for 5 min at 20^{0}C. Blood plasma that has been separated from the red blood cells were taken using a pipette, and then put into a new Eppendorf tube and stored at 4°C until analysis. Protein polimorphism analysis was conducted according standard protocol. Blood plasm be then analyzed using PAGE-TYLE (Polyacrilamide Gel Electrophoresis-Thin Layer Electrophoresis) that was set up horizontally according to Ogita and Marker (1968).

Gel preparation., Gel that is used consists of two layers, namely gradient gels and "staking gel". The first stage of making a gradient gel topped with butanol to surface and wait for at least 2 hours in order to gel. After the butanol was removed and cleaned and then given staking gel and topped with a comb to make wells that will be used to put the sample and wait until it becomes a gel for 1 hour. Samples of blood plasma were taken as much as 20 ml and diluted 20x with distilled water and then given a buffer electrode with a ratio of 4: 1. Once the sample is ready and gel hardens, then put the sample wells that have been provided to gel.

Electrophoresis., Gel electrophoresis and electrophoresis apparatus filled with the prepared electrode buffer, and samples were diluted with distilled water as much as 20x incorporated into the gel mold that has made as many as 20 µ then in the running for 2-3 hours. Once this done, staining on the gel for 3 hours. The latter process is done washing gel (destaining) so that the protein bands can be seen. Polyacrylamide gel electrophoresis was used to determine the variations of pre albumin (Pab); Albumin (Alb), ceruloplasmin (Cp); transferrin (Tf), post transferrin (Ptf) and Amylase-1 (Amy 1).

Data Analysis

The data will collected and analyse by excell spreadsheet and using the tools of DISPAN program.

Genetic Frequency

Gen frequency calculated based on the formula of Warwick *et al*. (1990).

$$F_n = \frac{\text{locus An}}{\text{locus A1} + \text{locus A2} + \text{locus A3}}$$

Where F_{AN} = gen frequency of A at the locus –n

Genetic differentiation are determined by using heterozygosis (h) and average of heterozygosis ($\overline{H}$) according to Nei (1987), as follow:

$$h = 1 - \sum \overline{q_i^{\,2}}$$

$$\overline{H} = \frac{1 - \sum \overline{q_i^{\,2}}}{r}$$

Explanation: q_i = gen frequency of-i

h = Individual heterozygosis

r = Amount of loci observed

$\overline{H}$ = average heterozygosis

Estimation of genetic similarity (I) and genetic distance were done by formulation according to Nei (1987): Genetic similarity (I):

$$I = \frac{\sum x_{ij} y_{ik}}{\sqrt{\left(\sum X_{ij}^{2}\right)\left(\sum Y_{ik}^{2}\right)}}$$

Explanation:

Xij : gen frequency on loci-i section j

Yik : gen frequency on loci-i section

k Genetic Distance (D):

D = -In (I)

Genetic distance between population and average of heterozigoty was calculated using computer program. Heterozigoty data blood plasma from this research will be test using t-test that followed the directions from Steel and Torrie as seen below:

$$S^2 = \frac{(n_1 - 1)S_1^{\ 2} + (n_2 - 1)S_2^{\ 2}}{(n_1 + n_2) - 2} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

$$S_{y_1 - y_2} = \sqrt{S^2 \frac{n_1 + n_2}{n_1 n_2}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

$$t - hit = \frac{x_1 - x_2}{S_{y_1 - y_2}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3)$$

Explanation:

X_1 : average calculation of T_1
X_2 : average calculation of T_2
: standard deviation of T_1 and T_2
S^2 : combination of standard deviation
n_1 and n_2 : calculation of repeated T_1 and T_2

IV. RESULTS AND DISCUSSION

Genetic Diversity of Local Chicken from Blood Protein

Electrophoresis result of blood plasma using an polyacrilamide gel showed 6 loci of protein that was polymorphic which is pre-albumin (Palb), albumin (Alb), Transferin (Tf), Ceruloplasmin (CP), Post-tranferin (Ptf) and Amylase-1 (Am-1). This result will be illustrated in the following figure.

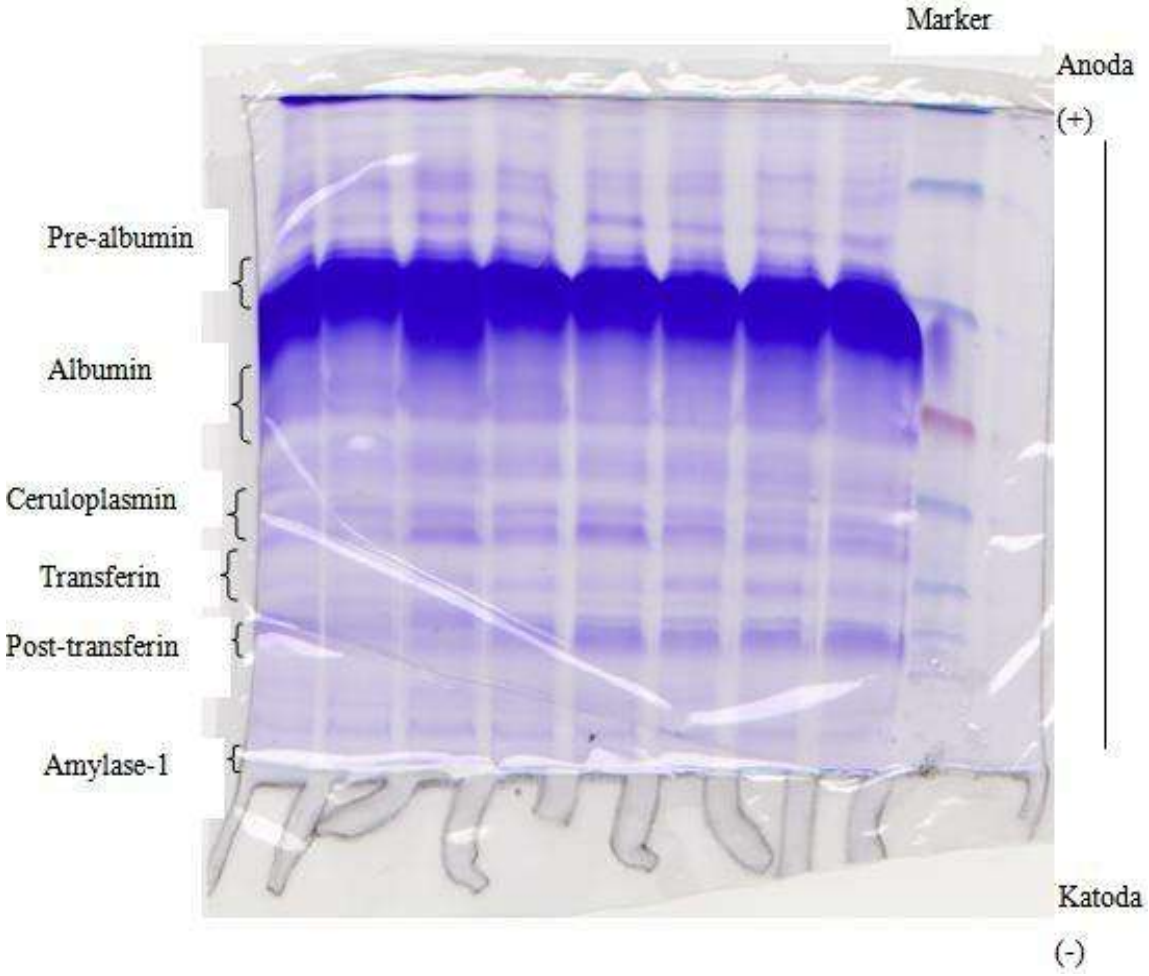

Figure 2. Protein ribbon pattern from blood plasma analysis

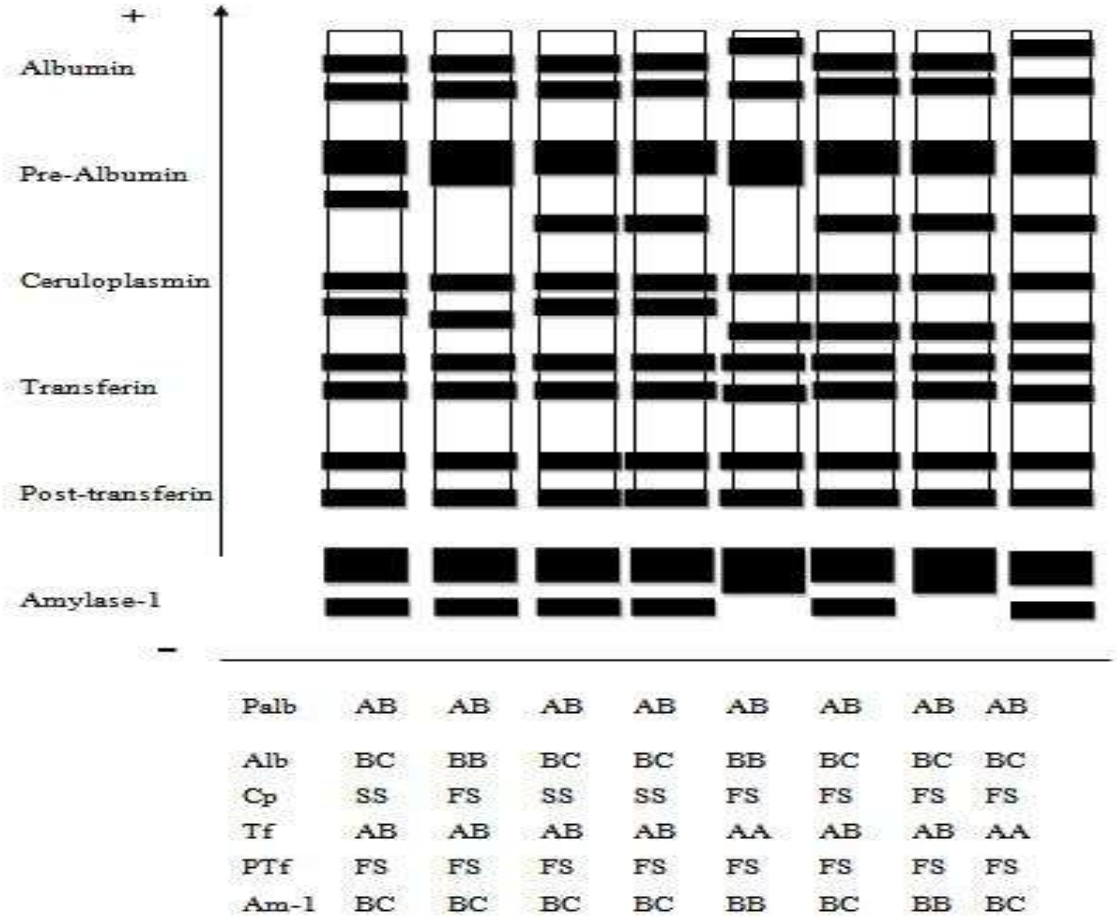

Figure 2. Composition/Scatter diagram of Protein Banding Pattern

Pre-albumin (Palb)

Based on observation on pre-albumin loci from Kedu chicken (KJ), Lurik chicken (LU) and Lingnan Chicken (BM) and female chicken (FIB), can be assuming that pre-albumin loci has a homozygot and heterozygot genotype. Homozygot genotype was marked as a $Palb^{A}$ and $Palb^{B}$, while the heterozygot genotype was marked as a $Palb^{AB}$. Characteristic of this fenotype was thick and thin and also have a different movement to the positive pole. Result from observation showed that Kedu Chicken (KJ), Lurik Chicken (LU), Lingnan Chicken (BM) and Female of Chicken (FIB) have two different allele, which is $Palb^{B}$ and $Palb^{AB}$. Table below showed that in general, allele $Palb^{AB}$ has a higher score compared to allele $Palb^{B}$. The highest genetic frequency score was in Kedu Chicken (KJ), Lurik Chicken (LU), and Lingnan Chicken (BM) with 0,5 for allele A. The highest genetic frequency score for allele B was in Female of Chicken (FIB) with 0,53. Lumataw (1993) showed the result of Pre-Albumin in Philipine Local Chicken. In Philipine local chicken, Lumataw (1990) found two alleles in Pre-Albumin loci where allele B have a higher genetic frequency compared to Allele A.

Table 1. Genotype Distribution of Pre-Albumin of Local Crosbred Chicken

Breed of Chicken	Sample	Genotype			Frequency	
		$Pa^{A}Pa^{A}$	$Pa^{A}Pa^{B}$	$Pa^{B}Pa^{B}$	A	B
Male Kedu Chicken (KJ)	5	0	5	0	0,5	0,5
Female F1	17	0	16	1	0,47	0,53
Lurik Chicken (LU)	9	0	9	0	0,5	0,5
Lingnan Chicken (BM)	10	0	10	0	0,5	0,5
Total	41	0	40	1	0,49	0,51

Albumin

Table 2 showed the analysis result for albumin loci from Kedu Chicken (KJ), Kedu *Lurik* Chicken (LU), Lingnan Chicken (BM), and Female of Chicken (FIB). As shown in Table 2, the result from albumin loci illustrated that genotype of local chicken was homozygote and heterozygote. The homozygote genotype was Alb^{B} and Alb^{C}. While the heterozygote genotype was marked as Alb^{BC}. The highest frequency for allele B was

in Kedu Chicken (KJ) which is 1 and the lowest frequency (0,56) for the same allele was in Female of Chicken (FIB). Allele C with the highest frequency was in Female of Chicken (FIB) which is 0,44 while the lowest frequency (0,39) was in Lurik Chicken (LU). Characteristics of fenotype in albumin loci were so easy to recognize since they have a wider thickness than other loci. The other characteristics, that make fenotype in albumin are so easy to recognized, is they have two protein bands with the same thickness. The speed of movement from this fenotype in albumin loci was to different positive pole.

This result didn't go along with the results that Hashiguchi et al (1982) found which stated indonesia local chicken showed two genetic frequency on albumin loci which is Alb^A and Alb^B. Hashiguchi et al (1982) stated that the highest frequency is in Alb^A while Maeda (1999) stated that the highest frequency is in Alb^B. Sartika et al (1997) found that albumin loci, there are three autosomal codominants allele which is ALB^A, ALB^B, and ALB^C. Hashiguchi (1982) found four alleles in Indonesian Local Chicken which is ALB^A, ALB^B, ALB^C and ALB^D.

Table 2. Genotype Distribution of Albumin of Local Crosbred Chicken

Breed of Chicken	Sample	Genotype			Frequency	
		Alb^BAlb^B	Alb^BAlb^C	Alb^CAlb^C	B	C
Male Kedu Chicken (KJ)	5	5	0	0	1	0
Female F1	17	2	15	0	0,56	0,44
Lurik Chicken (LU)	9	2	7	0	0,61	0,39
Lingnan Chicken (BM)	10	2	8	0	0,6	0,4
Total	41	11	30	0	0,63	0,37

Ceruloplasmin

Result from observation in ceruloplasmin loci showed that ceruloplasmin loci was controlled by two different allele, which is F and S. Ceruloplasmin loci ini Kedu Chicken (KJ), Lurik Chicken (LU), Lingnan Chicken (BM) and Female of Chicken (FIB) has a homozygote fenotype and also a heterozygote fenotype as showed in Table 3.

Table 3. Genotype Distribution of Ceruloplasmin of Local Crosbred Chicken

Breed of Chicken	Sample	Genotype			Frequency	
		Cp^FCP^F	Cp^FCp^S	Cp^SCp^S	F	S
Male Kedu Chicken (KJ)	5	0	1	4	0,1	0,9
Female F1	17	5	4	8	0,41	0,59
Lurik Chicken (LU)	9	3	3	3	0,5	0,5
Lingnan Chicken (BM)	10	4	4	2	0,6	0,4
Total	41	12	12	17	0,44	0,56

Characteristics of allele Cp^F was move faster to positive pole than allele Cp^S. Homozygotes from this loci showed in observational result that indicate Kedu Chicken (KJ), Lurik Chicken (LU), Lingnan Chicken (BM) and Female of Chicken (FIB) have a genotype of Cp^F and Cp^S. Heterozygotes from this loci also showed in observational result with allele Cp^FCp^S. Highest frequency was in Lingnan Chicken (BM) with 0,6 and the lowest was in Kedu Chicken (KJ) with 0,1 for allele F. Allele S, in the other hand, have the highest frequency in Kedu Chicken (KJ) which is 0,9 and the lowest frequency from Lingnan Chicken (BM) which is 0,4.

Transferrin

Identification of tranferrin loci illustrated two allele which is Tf^A and Tf^B. Frequency from allele Tf^A is than allele Tf^B as showed in Table 4.

Table 4. Genotype Distribution of Transferrin of Local Crosbred Chicken

Breed of Chicken	Sample	Genotype			Frequency	
		Tf^ATf^A	Tf^ATf^B	Tf^BTf^B	A	B
Male Kedu Chicken (KJ)	5	0	5	0	0,5	0,5
Female F1	17	0	15	2	0,44	0,56
Lurik Chicken (LU)	9	0	9	0	0,5	0,5
Lingnan Chicken (BM)	10	2	8	0	0,6	0,4
Total	41	2	37	2	0,5	0,5

Table 4 illustrated that the frequency of allele A and allele B in tranferrin loci were at the same point which is 0,5. The characteristics itself from the tranferrin loci were Tf^{A} move faster to positive pole compared to Tf^{B}. Total amount of sample showed that in this research, most of sample were heterozygotes ($Tf^{A}Tf^{B}$) which is 37 sample and the rest were homozygotes. This result has a difference with the one that Tanabe et al (1999) found. Tanabe et al (1999) found that allele B is more common among chicken especially chicken from Mongolia, but in this study, the more common allele was alelle A. Further, Khan and Singh (1990) explained that mixed between two populations with different genetic frequency can change the specific frequency of gen.

Sartika et al (1997) noted in their research that the genotype of Indonesian Local Chicken which is kampung chicken, Pelung Chicken, and Sentul Chicken in Transferrin loci were controlled by three kind of fenotype. These genotype are TF^{A}, TF^{B} and TF^{C}. Ardiningsasi et al (1995) also noted that in Kedu Chicken, the genotype was controlled by three autosomal codominants allele and the highest gen frequency was in TF^{C}. This findinds in this trasferrin loci was same as Darwati (1995) found. Darwati (1995)found that in Indonesian Local Chicken there are only two kind of allele which is TF^{A} and TF^{B}.

Post-Transferin

Table 5 illustrated that post-transferin loci has a homozygote genotype in form of allele F and S. Result from observation showed that there are two kind of allele that can be found in this research's sample. Those allele were Ptf^{F} and Ptf^{S}.

Table 5. Genotype Distribution of Post-transferrin of Local Crosbred Chicken

Breed of Chicken	Sample	Genotype			Frequency	
		$Ptf^{F}Ptf^{F}$	$Ptf^{F}Ptf^{S}$	$Ptf^{S}Ptf^{S}$	F	S
Male Kedu Chicken (KJ)	5	0	0	5	0	1
Female F1	17	3	10	4	0,47	0,53
Lurik Chicken (LU)	9	0	6	3	0,34	0,66
Lingnan Chicken (BM)	10	4	4	2	0,6	0,4
Total	41	7	20	14	0,41	0,59

Allele S has a higher frequency than allele F which is 0,59 compared to 0,41, respectively. The different frequency of allele F and Allele S in sample of this research can be caused by selection, mutation, mixed population, in-crossbreed, out-crossbreed or genetic drift (a sudden change of genetic frequency) (Noor, 2000). The highest genetic frequency was in Lurik Chicken (LU) and the high differences between allele F and S in this study can be the result of crossbreed. The purpose of crossbreed itself, was to produce a new strain of chicken that have a better qualification whether it is have a high level of egg productivity or else. Darwati (1995) found that in Indonesian Local Duck, there are three alleles which is $PTF\text{-}I^{A}$, $PTF\text{-}1^{B}$ and $PTF\text{-}I^{C}$

Amylase-1

Result from observation showed that amylase-1 loci have two kind of allele which is $Am\text{-}1^{B}$ and $Am\text{-}1^{C}$. From characteristics point of view, $Am\text{-}1^{B}$ has a faster movement to positive pole rather than $Am\text{-}1^{C}$. Table below also indicated that most of the sample of this research has a heterozygote fenotype which is $Am\text{-}1^{BC}$. For the same reason as a previous loci, a different amount of heterozygotes chicken and homozygote chicken in this research can be caused by selection, mutation, mixed population, in-crossbreed, out-crossbreed or genetic drift (a sudden change of genetic frequency) (Noor, 2000). The highest frequency was from Lurik Chicken (LU) in allele C (0,66) and the lowest was 0,34 also from Lurik Chicken (LU).

Table 6. Genotype Distribution of Amylase-1 of Local Crosbred Chicken

Breed of Chicken	Sample	Genotype			Frequency	
		$Am\text{-}1^{BB}$	$Am\text{-}1^{BC}$	$Am\text{-}1^{CC}$	B	C
Male Kedu Chicken (KJ)	5	0	5	0	0,5	0,5
Female F1	17	2	13	2	0,5	0,5
Lurik Chicken (LU)	9	0	6	3	0,34	0,66
Lingnan Chicken (BM)	10	2	8	0	0,6	O,4
Total	41	4	32	5	0,49	0,51

Heterozigosity of Local Chicken

Heterozigosity was one of the parameter that can be used to determined genetic diversity among population. Average heterozigosity from four kind of chicken which is Kedu Chicken (KJ), Female of Chicken (FIB), Lurik Chicken (LU) dan Lingnan Chicken (BM) was analysis using blood protein that showed in Table 7.

Table 7 showed that there are differences in heterozigosity among Kedu Chicken (KJ), Female of Chicken (FIB), Lurik Chicken (LU) dan Lingnan Chicken (BM). Average value of heterozigosity that was highest was in Female of Chicken (FIB) wih 0,490, Lurik Chicken (LU) with 0,487, Lingnan Chicken (BM) with 0,486 and last one was in Kedu Chicken (KJ) with 0,340. The different in heterozigosity caused by differences between chicken breed that used in this research. This assumption was emphasized by Ardiningsi (1997) that stated differences between heterozigosity among population can be caused by the differences of the spesies in this population.

The highest score of heterozigosity, that was earned from Female of Chicken (FIB), can be caused by this kind of chicken was a result from crossbreed. Banker and Manwell (1986) explained that heterozigosity can be influenced by a lot of factors such as overdominant gen (positive heterosys), the differences of genetic frequency between male and female, and also can be influenced by assortive mating. The high score obtained from heterozigosity can be fortune since the fartest kinship between species then the possibility of inbreeding can be lower. The lower possibility of inbreeding can lead to lower access of resesive allele that can bring deficiency for the next generation. The high score of heterozigosity was expected to form a new kind of species that have higher productivity compared to previous species (Hardjosubroto, 1994).

Table 7. Average Heterozigosity from Indonesian Local Chicken

Breed of Chicken	Amount of Breed	H ─
Male Kedu Chicken (KJ)	**5**	**0,340**
Female of Chicken (FIB)	**17**	**0,490**
Lurik Chicken (LU)	**9**	**0,487**
Lingnan Chicken (BM)	**10**	**0,486**

From table 7 above, the highest score of heterozigosity was in lingnan chicken (0,486) and the lowest score of heterozigosity was Kedu Chicken (0,340). This findings were contradictionary because in the beginning of the research, there are assumptions that Kedu Chicken (KJ) was a crossbreed chicken, therefor the score of heterozigosity should be higher than any other kind of chicken in this research. In the contrary, Lingnan Chicken (BM) was a pure breed chicken so the score of heterozigosity should be lower than any other kind of chicken in this research.

Male of Kedu Chicken (KJ) get the lowest score of heterozigosity can be caused from inbreeding. Inbreeding can lead to higher possibility of homozygot in allele and lowering down the possibility of heterozygosity (Legates and Warwick, 1990). Reason of low score in heterozigosity on Kedu Chicken can be caused by habit of the people around poultry breeding in Temanggung, Central Java that more likely to inbreed Kedu Chicken. The in breed of Kedu Chicken can produce a lot of Cemani chicken that were famous among Javanese people.

On the other hand, the highest score of heterozigosity came from Female of Chicken (FIB) with 0,490. This score can be earned because Female of Chicken (FIB) was a crossbreed chicken, that why they have a highest score in heterozigosity. The high score of heterozigosity were profitable since as far as the kinship, the possibility of inbreeding can be lowering down and this can be lead to lower possibility of disablement for next breed of the chicken. Hardjosubroto (1994) claimed, the high score of heterozigosity then the possibility of creating a new race that have a higher score of productivity will be more possible compared to the ancestry.

Heterozigosity of Indonesian Local Chicken from blood protein analysis

Heterozigosity of Indonesian local chicken from blood protein polymorphisms can be showed on table 8 below:

Table 8. Calculation of Genetic Diversity (h) from blood protein

Breed of Chicken	Protein	P-Alb	Alb	Cp	Tf	P-Tf	Amy-1
Male Kedu Chicken (KJ)		0,500	0,500	0	0	0,500	0,500
Female F1		0,498	0,493	0,455	0,493	0,498	0,500
Lurik Chicken (LU)		0,500	0,476	0,471	0,500	0,500	0,475
Lingnan Chicken (BM)		0,500	0,500	0,500	0,480	0,500	0,480

From Table 8, the result of heterozigosity from blood polymorphisms showed that there are locis of protein that polymorph. Those are protein loci of pre-albumin, albumin, ceruloplasmin, transferrin, post-transferrin and amylase-1. This table also provide us with information about egg productivity among Kedu Chicken (KJ), Female of Chicken (FIB),Lurik Chicken (LU) and Lingnan Chicken (BM) that can be conclude from transferrin loci. The score of heterozigosity in transferrin loci describe that Female of Chicken (FIB),Lurik Chicken (LU) and Lingnan Chicken (BM) have a higher egg productivity compared to Kedu Chicken (KJ). Egg

productivity in chicken was controlled by heterozigosity of allele in tranferrin loci, which mean the higher score of heterozigosity in transferrin loci, the higher egg productivity in chicken.

Result from statistical analysis showed that there are significance differences between protein loci in blood polymorphisms. It means that the sixth of blood protein which is pre-albumin, albumin, ceruloplasmin, transferrin, post-transferrin and amylase-1 can be used to observed the genetic diversity through electrophoresis. Warwick et al (1990) also stated, the protein that contained in blood were transferrin, albumin, hemoglobyn, and other blood enzymes. Most of them can be used to determine the genetic diversity between races.

Heterozigosity on blood protein between Kedu Chicken, Female of Chicken, Lurik Chicken and Lingnan Chicken from statistical analysis, showed no significance differences with P>0,05. The lack of genetic diversity among chickens used in this research lead us to a conclusion that there are no significance differences in genotype distribution among four kind of chickens used in this research. This could mean that all of the chickens in this research came from the same ancestors or have a closed relation from each other. Although there are no differences between fourth races of chicken in genotype distribution, this result may not represent the real condition due to lack of sample that used in this research.

V. CONCLUSION AND SUGGESTION

Based on this research was concluded that:

1. The sixth kind of blood protein which is pre-albumin, albumin, and ceruloplasmin, transferrin, post-transferrin and amylase-1 can be used to determine and evaluated the genotype distribution among chicken raised in Poultry Breeding Center Temanggung. The characteristics of each loci were $PALB^{A}$ and $PALB^{B}$ in pre-albumin loci, ALB^{B} and ALB^{C} in albumin loci, CP^{F} and CP^{S} in ceruloplasmin loci, TF^{A} and TF^{B} in tranferin loci, PTF^{S} and PTF^{F} in post-tranferrin loci, AM-1^{B} and AM-1^{C} in amylase loci.
2. There were no differences genotype distribution between materials used with lowest average heterozigosity in male Black Kedu chicken.

Suggestion

Crossbreed between other kind of Indonesian local chicken was important to maintenance the heterozigosity of the next breed that can guarantee higher productivity. But, in this research, I am suggesting to keep the homozygotes in male Black Kedu Chicken (KJ) since has the lowest score in heterozygosity.

REFERENCES

[1]. Ardiningsasi, S.M., A.M. Umiyati, and S.K. Irene. 1995. Tinjauan genetika pada ayam Kedu melalui pengamatan karakteristik polimorfisme albumin (Alb) and transferrin (Tf) darah. Pros. Sem. Nas. Sains dan Teknologi Peternakan, Balistik. Bogor

[2]. Ardiningsasi, S. M. 2000. Kajian Genetika pada Ayam Kampung di Jawa dan Bali berdasarkan dari Protein Polimorfisme Protein Putih Telur. Jurnal Peternakan Tropis. 25: 100-104.

[3]. Ashton G.C. 1964. Serum albumin polymorphism in cattle. J. Anim. Genet. **50**: 1421-1426.

[4]. Brahmantiyo, B., L.H. Prasetyo, A. R. Setioko, dan R.H. Mulyono. 2003. Pendugaan jarak genetik dan faktor peubah pembeda galur itik Alabio, Bali, Khaki, Campbell Mojosari dan Pegagan melalui analisis morfometrik. JITV. **8** (1): 1-7.

[5]. Brodacki A., Gluchowski W., Smalec E., 1986. Preliminary report on blood serum polymorphism in geese. 7th Europ. Poult. Conf., Paris 24–28 July 1986: 95–98.

[6]. Brodacki A., & Smalec E. 2001. Genetic Polymorphism of slow-migrating prealbumin in geese. EJPAU. 4(2): 10-17.

[7]. Budipurwanto, T. 2001. Studi Tentang Fenotip Ayam Buras Berdasarkan Sifat Kuantitatif dan Kualitatif. Fakultas Peternakan Universitas Diponegoro, Semarang. (Tesis).

[8]. Darwati, S. 1995. Studi fenotopik ayam Kampung, Pelung, dan Kude serta respon terhadap vaksinasi tertelo. Thesis S2. Program Pascasarjana, IPB. Bogor.

[9]. Dobzhansky, T. 1970. *Genetics of the evolutionary process*. Columbia University Press, New York.

[10]. Feldhamer, G. A., L. C. Drickamer, S. H. Vessey, & J. F. Merrit. 1999. Mammalogy Adaptation, Diversity and Ecology. McGraw-Hill Companiess, Boston.

[11]. Hamilton, J. A., & Benson, M. D. 2001. Transthyretin: A review from structural perspective. Cellular and Mollecular Life Science. 58: 1491-1521

[12]. Hardjosubroto, W. 1994. Aplikasi Pemulibiakan Ternak di Lapangan. PT. Gramedia Widiasarana Indonesia. Jakarta.

[13]. Harris, H. 1994. Dasar-dasar Genetika Biokemis Manusia. Edisi ke-3. Terjemahan: Abdul Salam & M. Sofro. Gadjah Mada University Press, Yogyakarta.

[14]. Hubbard, G. R. 1999. Ceruloplasmin loci at Tibet. J. Anim. Genet. **77** (1) : 167-169.

[15]. Ismoyowati. 2008. Kajian deteksi produksi telur itik Tegal melalui polimorfisme protein darah. J. Animal Production **2** [10]: 122-128.

[16]. Johari, S. Sutopo, E. Kurnianto, & E. Hasviara. 2008. Polimorfisme protein darah ayam Kedu. J. Indon. Trop. Anim. Agric. **33** [40]: 313-318.

[17]. Johari, S. 1999. Polimorfisme protein darah ayam Kampung di Jawa Tengah. Fakultas Peternakan Universitas Diponegoro, Semarang.

[18]. Hashiguci, T., T. Nishida, Y. Hayashi. And S. S. Mansjoer. 1982. Blood Protein Variation of the Native and the Jungle Fowl in Indonesia. The Report by Grant-in-Aid for Overseas Scientific Survey in 1981: 97-109.

[19]. Kanda Y., Goodman D. S., Canfield R. E. and Morgan F. J. (1974) The amino acid sequence of human plasma prealbumin. J. Biol. Chem. **249:** 6796–6805

[20]. Katsumata, M., T. Amano, S. Suzuki, K. Nozawa, H. Martojo, I.K. Abdulgani and H. Nadjib. 1981. Morphological characters and blood protein gene constitution of Indonesian goat. J. Rep. Soc. Res. Native Livestock **2**: 55-68.

[21]. Khana, N. D. 1973. Blood protein in the donkey. J. Anim. Genet. **80** (1): 5-7.

[22]. Khan, I., Khatun, J., Bhuiyan, A., and Sharmin, R. 2006. Production performance of Fayoumi Chicken under Intensive Management. Pakistan Journal of Biological Sciences, **9** (2): 179-181

[23]. Khan, F. And Singh, A. 1990. Principles of Genetics and Animal Breeding. Jaypee Brother Medical Publishers. New Delhi.

[24]. Lumataw, S. 1993. Protein in Polymorphisms in Phillipine Native Chicken and Six Exotic Breed. University of Phillipine. Phillipine (Thesis).

[25]. Maeda, Y., Yamamoto and T. Nishida. 1999. *Protein Polymoprhisms of the Native Chicken and Red Jungle Fowl in Nepal in Morphologi and Genetical studies on the Native Domestic Animals and Their Wild Form in Nepal*. University of Tokyo, Japan.

[26]. Nataamidjaya, A. G. 2003. *The Native Chicken Of Indonesia.* Balai Penelitian Dan Pengembangan Pertanian Peternakan. Departemen Pertanian. Jakarta. 1-6

[27]. Natalia, L. And A. Priadi. 2005. Penggunaan Probiotik untuk Pengendalian Clostridal Necrotic Enteritis pada Ayam Pedaging. JITV. **10**(1): 71-78.

[28]. Nei, M. 1987. *Molecular Evolutionary Genetics. Columbia University Press*. New York.

[29]. Noor, R. R. 2000. *Genetika Ternak. Penebar Swadaya*, Jakarta.

[30]. Nozawa K., T. Kenji, M. Yoshizane, T. Yuichi, Z. Tsesrejavyn, T. Horloojau, and T. Tsendsuren. 1994. Morphology and gene constitution of the Indonesian horses. **In**: The Origin and Phylogeny of Indonesia Native Livestock. Ed ke-2. Tokyo: The Research Group of Overseas Scientific Survey. Japan. pages 9-30.

[31]. Rasyaf, M. 1989. *Pengelolaan Produksi Telur*. Kanisius. Yogyakarta

[32]. Sartika, T., Mulyono, R.H., Mansyoer, S.S., Purwadaria, T, Gunawan, B., Nataamijaya, A.G., and Diwyanto, K. 1997. Penentuan jarak genetik pada ayam lokal melalui polimorfisme protein darah. Sem. Nas. Peternakan and Veteriner. Fakultas Peternakan Institut Pertanian Bogor, Bogor.

[33]. Simm, G. 2000. *Genetic Improvement of Cattle and Sheep*. Farming Press Miller Freeman Ltd., UK.

[34]. Stansfield, W.D. & S. L. Elrod. 2002. *Genetika. Edisi ke-4*. Terjemahan: Damaring Tyas W. Erlangga, Jakarta.

[35]. Sulandari, S., M. S. A. Zein., S. Paryanti, T. Sartika, M. Astuti, T. Widjastuti, E. Sudjana, S. Darana, I. Setiawan, & D. Garnida. 2007. *Sumberdaya genetik ayam lokal Indonesia. Keanekaragaman Sumberaya Hayati Ayam Lokal Indonesia: Manfaat dan Potensi*. Pusat Penelitian Biologi Lembaga Ilmu Pengetahuan Indonesia, Jakarta. Hal: 45-67.

[36]. Sutopo, K. Nomura, Y. Sugimoto and T. Amano. 2001. *Genetic relationships amongs Indonesian Native Cattle*. J. Anim. Genet. **28**: 3-11.

[37]. Tsunoda, K., O. Hisota, A. Takashi, K. Kazuhito, N. Takao, Y. Takahiro, Y. Yoshio, X. Vo-tong dan Ba Loc Chau. 1998. Morphological and genetic characteristics of sheep raised by the cham tribe in Vietnam. J. Rep. Soc. Res. Native Livestock **17**: 63-73.

[38]. Tsunoda, K., K. Nozawa, M. Yoshizane, T. Yuichi, T. Tuya, B. Munkhtuya, T. Horloojav, T. Zanchic, and B. Dashnyam. 1999. External morphological character and blood protein and non-protein polymorphisme of native sheep in Central Mongolia. J. Rep. Soc. Res. Native Livestock **17**: 73-82.

[39]. Warwick, E. J., J. M. Astuti & W. Hardjosubroto. 1990. *Pemuliaan Ternak*. Cetakan 4. Gadjah Mada University Press. Yogyakarta.

[40]. Wulandari, A. R. 2008. Studi tentang keragaman genetik melalui polimorfisme protein darah dan putih telur pada tiga jenis ayam Kedu periode "layer". Tesis. Program Pascasarjana Fakultas Peternakan, Universitas Diponegoro, Semarang.
[41]. Wyne, R.Z., H. Shelwyn and T. Robert. 1990. Elektroforesis DNA: Allels of Blood protein. Wallingford, United Kingdom.
[42]. Yuwono, T. 2005. Biologi Molekuler. Penerbit Erlangga, Yogyakarta.