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

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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
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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.**

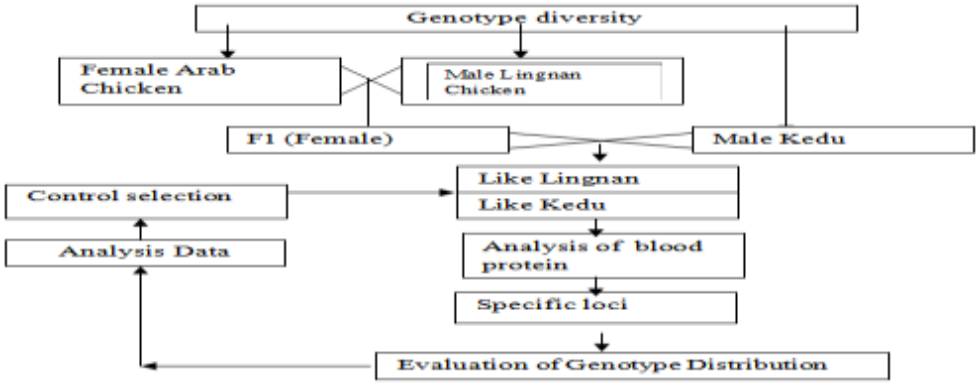

Illustration 1. Scientific Framework of Research

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^{o}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).

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

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:

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

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

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

Explanation:
X_1 : average calculation of T_1
X_2 : average calculation of T_2
S_1^2 and S_2^2 : 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

Research about genetic variation of Arabic, Arabic cross, Lingnan and and Kedu Lurik chicken in Temanggung using electrophoresis methods giving result as follow: Polymorphism of Blood Protein Electrophoresis results of blood plasma can be seen on Illustration 2. Sample molecule move based on weight of molecule.

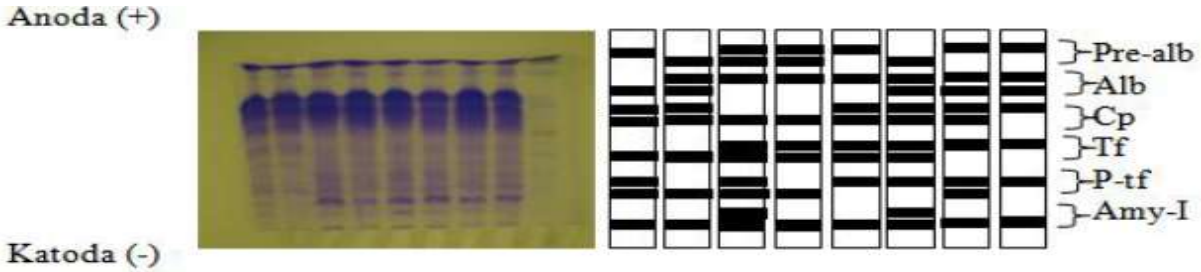

Illustration 2. Electrophoresis Result and scater Diagram of Blood Protein

According to Warwick et al. (1990) protein polymorphism is different of biochemical properties genetically regulated and are found in body fluids and fat cells. One way to identify a blood plasma protein polymorphism is the technique electrophoresis (Tsunoda et al., 1998). According to Lestari et al. (1998), to identify genetic traits can be approached through the study of blood protein polymorphism, because proteins polymorhism can separates based on the size. According to Oktariani and Pristiwindari (2007), a blood plasma protein that indicate the presence of polymorphisms (controlled by two or more alleles at a particular gene locus). Illustration 2 shows that at each locus have different zones according to the direction of the velocity of the molecular weight. This suggests that the compounds will move in solution as a result of the nature of the opposite polarity. Results of electrophoresis analysis of blood plasma in this study found several loci those were pre - albumin protein, albumin, cerruloplasmin, transferrin, transferrin and amylase-I. A study conducted by Malihatun (2009) on the chicken Kampung and Kedu found 7 loci, pre-albumin protein, albumin, post-transferrin, transferrin and three other locus remains unknown but showed a different pattern of proteins. Elektrophoresis is one of the techniques were use to look at the DNA, this technique can separates DNA molecule size. Scientific dyes can also be separated by electrophoresis. Like DNA, most scientific dyes are negatively charged. Scientific dyes are often used in simulating DNA, because can be visualize DNA. According to Yuwono (2005) Electrophoresis is separation of charged ions based on their migration rate in electric field.

Plasma Pre-Albumin

Observations in this study indicate that pre-albumin (P-alb) loci move faster towards the anode than albumin (Alb), Pre-albumin (P-alb) has a smaller molecular weight than albumin. From the analysis of blood plasma in the medium polyacrylamide gel electrophoresis, the locus pre-albumin in this study obtained in the whole population that is controlled by allele A and allele B as homozygote genotype AA, BB and AB heterozygote genotype. The previous study conducted on Pelung chicken and Sentul Race chicken that pre-albumin locus controlled by two alleles, namely Palba and Palbb respectively. In accordance with Tehupuring (1999) that the birds Wren Java pre-albumin locus is controlled by allele A and allele B.

Table 1. Gene Frequency of Pre-Albumin (P-alb) Blood of Arabic cross, Kedu, Lingnan and Arabic Rooster

Type of Chicken	Total	Genotip			Frequency of Gen	
	Sample	AA	AB	BB	A	B
Arabic cross	15	8	5	2	0.70	0.30
Kedu	20	5	9	6	0.48	0.52
Lingnan	5	2	3	-	0.70	0.30
Arabic rooster	5	-	5	-	0.50	0.50
Total	**45**	**15**	**22**	**8**		

Results of statistical tests on the distribution of pre-albumin locus genotype (P-alb) and the frequency of gene A and B in Arabic cross, Kedu, Lingnan and Arabic rooster showed no differences (P>0.05) It could be happened because the Arabic cross, Kedu, Lingnan and Arabic rooster is still in one species and then in the population may occurs selective mating, so that genes that appear in this population are all the same and polymorphic because more than one allele obtained. Based on Table 1 shows that the frequency gene pre albumin A on the Arabic cross and Lingnan is greater (0,70) than on the Kedu (0.48) and Arabic rooster (0.50). Whereas, the frequency gene pre albumin B allele on Arabic cross and Lingnan were similar (0,30).

Plasma of Albumin (Alb)

Electrophoretic analysis of blood plasma in this study showed that albumin has different characteristics compared to another protein performan. Albumin observed have a different shape that is thicker than the other bands. The thickness of the band formed from protein bands indicate the weight amount of protein contained. On Kedu chicken easily recognized that albumin has a greater thickness than the other bands. According to White et al. (1973), albumin has a molecular weight of 69,000 g/mol. In contrast to research conducted by Ismoyowati (2008), that Kampung chicken of albumin protein has a molecular weight around 52,000 Dalton. From the results of the blood plasma electrophoresis in this study identified that albumin locus is controlled by two alleles, A and B, which form three kinds of genotypes, namely AA, AB and BB. According to the research Ismoyowati (2008) on the identification of three loci Kampung chicken obtained combinations allele form those were albaa, albab, Albac, albbb and albbc. A study conducted by Malihatun (2009) on the Kampung and Kedu chicken showed that albumin locus is controlled by a single allele that form the AA genotype. Previous research conducted at a local chicken showed that albumin is controlled by three allele alba, albb and albc. Johari et al. (2008) stated that albumin locus allele is controlled by two alleles of A and B in Kedu chickens.

Table 2. Gene Frequency of Albumin (Alb) Blood of Arabic cross, Kedu, Lingnan and Arabic Rooster

Type of Chicken	Total	Genotype			Frequency of Gen	
	Sample	AA	AB	BB	A	B
Arabic cross	15	5	7	3	0.57	0.43
Kedu	20	3	11	6	0.42	0.58
Lingnan	5	-	3	2	0.30	0.70
Arabic Rooster	5		5		0.50	0.50
Total	**45**	**8**	**26**	**11**		

Statistical tests on the distribution of genotypes of locus albumin (Alb) and gene frequencies A and B of Arabic cross, Kedu, Lingnan and Arabic Rooster showed no differences (P>0.05). It is suggested that there is no genetic variation between each of chicken breed. Based on Table 2 showed that gene frequency albumin A of Arabic cross is greater (0.57) than Kedu (0.43, Lingnan (0,30) and Arabic rooster (0.50). Research Utomo (1999) states that the highest gene frequency at A Albumin allele found in local chicken (buras), that is equal to 0.78. Gene frequency albumin B on this research showed that Lingnan chicken is greater (0,70) than other chickens breed.

Plasma of Cerruloplasmin (Cp)

Ceruloplasmin (Cp) on the Arabic cross, Kedu, Lingnan and Arabic rooster having three genotypes FF, FS and SS. There are two alleles of F and S genotypes and heterozygous genotype is more common. This research similar with Johari et al. (2008), that ceruloplasmin alleles in Kedu chickens have type FF and SS, also heterozygous characteristics of FS.

Table 3. Gene Frequency of Cerruloplasmin (Cp) of Arabic cross, Kedu, Lingnan and Arabic Rooster

Type of Chicken	Total	Genotip			Frequency of Gen	
	Sample	FF	FS	SS	F	S
Arabic cross	15	1	11	3	0.43	0.57
Kedu	20	2	14	4	0.45	0.55
Lingnan	5	-	4	1	0.40	0.60
Arabic Rooster	5	1	4	-	0.60	0.40
Total	**45**	**4**	**33**	**8**		

Statistical analysis on the distribution of cerruloplasmin (Cp) and gene frequencies F and S of Arabic cross, Lingnan, Kedu and Arabic rooster showed no differences (P>0.05). It is shows that the blood protein can be used to observe genetic diversity through electrophoresis. According to Harper et al. (1980), electrophoresis is a method for separation particles or components according to their electrical charge. The components used are proteins or polynucleotide acids derived from blood and biological solution or a network, where the ions change depending on the pH of the solution to be analyzed.

The results of gene frequency of F allele presented on Table 3 showed that Arabic Rooster is the greatest (0.60) among all the samples. In order was Kedu (0.45), followed by Arabic cross (0.43) and Lingnan (0.40). On the other hand, Gene frequency of S allele showed that Lingnan be the highest (0.60) followed by

Arabic cross (0.57), Kedu (0.55) and Arabic Rooster (0.40). Gene frequency of S allele on this research is showing greater in Lingnan (60) than other chickens.

Plasma of Transferin (Tf)

Transferin (Tf) on the Arabic cross, Kedu, Lingnan and Arabic rooster found two alleles, those were A and B allele. Based on Deza et al. (2000), chicken in Central Argentina, locus of transferrin (Tf) have 2 alleles, there are A and B. Research by Guney et al. (2003) in Damascuz chicken, the transferrin locus (Tf) have type of BD, CD, AD, CC, AC, BC and DD genotype. The results of transferin (Tf) on this research can be seen in Table 4.

Table 4. Gene Frequency of Transferin (Tf) of Arabic cross, Kedu, Lingnan and Arabic Rooster

Type of Chicken	Total	Genotype			Frequency of Gen	
	Sample	AA	AB	BB	A	B
Arabic cross	15	5	7	3	0.57	0.43
Kedu	20	1	16	3	0.45	0.55
Lingnan	5	-	4	1	0.40	0.60
Arabic Rooster	5	-	4	1	0.40	0.60
Total	**45**	**6**	**31**	**8**		

Statistical analyses on the distribution genotypes of transferin (Tf) and gene frequencies A and B of Arabic in Arabic cross, Kedu, Lingnan and Arabic rooster showed no differences (P>0,05). This shows there is no genetic variation between each of chicken. Result of tranferrin loci is presented on Table 4. In this research showed that gene frequency of A allele in Arabic cross is greater (0.57) than Kedu (0.45), Lingnan (0.40) and Arabic rooster (0.40). Whereas the gene frequency of B allele on this research showing that Lingnan and Arabic rooster is greater (0.60) than other chickens. This research showed not similar with Utomo (1999) in Kampung chicken that the frequency of transferrin (Tf) is the highest in the A allele, that is equal to 0.406. According to Wiwanitkit et al. (2007), transferrin (Tf) is a glycoprotein that can be found in chicken Thailand, from the extracellular compartment into the cells.

Plasma of Post-Transferin (Ptf)

Result of post transferin (Ptf) loci on this research presented on Table 5. Locus post transferrin (P-tf) composed by F and S. According to (Gahne et al., 1977), post transferin on the India local cattle controlled by F and S allele

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. Gene Frequency of Post Transferin (Ptf) of Arabic cross, Kedu, Lingnan and Arabic Rooster

Type of Chicken	Total	Genotype			Frequency of Gen	
	Sample	FF	FS	SS	F	S
Arabic cross	15	4	7	4	0.50	0.50
Kedu	20	3	11	6	0.42	0.58
Lingnan	5	-	4	1	0.40	0.60
Arabic Rooster	5	-	2	3	0.20	0.80
Total	**45**	**7**	**24**	**14**		

Statistical analysis the distribution genotypes of locus transferin (Tf) and gene frequencies of A and B allele in Arabic cross, Kedu, Lingnan and Arabic rooster showed no differences (P>0,05). It means no genetic variation between each of chicken in this study. According to Table 5 showed that the allele F in Arabic cross is greater (0.50) than Kedu (0.43), Lingnan (0.40) and Arabic rooster (0.20). Gene frequency of S allele on this research showing that Arabic rooster (0.80) more higher than other chickens. The frequency of S allele are more higher than F gene frequency. It is similar with research by Butkauskas et al. (2004), that gene frequency in the S allele (0.53) is higher than the F allele (0.41) in Cross White Loman chickens. The difference in the high and low frequency alleles of genes on the F and S chicken cross white Lohman because the result of new strains. This implies also that it is true in this research that the bigger difference in frequency is better, because it affects the quality of the newly bred chickens (crossbred) which in this case gave a result to have produced a new strain and high sales value chicken. According to Butkauskas et al. (2004) those condition supposed be influenced by the frequency of genetic selection, mutation, population mixing, inbreeding, out crossing and "genetic drift"(sudden change of gene frequency).

Plasma of Amylase-I (Amy-I)

Amylase is protein enzyme on the blood have helping work of metabolism on the body. Amylase also affecting degeneration of cell. Amylase can be used to knowing gen population by locus genes with blood of protein. Tape protein of amylase on the chicken in Vietnam has named Amy1dan Amy2. Frequency of Amy1 higher than Amy2 (Nozawa et al., 1998). The results of amylase-I on this research with electrophoresis methods showing two alleles (F and S), be presented in Table 6.

Table 6. Gene Frequency of Amylase-I (Amy-I) Blood of Arabic cross, Kedu, Lingnan and Arabic Rooster

Type of Chicken	Total	Genotype			Frequency of Gen	
	Sample	FF	FS	SS	F	S
Arabic cross	15	6	5	4	0.57	0.43
Kedu	20	4	13	3	0.52	0.48
Lingnan	5	-	4	1	0.40	0.60
Arabic Rooster	5	-	4	1	0.40	0.60
Total	**45**	**10**	**26**	**9**		

Heterozygosity and Phenogram between Breed

According to Warwick et al. (1990), high heterozygosity values are very favorable, because the more distant kinship, the smaller pressumed of the possibility inbreeding and the emergence of a recessive allele that can carry a relatively low defect. According to Johari et al. (2008), the higher value of heterozygosity will perform on genetic diversity. As a consequence the lower value of heterozygosity, the lower genetic diversity will be occured. Based on Table 7 showed that all breed of chicken seemed to be in around heterozygosity, those were between 0,460 to 0,494.

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. Heterozygosity of Arabic Cross, Kedu, Lingnan and Arabic Rooster

Type of Chicken	h						$\bar{H}$
	P-alb	*Alb*	Cp	Tf	Ptf	Amy-I	
Arabic cross	0,420	0,490	0,491	0,491	0,500	0,490	0,480
Kedu	0,499	0,487	0,495	0,495	0,487	0,499	0,494
Lingnan	0,420	0,420	0,480	0,480	0,480	0,480	0,460
Arabic rooster	**0,500**	**0,500**	**0,480**	**0,480**	**0,320**	**0,480**	**0,460**

The study also describe phenogram based on the gene frequency observed at all loci. According to Illustration 3 informed that Kedu chicken and Lingnan were closed and being one big cluster with Arabic cross. Whereas, the Arabic rooster separated from the grup of Kedu, Lingnan and Arabic cross chicken. This result was not surprisingly because the alllele frequency between those three grup of chicken were seemed similar.

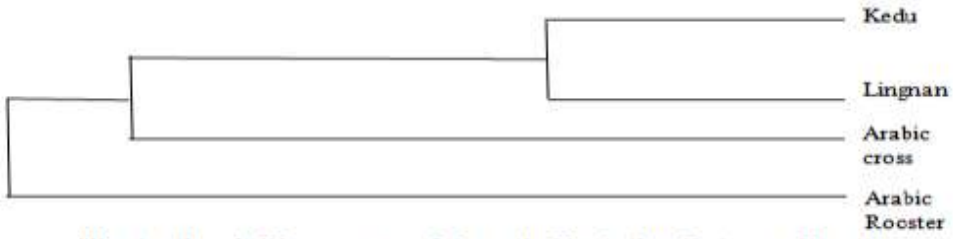

Illustration 3. Phenogram of Genetic Variation Between Breed

V. CONCLUSION

Based on the results of research on Genetic Variation of Arabic, Arabic cross, Lingnan and Kedu chicken in Poultry Breeding Center Temanggung Central Java," it could be concluded that:

1. Each breed of chicken found in the similarity alleles at all loci.
2. There were no significant genetic variation between Arabic, Arabic cross, Lingnan and Kedu chicken crossbreed.

Suggestion

It is therefore suggested and strongly recommended to consider that Arabic and Lingnan as a reference for breeding with other chickens to produce a new strain in order to get a certain purpose.

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