Characteristics of immunocompetence in Indonesian chickens

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Abstract. The concentration of IgY in the body is often associated with resistance to bacterial infections, whereas ND specific antibody titters are indicated as resistance to viral infections. The aim of this study was to determine the characteristics of IgY and ND titters in newly released chickens, and to compare them with native chickens spread in the community. This research used 286 IPB-D1 chickens, Jatiwangi Sentul chickens, and Sensi-1 chicken. IgY concentration were analyzed using the ELISA method, and ND titters were analyzed using the HI test. The results of the study showed high IgY concentrations were found in IPB-D1 chickens (10.10) and Sensi-1 chickens (10.63), while moderate IgY concentrations were found in jatiwangi sentul chickens (8.63). The results of ND titters showed that the chicken IPB-D1 (2.08) and Sensi-1 chicken (2.54) were higher than Sentul Jatiwangi chickens (0.70). It could be concluded that IPB-D1 chickens and Sensi-1 chickens are more resistant to disease infections than other chickens. The disease resistance selection program in Sentul Sensi-1 chickens, and the utilization of the heterosis effect through the crossing program in chickens IPB-D1 has been shown to increase good disease resistance indicators. Selection and crossbreeding programs in IPB-D1 and Sensi-1 chicken have been proven to increase resistance characteristics.

1. Introduction
IPB-D1 chicken is a composite chicken from a cross between F1 male (Pelung x Sentul) and F1 KM female (Kampung x parent stock Cobb). The chickens are crossed with each other for five generations. IPB-D1 chickens reach 1.5 kg / head body weight at 12 weeks and are almost the same as broilers at 5 weeks) [1]. The selected Sentul chickens (Sensi-1) results from selection of native Sentul chickens with a body weight of 900 grams / head at 10 weeks of age by IRIAP (Indonesian Research Institute for Animal Production) and has been selected for disease resistance by the Molecular Genetic Laboratory, IPB University. Sentul Jatiwangi chicken is a native chicken from Jatiwangi area that has not been crossed or selected.

IPB-D1 chicken and Sentul Sensi-1 chicken are new families that have been released by the Ministry of Agriculture of the Republic of Indonesia through SK No.693/KPTS/PK.230/M/9/2019, and No.39/KPTS/PK.020/1/2017. Release of the two chicken families above to the farmer will be traditionally maintained, in an uncontrolled and unhygienic environment. This maintenance condition demands high chicken disease resistance. The use of original Sentul chicken in East Java is intended to...
determine the nature of the resilience of native chickens found in the community, and can be used as a picture of the comparison with the nature of resistance in the new chicken families.

Dangerous diseases that often infect chickens are pullorum disease caused by bacterial infection S. pullorum and New Castle (ND) disease caused by viral infections [2]. The disease is pathogenic which causes very high mortality [2-3]. Yolk immunoglobulin (IgY) is a specific total antibody which can be used as a general immune parameter. The concentration of IgY in the body is often associated with resistance to bacterial infections [4]. The body's resistance to viral infections is indicated by levels/ND specific antibody titter in blood cerium [5].

Information on Indonesian chicken disease resistance is still limited. This research was conducted to determine the characteristics of total IgY antibody titters and ND antibody titters in IPB-D1 chickens and Sentul Sensi-1 chickens and to compare them with native Sentul chickens from Jatiwangi that were spread in the community. Another benefit that can be used is as a reference for selection and crossing programs to increase disease resistance in chicken.

2. Materials and methods

2.1. Animal
This research used 286 IPB-D1 chickens, native Sentul chickens from Jatiwangi, and selected Sentul chickens (Sensi-1 Agrinak). Chickens are placed in semi-intensive cages (colonies). The size of the cage is 9 m² and contains five chickens. The cage is equipped with a feeder tray, drink, and litter. Feed is given twice a day and drink given ad libitum. The feed used is commercial feed with crude protein content of 22%, and mixed feed with crude protein 17%. During maintenance of vaccinated chickens at the age of one day and three days. Blood samples were collected at five weeks and 21 weeks.

2.2. Enzyme-linked Immunosorbent Assay Test (ELISA)
Elisa test is used to determine the concentration of IgY antibodies. IgY analysis used the indirect elisa method [6]. Elisa test using chicken blood serum. The first step was coating with 2.5µg/ml IgY antigen as a catching antibody. The second step was blocking with BSA1% 100µl/hole. Then, the serum sample is diluted 1:100 and inserted 2.5 µl/hole. The third stage was included IgG as a 100 µl / well detection antibody, then secondary antibody (IgG HRP anti-rabbit) was added 50 µl / well. Optical density (OD) is read using an ELISA reader. Data were analyzed descriptively based on the average IgY concentration.

2.3. Hemagglutination Inhibition Test (HI)
The HI test used to count antibodies and determine the correlation between antibody titters and body resistance [7]. The principle of HI testing is to inhibit agglutination of red blood cells by a virus, due to the binding of the virus by specific antibodies [8]. Paramyxovirus stimulates the formation of specific antibodies that inhibit blood agglutination [9].

The first stage of the HI test was a microplate U filled with standard 25µL viral suspension (4 HA) in all wells. 25µL of the serum to be tested was added and homogenized in the first well. A total of 25µL of standard and serum virus mixture in the first well was transferred and homogenized into the second well and so on until the last well. The microplate is shaken, and incubated at room temperature for 15 minutes. As much as 25µL 0.5% red blood cell suspension (as an antibody) was added to the whole well, the microplate was shaken, and re-incubated for 30 minutes. The results are read if erythrocytes in the control well have settled [10]. Positive antibody titters for ND disease when hemagglutination occurs at 1/16 dilution using 4 HAU antigens [11].

3. Results and discussion

3.1. Immunoglobulin Yolk (IgY)
The immune system in poultry consists of two types, non-specific immune responses and specific immune responses. Non-specific immune responses do not require recognition of antigens (skin, mucosa, and macrophages) [12]. The immune system is then replaced with specific immunity (adaptive immunity) in the form of a humoral immune response (antibody) and cellular immune response (infection and vaccination).

Humoral immunity response plays an important role in the resistance of infectious diseases. Antibody concentrations can be increased by vaccination. In extensive maintenance, antibodies are formed through direct exposure to the disease. Table 1 presents the results of the analysis of the concentration of IgY in Indonesian local chickens.

| Chicken Families | Concentrations of IgY (mg mL⁻¹) |
|------------------|---------------------------------|
|                  | 5 weeks old | 21 weeks old |
| IPB-D1           | 3.76±1.77 (42) | 10.10±2.44 (154) |
| Sentul Sensi-1   | 6.70±2.34 (80) | 10.63±2.37 (25) |
| Sentul Jatiwangi | -            | 8.68±2.64 (93) |

Concentration of Immunoglobulin Yolk (IgY) in blood serum 5-15mg mL⁻¹ and in egg yolk 15-25mg mL⁻¹ [13-14]. Based on the result, concentration of IgY in Sensi-1 chicken were higher than IPB-D1 at five weeks. The results of IgY concentration at 21 weeks showed that the Sentul Sensi-1 chicken was higher than the IPB-D1 chicken and Sentul Jatiwangi chicken.

High concentration of IgY in Sentul Sensi-1 chicken at five weeks is due to the parents has been selected on high IgY concentration. As the research conducted by [15], IgY is a maternal antibody inherited from progeny through blood serum transferred to egg yolk. IgY concentrations decrease with age and increase when there is exposure to the disease [16].

Based on the results of several literature studies, the concentration of IgY of IPB-D1 chicken and Sensi-1 chicken were higher than that of native chicken (10.07 mg mL⁻¹), purebred chicken (7.89 mg mL⁻¹) [17], and Sentul chicken (9.55 mg mL⁻¹) [18]. The concentration of IgY Sentul jatiwangi chickens is lower than Sentul chickens. Which data shows that purebred chicken have the lowest IgY concentration compared to other local chickens.

The production of antibodies in chickens is influenced by the environment (maintenance management, disease exposure) and genetics. Based on the results of the study, with the same maintenance management, Sentul Sensi-1 chicken that has been selected parents have higher IgY concentrations compared to other local chickens. Whereas the chicken of IPB-D1 showed low IgY concentration at 5 weeks and increased until the concentration was almost the same as the sensi-1 chicken at 21 weeks. IPB-D1 chicken is a composite chicken from a crossing of 4 chicken families, where the effect of heterosis on the immune response is quite good as evidenced by the rapidly increasing IgY concentration from the age of 5-21 weeks.

Sentul Jatiwangi chickens showed lower antibody response compared to IPB-D1 chickens and Sentul Sensi-1 chickens. The production of Sentul Jatiwangi’s antibody is influenced by genetics, where the chicken has not been selected and is still the original Sentul chicken from Jatiwangi.

3.2. Newcastle Disease titter
Newcastle disease (ND) is a disease that often attacks local chickens. The immune response can be measured by specific antibody titters. The following data (table 2) is the results of the ND titter antibodies of the IPB-D1 chicken, Sentul Sensi-1 chicken, and Jatiwangi Sentul Chicken.
Table 2. ND titters in IPB-D1 chickens, Sentul Sensi-1 chickens, and Jatiwangi Sentul chicken

| Chicken Families         | ND Titter (log 2)       | 5 weeks old | 21 weeks old |
|--------------------------|-------------------------|-------------|--------------|
| IPB-D1                   | 1.62±1.19 (42)          | 2.08±1.35 (24) |
| Sentul Sensi-1           | 1.57±1.53 (80)          | 2.54±1.16 (39) |
| Sentul Jatiwangi         | 0.70±0.72 (93)          | -           | 0.70±0.72 (93) |

Based on the result, Sentul Sensi-1 chicken had higher ND titters compared to IPB-D1 chickens at 21 weeks. At 5 weeks the titter was not significantly different between the IPD-D1 chicken and Sentul Sensi-1 chicken. Significant difference in ND titter occurs in different age. Based on research by [19], factors that influence antibody titters are health status, level of viral infection and time of infection. The formation of antibodies requires 6-10 days after infection and reaches the peak of antibody production in 3-4 weeks, after which it decreases for up to 3-4 months and is not detected after 1 year [20].

Based on research by [21] in local Vietnam chickens, the average ND titter was 1.6 and vaccinated chickens were 2.6. Meanwhile, research by [22] in laying hens, ND antibody titter was 2.27. ND titters in IPB-D1 chickens and Sentul Sensi-1 chickens are higher compared to native laying hens and Jatiwangi Sentul chickens.

Indonesian local chickens have good potential in developing disease-resistant chickens. The genetic potential is still very possible to be developed by disease-resistant local chickens using the antibody titter indicator as an indicator of selection. The selection program can be maximally carried out by conducting the optimum vaccination so that the chicken's response to the disease can be identified maximally and selected chickens that have superior genetic makeup. The superiority of local chickens that are more responsive to the presence of disease agents makes the advantages and ease in carrying out genetic improvement in the quality of local Indonesian chickens.

4. Conclusion
Selection of disease-resistant local chicken strains can be made using selection indicators in the form of IgY concentrations and ND titters. As the results of the study show that two new families of chickens released have indicators of better disease resistance compared to other chickens. The disease resistance selection program in Sentul Sensi-1 chickens, and the utilization of the heterosis effect through the crossing program in chickens IPB-D1 has been shown to increase good disease resistance indicators.

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