Hematological parameters and antibody titers to new castle diseases and avian influenza on extensive and semi-intensive system

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Abstract. The study evaluated the hematological parameters and antibody titers against Newcastle Disease (ND) and Avian Influenza (AI) on native chickens under extensive and semi-intensive rearing in Central Java, Indonesia. The target of the study was native chickens in Banyumas and Kebumen areas. The chickens were reared extensive and semi-intensively. The survey method was carried out by drawing 324 chicken blood samples for hematological tests and measuring antibody titer to ND and AI. The data obtained were analyzed using a general linear model (GLM) with the Systat ver.13 program. The results showed that native chicken in extensive and semi-intensive systems had different physiological statuses ($P<0.05$). The number of erythrocytes, hemoglobin levels, hematocrit values, total plasma protein levels, and heterophil-lymphocyte ratios was higher on semi-intensive than extensive system. However, the number of leucocytes was relatively high the same ($P>0.05$). In extensive system rearing, the number of native chickens with effective antibody titers against ND and AI was higher than the semi-intensive. The study concluded that native chickens reared in semi-intensive systems had a healthier physiological status than chickens in extensive systems. However, the effective antibody titers against ND and AI in the extensive and semi-intensive systems were still very low.

1. Introduction

Native chickens can be found all over Indonesia but are mostly reared in the rural areas under an extensive system (free-roaming) or semi-intensive systems (enclosed captivity). The majority of the rural community prefer the semi-intensive system; the chickens were allowed to roam free in the morning and kept in the cage in the evening. The extensive system omits the management of caging, feeding, and animal health. Accordingly, different environmental conditions and farming management result in contrasting physiological conditions and disease incidence in chickens.

The minimum handling of native chicken farming may cause a poor state of body, which is responsible for disease and mortality incidences. Furthermore, the mortality of native chickens is affected by extreme environmental conditions, diseases, anti-nutrition substances in their feed, and competition for feed. The most prevalent diseases in the poultry industry across the globe are Newcastle Disease (ND) and Avian Influenza (AI) [1]. The cause of Newcastle Disease is the Avian Paramyxovirus type 1 from the Paramyxoviridae family, while AI is due to the infection of influenza virus type A from the Orthomyxoviridae family [2,3]. The most severe ND and AI diseases are listed in the Office
International des Epizooties [4]. Vaccination is a successful intervention strategy in controlling ND and AI [5].

Varied environmental conditions due to topographical factors may affect the physiological status of the livestock. Also, discrepancies in altitude significantly affect the temperature, level of oxygen, and environment humidity. A high temperature would negatively affect both physiological conditions and production performance. Banyumas district is an area in Central Java situated in the highland, while Kebumen district is in the lowland. Both districts have relatively high population of native chickens. This study aimed to investigate the physiological condition of native chickens based on the hematology parameters and antibody titers of AI and ND in native chickens farmed in extensive and semi-intensive systems.

2. Materials and methods

A survey through direct observation was conducted to examine the poultry conditions. Blood samples were drawn for regular blood examination (hematological parameter), and we performed the antibody titers of New Castle disease (ND) and Avian Influenza (AI) to all native chicken samples kept across Banyumas and Kebumen districts. The number of chicken farmers in Banyumas who managed extensive and semi-intensive systems was 36 and 44 farmers, respectively, while in Kebumen was 42 and 40, respectively. We sampled two native chickens aged 16-20 weeks from each farming, accounted for a total of 324 blood samples. Hematological parameters were examined using a hematology analyzer to the total erythrocyte, leukocyte, differential white blood cells, hemoglobin, hematocrit, and total plasma protein. We applied the HI method for antibody titer against ND and AI viruses. The obtained data of hematological parameters were presented in a tabular form and subjected to the analysis of variance based on the General Linear Model (GLM) using the Sysstat program ver.13. Data of antibody titer against ND and AI were processed in a descriptive analysis.

3. Results and discussion

The farming systems of native chickens may include traditional/extensive and semi-intensive systems. These activities are the local farmers’ second job, so they seldom provide proper feed but rather agricultural waste, such as rice bran, and household leftovers like vegetables and stale rice. Similarly, the housing is not the main priority; some poultry is put in a poor cage near the home kitchen or left to perch on the tree branches at night. Meanwhile, farmers in the semi-intensive system provide proper cages and separate the hens from the hatchlings. During the brooding period, chicks are fed complete feed.

In extensive and semi-extensive systems of native chicken farming, it is difficult to control the health status and development of the chickens. The native chicken farmers rarely perform preventive actions to minimize diseases, such as vaccination, thus exposing the chickens to various diseases. Health is a determining factor in the success of native chicken farming. One of the parameters of the health status of native chickens is hematological profile [6,7]; because blood is a physiological parameter reflecting the conditions of the poultry. Furthermore, the blood profile, total leucocyte, erythrocyte, hemoglobin, PCV, TPP, and H/L may describe the health status of the chickens and the level of body immune [8–10].

Leucocyte is an-active component of body immune system, is formed partly in the backbone marrow and the lymphoid organs like thymus, bursa of Fabricius in poultry, and spleen. Leucocyte maintains body immune and kills bacteria or viruses attempting to enter the body [11]. Erythrocyte is red blood cells that contain hemoglobin as the transport of oxygen from lungs to body cells and carbon dioxide from the cells to the lungs. Hemoglobin is the erythrocyte pigment that in blood that consisted of conjugated protein and simple protein. Meanwhile, protein hemoglobin is hemoglobin in a cell form whose red color is the hemes of iron atoms. PCV (Packed Cell Volume) is the percentage of cells in the blood, TPP refers to the total protein plasma in the blood, and H/L is the ratio of heterophil to lymphocyte
The mean value of blood physiology of the native chickens in the extensive and semi-extensive maintenance systems is presented in Table 1.

**Table 1. Mean value of hematology**

| Maintenance systems | Erythrocyte (mil/µl) | Leukocyte (./µl) | Hb (g/dL) | PCV (%) | TPP (g/dl) | H/L |
|---------------------|----------------------|-----------------|-----------|----------|------------|-----|
| Extensive           | 2.2411a              | 10,014.473a     | 7.3368a   | 21.684a  | 2.4526a    | 0.5769a |
| Semi-intensive      | 2.4757b              | 10,135.7143a    | 7.8000b   | 23.8571b | 2.8333b    | 0.7328a |

Note: Values followed by different superscripts within column showed highly significant difference (P<0.01).

The statistical analysis showed that the mean value of erythrocyte, leukocyte, Hb, PCV, TPP, and H/L of native chickens in Banyumas and Kebumen districts were not significantly different (P>0.05). However, a higher mean value of blood physiology was observed among native chickens in Banyumas than in Kebumen. It was in line with Ismoiyowati et al. [7] that environmental factors, i.e., temperature and humidity contribute to the discrepancy of animal physiology.

The mean value of erythrocyte, Hb, PVC, and TPP of native chickens kept in different maintenance systems showed a highly significant difference (P<0.01), but the leukocyte and H/L were not significantly different (P>0.05) (Table 1). The contributing factors to different physiological statuses among chickens include age, farming activity, and feed composition [13]. The quality and quantity of feed offered to native chickens in semi-intensive systems are better than those in the extensive system. In contrast, extensive farming allows chickens to forage and be more active outside of the cage than those in the semi-intensive.

Newcastle disease (ND) and Avian Influenza (AI) are the common diseases of native chickens. Avian Influenza (AI) is caused by the H5N1 subtype of AI virus that remains the most dangerous viral disease that costs a massive economic loss due to high mortality and declining egg production [14]. Meanwhile, ND disease has spread across Indonesia and causes an enormous deficit due to a high rate of morbidity and mortality (50–100%) due to viral infection of velogenic ND [15]. Vaccination is a preventive measure that activates immunity against the virus of AI and ND diseases. Poultry immunity against AI and ND viruses can be detected from the antibody of the poultry serum through a serology test, such as hemagglutination inhibition assay (HI). Table 2 presents the results of the HI assay of antibody titers of AI and ND in this study.

**Table 2. Percentage of antibody titers of AI and ND from HI assay on native chickens kept in extensive and semi-intensive maintenance systems.**

| Variables    | Banyumas | Kebumen |
|--------------|----------|---------|
|              | Extensive | Semi-intensive | Extensive | Semi-intensive |
| ND antibody titer | (%) | (%) | (%) | (%) |
| 0            | 33.33 | 27.27 | 65.00 | 90.00 |
| <26          | 50.00 | 59.09 | 20.00 | 0.00 |
| ≥26          | 16.67 | 13.64 | 15.00 | 10.00 |
| AI antibody titer | (%) | (%) | (%) | (%) |
| 0            | 38.89 | 40.91 | 60.00 | 70.00 |
| <24          | 55.56 | 54.55 | 40.00 | 30.00 |
| ≥24          | 5.56  | 4.55  | 0.00  | 0.00  |

The result showed that native chickens in Banyumas and Kebumen kept in the extensive and semi-intensive systems had relatively similar antibody titers: very low effectiveness against ND and AI diseases (Table 2). This result confirmed by Ismoiyowati et al. [16] on Tegal ducks and Magelang ducks farmed in different locations showed similar antibody titers against AI disease. The AI virus can be transmitted directly or indirectly through materials or equipment exposed to the virus. The contributing factors to the stability of the AI virus include environmental conditions, such as heat and drought. The AI virus can be activated at 40°C for 15 minutes [17].
World Organization of Animal Health (OIE) suggests the protective level of antibody against Avian Influenza (AI) diseases and ND be $\geq 2^4$ and $\geq 2^6$, respectively, thus the low percentage of antibody titer protection among native chickens in both extensive and semi-intensive systems (Table 2). This result is due to the absence of a vaccination program initiated by either the government (Agency of Husbandry) or the farmers (independently). The low level of protective antibody titers showed that the native chickens in both districts have been naturally infected with AI or ND viruses. The prevalence of AI disease is considered high when produced 2.5% from the total blood serum [18]. The antibody titer is not always protective because it will diminish after a period, and the decrease rate is affected by the disease itself or the animal condition [19]. Therefore, a proper vaccination will enhance the formation of optimum antibody titers [7].

4. Conclusions
Native chickens in the semi-intensive farming system show better hematological status than those in the extensive system. The level of antibody titers against AI and ND in both systems, however, remains considerably low.

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