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Detection of Newcastle Disease Egg Yolk Titer and Phenotypic Correlation in IPB-D2 Chickens

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A B S T R A C T

Maternal antibodies in egg yolks provide the initial protection for embryos after hatching. Newcastle disease virus is one of the viruses that causes mortality in chickens of all ages. This study aimed to analyze the Newcastle disease virus titer in the egg yolks of IPB-D2 chickens (Institut Pertanian Bogor-D2). Seventy-two eggs were collected from 12 IPB-D2 chickens with two repetitions over three periods, using a hemagglutination test for Newcastle disease titer. The hemagglutination test results were analyzed using geometric mean titers and descriptive analysis. The protection levels for Period I were 55%, Period II was 71%, and Period III was 70%, respectively. The egg weight was 40.11±3.61 grams, the hatch weight was 32.12±4.62 grams, and the body weight at four weeks was 288.2±59.42 grams, respectively. The phenotypic correlation of Newcastle antibody in egg yolks with egg weight and hatch weight showed significant differences but there was not statistically significant with body weight at four weeks.

Keywords: *Egg yolk, Newcastle disease, Correlation, Phenotypic.*

1 Introduction

Newcastle Disease (ND), caused by Avian Paramyxovirus Serotype 1 (APMV-1), belongs to the Avulavirus genus in the Paramyxoviridae family. Clinically, the severity of ND varies from mild disease without clinical symptoms to severe infections with mortality rates of up to 100% (1). Egg yolk serves as the first antibodies used to protect against ND virus and as a food reserve when embryos hatch. Chicks from immunized parents have high maternal

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antibodies that protect them from both lethal viruses and vaccine viruses (2). Previous studies consistently indicate that some live vaccines cannot be administered *in ovo*, mainly because vaccine viruses cause significant embryo mortality, decreased hatchability, or clinical disease development after hatching (3). Newcastle disease can threaten poultry life, thus affecting farmers' well-being.

IPB-D1 chickens (Institut Pertanian Bogor-D1) are a new strain of local chickens based on Decree No. 693/KPTS/PK.230/M/9/2019 resulting from crossbreeding between F1 males (Pelung x Sentul) and F1 females (Kampung x Ras pedaging parent stock Cobb) with genetic compositions of 25% each (4). Developing the IPB-D2 strain from its parent IPB-D1 for use as a female strain is one way to increase the number of local chicken breeds in Indonesia. IPB-D2 chickens have better disease resistance than their IPB D1 ancestors, representing one of the innovations in local chicken breeds developed by the Bogor Agricultural Institute (IPB). IPB-D2 chickens were selected based on IgY concentration ≥9.55 mg mL-1 and ND antibody titer ≥ 3 Log2 HI units (5). The immune response of chickens can be detected serologically by examining chicken serum with the HI haemagglutination test (1).

The importance of chicken husbandry is closely related to the level of chicken resistance to diseases. Therefore, developing potential IPB-D2 chicken strains is an effort to improve the performance of local Indonesian chickens, ensuring resistance to diseases, adaptability to community husbandry practices, and rapid growth. Improving chicken immunity through vaccination is only temporary and cannot be genetically inherited; thus, genetic improvement is necessary.

Estimating correlation values is important for early selection. Genetic information is needed to determine the genetic quality of livestock, which will be used as a consideration in selection and crossbreeding (6). Phenotypic correlation is one way to explore the genetic information possessed. The correlation coefficient values indicate the relationship's closeness or correlation between variables. Correlation is categorized as high if the Pearson correlation coefficient is 0.5-1.0, medium if it is 0.25-0.50, low if it is 0.05-0.25, and very low if it is 0.00-0.05 (7).

2 Materials and Methods

2.1 Experimental Design

Nineteen 27-week-old chickens, comprising 7 males and 12 females, were kept in a 1:2 mating scheme. Each male and female was fitted with a wing tag. Eggs from each female were sampled for yolk twice during three periods; all chickens were not vaccinated. Observed variables included hemagglutination titer of egg yolks, egg production, and hatch weight. Feed and water were provided ad libitum. The cage size for each group of males and females was 4 × 5 meters. The female chickens were fed layer feed, while the chicks were fed starter feed.

2.2 Sampling

Yolk testing was performed on each female. The yolk was separated from the egg white using filter paper in a petri dish. Yolk was extracted with a 25 µL micropipette, transferred to a microtube, homogenized, and centrifuged at 4°C for 30 minutes. The supernatant from the yolk was transferred to a microtube and coded according to the hen's number. Egg weight, hatch weight, and 4-week-old body weight were measured.

2.3 Hemagglutination Test

Hemagglutination is a test to determine egg yolks' antibody levels against Newcastle disease antibodies. The hemagglutination test began by adding 25 µL of PBS solution to each microplate well, diluting until well 11, and then adding 4 HAU antigens to all wells except well 12 and equilibrating at room temperature for 30-40 minutes. 25 µL of 1% RBC (red blood cell) was added to all wells, mixed, and incubated for 30 minutes. Positive serum contained antibodies indicated by red blood cell precipitation.

2.4 Statistical Analysis

Antibody titer data for each individual in the group were averaged using geometric mean titer (GMT). Descriptive analysis was performed on production performance data, including population mean, standard deviation, and Pearson coefficient of variation. The phenotypic correlation between ND titer and production performance was estimated using correlation analysis, allowing for linear relationships between the two variables.

3 Results

The results of Newcastle disease antibody titers in egg yolks for 3 periods are presented in Table 1. The significant difference test results showed no significant difference (p>0.05). The phenotypic correlation of Newcastle disease

antibody with egg weight, hatch weight, and body weight at 4 weeks is presented in Table 2.

Table 1. Titer hemagglutination antibodies Newcastle disease in egg yolk

Period	n	Non-protective	protective	description
I	24	45%	55%	NS
II	24	29%	71%	NS
III	24	30%	70%	NS

GMT: geometric mean titer LOG 2, n: total sample, significant level=0.05

Table 2. Correlation between titer of HI antibodies in egg yolks

Variable	Mean ± SD	Correlation
Egg weight (g)	40.11 ± 3.61	0.74^{s}
Doc weight (g)	32.12 ± 4.62	0.01^{s}
Body weight 4 week (g)	288.2 ± 59.42	0.043^{ns}

g: gram; ^{s}: significant; ^{ns} non significant, significant level=0.05

4 Discussion

The results of three measurements of antibody titers in egg yolks showed different protection percentages, likely due to lack of vaccination. Variation in antibody titer values can occur due to several factors, including the amount of virus infected and varying individual immunity levels. Antibodies play a role in neutralization by binding and preventing ND virus attachment to host cells (8). If chicken antibody titers test positive and increase to 2^4 or higher, the chicken is considered to have protective immunity against ND. Chickens with antibody titers less than 2^4 are not protected from ND (9). Unvaccinated egg embryos had an ND titer value of 2.3 ± 0.07 with an 80% hatchability rate. In vivo, vaccination of 13-day-old embryo eggs showed higher ND titer values but higher mortality compared to those vaccinated at 18 days (10). Chickens develop antibodies in response to exposure to antigens, known as active immunity. Conversely, chickens receiving antibodies from their parents via eggs are said to have passive immunity (3). The low percentage of protection in IPB-D2 chicken egg yolks is likely because these chickens have not been infected with the ND virus. Thus, no antibodies are present in their bodies, or the antibody levels are still very low, which is insufficient to yield positive results in the HI test.

Low antibody titers can be caused by excessive heat stress from the environment, making the chickens' situation stressful. Heat stress is a disorder resulting from environmental air temperatures exceeding normal levels (>28°C), leading to an imbalance between chicken production and body heat dissipation (11). Vaccines made from live or inactivated lentogenic strains have not successfully controlled the spread of virulent viruses (12). Stress indicators, including plasma corticosterone concentration and heterophil/lymphocyte ratio, can also contribute to low antibody titers (13). The most effective approach to managing the Newcastle disease virus (NDV) is through prevention of its spread, and vaccines have not been successful in achieving this objective. Implementing

biosecurity protocols is essential to prevent the introduction and transmission of NDV and other poultry diseases.

Maternal antibodies are transferred from the hen to the chicks until they are three weeks old. Vaccination on the first day is less effective. Maternal vaccination results in the inability of B cells to produce antibodies at an early age, and the evolution of the immune system creates effective antibodies against Newcastle disease (13). Breeding strategies can be used to study potential genes that influence Newcastle disease antibodies. A region of about 100 Mb from the proximal end of chicken chromosome 1, including the *ROBO1* and *ROBO2* genes, is involved (14). This research paves the way for further studies on the host immune response to NDV.

The egg weight of IPB-D2 chickens is lower than that of Sentul chickens, ranging from 47-to 49 grams. This difference in egg weight is suspected to be due to egg collection at different ages of the hens (15). As the age of the hens increases, the egg weight also increases, indicating that the weight of day-old chicks (DOC) and 1-month-old Sentul chickens is 30.467 grams and 250.34 grams, respectively (15). This research found higher results than IPB-D2 chickens, likely because IPB-D2 chickens result from crossbreeding between strains.

Phenotypic correlation is one way to improve or select for better traits. Phenotypic correlation between egg weight, DOC weight, and Newcastle disease (ND) antibodies showed low correlation. DOC obtains nutrition from the yolk. The heavier the egg weight, the higher the nutrient content in the egg. These results differ from previous studies where HI antibody titers in egg yolks showed a positive correlation with titers in hens and a positive correlation between HI antibody titers in egg yolks and one-day-old chicks (16).

The high nutrient content in eggs provides a food reserve for embryo development. Yolk contributes approximately 30% of the egg weight and is rich in various fatty acids, vitamins, minerals, and proteins, important nutritional sources for embryo development (17). Chicken embryo development can be divided into three stages: the first stage of embryogenesis (0-7 days after embryo hatching), the second stage of embryo development (8-14 days after embryo hatching), and the third stage (15-21 days) for rapid nutrient absorption for hatching (18). These nutrients are required during incubation for embryo tissue growth and as an energy source.

Phenotypic correlation of ND in egg yolks with 4-week-old body weight showed no significant difference. This is likely because after hatching, DOC obtains nutrition not from the egg yolk but from feed. Other research results showed no correlation between Newcastle disease antibodies and body weight at 16 weeks (19). Early feeding of chicks after hatching stimulates the development of their digestive tract, accelerating the morphological growth of the small intestine, as evidenced by the faster increase in body weight (20). During the post-hatch period, chicks must transition from metabolic dependence on egg yolk to feed utilization. Chicks fed soon after hatching experienced increased body weight and small intestine weight within 48 hours after hatching, while those not fed experienced decreased body weight (21).

This research showed a positive and significant correlation between Newcastle antibodies in egg yolks with egg weight and hatch weight but not with body weight. Breeding strategies that select for egg weight and hatch weight can be used for genetic improvement, although further molecular testing is needed. Monitoring passive antibodies in chickens can be done by testing Newcastle disease antibody levels through egg yolks, thereby reducing chicken stress levels. NDV challenge tests can be conducted to determine the difference in response to NDV between IPB-D2 chickens.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

Author Contributions

All authors contributed to the original idea and study design.

Data Availability Statement

Data are available from the corresponding author upon reasonable request.

Ethical Considerations

A clearance test was conducted at the Faculty of Medicine Andalas with number 19/UN.16.2/KEP-FK/2024.

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