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Productivity of IPB-D2 and IPB-D3 Chickens with Repeatability of Newcastle Disease Antibody Titer

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ABSTRACT

ND virus causes mortality in poultry. Passively acquired maternal antibodies inhibit immunoglobulin formation. Repeatability is a genetic parameter that determines the inheritance of traits from elders to chicks. IPB-D2 chickens were selected for disease resistance, and IPB-D3 chickens were selected for weight gain. This study aimed to evaluate the productivity of IPB-D2 and IPB-D3 chickens and assess the inheritance of Newcastle Disease (ND) antibody traits in 36 IPB-D3 and 21 IPB-D2 chickens. The T-test was used to compare the group means of the two chicken breeds. Antibody titer measurements were based on the Geometric Mean Titer (GMT). Estimation of ND titer repeatability using within-class correlation. Fertility and hatchability differed significantly ($p < 0.05$). ND titer of IPB-D2 chicken and IPB-D3 chicken DOC LOG 2 GMT 1.61 ± 1.10 and 1.34 ± 0.95. The antibody titer of IPB-D2 and IPB-D3 chickens at 14 days of age was 1.02 ± 1.20 and 1.37 ± 0.95. The ND titer value in the egg yolk of IPB-D2 chicken was 4.02 ± 1.94, and in IPB-D3 chicken was 3.64 ± 2.54. The results showed the repeatability value of l antibodies in IPB D-2 chickens and IPB D-3 chickens in DOC 0.49±0.30, 0.42±0.33 and 0.39±0.28; 0.25±0.15, respectively. Fertility and hatchability of IPB-D3 chickens were better than those of IPB-D2 chickens. The yolk ND titer of IPB-D2 chickens was higher than IPB- D3 chicken. The ND titer reciprocity of IPB-D2 chickens was higher than that of the IPB-D3 chickens.

Keywords: IPB-D2 Chicken, IPB-D3 Chicken, Newcastle Disease, repeatability

INTRODUCTION

Developing candidate chicken strains IPB-D3 and IPB-D2 is one way to increase the potential of chickens in Indonesia. IPB-D3 chickens are children of IPB-D1 chickens, selected based on traits related to *tetelo* disease, growth, and egg production. Disease resistance to ND virus (*Newcastle* Disease) was measured against ND antibody titers. Setyawati *et al. (*2019) stated that IPB D-2 chickens were selected based on the concentration of IgY≥9.55 mg mL-1 and the antibody titer of ND≥ 3Log2 HI unit. IPB-D3 chickens were selected for rapid growth until the 2nd generation (Al Habib *et al.* 2020). IPB-D3 chickens are derivatives of IPB-D1 chickens with rapid body weight gain and are, therefore, suitable research objects. Salsabila *et al.* (2022) reported that the average weight of IPB-D3 chickens aged 12 weeks was 883 g.

The formation of this cluster was carried out to increase the importance of local chickens because IPB-D1 chickens have advantages in terms of good disease resistance to *Salmonella enteritidis infection* (Susanti *et al*. 2020) and good immunity against the ND virus in chickens vaccinated with the ND vaccine and chickens not vaccinated with the vaccine ND (Setyaningsih *et al.* 2020).

One factor affecting antibody titers is the parent antibody (Handayani *et al*. 2015). The formation of immunoglobulins can be inhibited by the passive formation of antibodies from the parent (Bagus *et al.* 2023). Antibody titers can be passed down from the elderly to the offspring and are controlled by polygens. Lestari *et al.* (2021) sequencing RNA analysis in IPB-D2 chickens revealed that several potential genes control ND antibody titers, such as ZBTB38, CCR9, and TLR2A. ND is acute in poultry, is transmitted quickly, and causes respiratory disorders that are often followed by neurological disorders. ND outbreaks often occur in groups of chickens that do not have immunity (Angreini *et al*. 2023).

Repeatability is a parameter that can be used to determine the reproducibility of a trait possessed by an individual during his or her lifetime (Darwati *et al.* 2019). According to Noor (2008), repeatability is between 0 and 1. Repeatability can be expressed as a measure of the degree of relationship between production in the first period and production in the next period of livestock that has more than one production record and can also be

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suspected of permanent environmental influences. This study aimed to analyze the productivity of IPB-D2 and D-3 chickens and the inheritance of maternal traits of ND antibodies.

METHODS

Time and Place of Research

This research was conducted from February 2023 to January 2024 at the Laboratory of Genetics and Livestock Breeding, Bogor Agricultural University. The samples were analyzed at the Virology Laboratory of the School of Veterinary Medicine, Bogor Agricultural University. The clearance test was performed at Andalas Faculty of Medicine (No. 19/UN.16.2/KEP-FK/2024).

Chicken Rearing

IPB D2 and IPB D3 chickens were maintained as many as 36 IPB D3 chickens consisting of 9 roosters and 27 hens with a mating ratio of 1:3. IPB-D2 chickens as many as 21 composed of 7 roosters and 13 hens with a mating ratio of 1:2. The chickens were reared without vaccination. Feed for laying hens during the production period, namely, 60% Saripakan layer, 20% bran, and 20% corn containing PK 15.261% and energy 2600 kcal/kg, chicks were given BR 511 feed with a crude protein content of 21–23% and energy of 2900 kcal/kg. Feed was provided ad *libitum*. IPB-D2 and IPB-D3 chicken coops were placed separately. Each chicken was placed on an individual coop.

Blood Sampling

Blood samples were collected from 21 IPB-D2 chickens and 36 samples from the blood of IPB-D3 broodstock chickens. Sampling of maternal and male blood at 27 weeks of age. Blood sampling for chicks was performed, and as many as three chickens were collected, each IPB-D2 and IPB-D3 chicken. 39 and 81 blood samples from IPB-D2 and IPB-D3 chicks were collected at 2 weeks and 27 weeks after hatching, respectively. Blood was taken from the brachialis vein using a syringe; then, the blood was placed into a cool box that contained an ice pack. The formed serum was collected from each sample, stored in a 1.5 mL microtube, and stored at -20°C until the hemagglutination test.

Egg Yolk Intake

A total of 72 egg yolk samples from IPB-D2 chickens and 156 eggs from IPB-D3 chickens were separated between the yolk and egg white and then placed on filter paper to remove egg white residues. The yolk was transferred to a 0.5 mL microtube with a micropipette, and 0.5 mL saline buffer phosphate was added and then centrifuged for 10 minutes at 40C. Supernatants were separated and placed in a microtube.

Hemagglutination Test (HI)

Hemagglutination inhibition is a serological test in the form of specific antibody inhibitors against the hemagglutination activity of ND virus antigens. The HI test began by inserting 25 µL of PBS solution on a microplate containing 25 µL of sample serum into the first well of the microplate, and a series of dilutions were carried out until the 11th well. ND 4 HAU antigen was added to every well except for the 12th well. The plates were incubated at room temperature for 30-40 minutes. A total of 25 µL of 1% RBC was added, mixed into each well, and incubated for 30 min at room temperature. Serums that are positive for antibodies are characterized by the inhibition of agglutination, such that red blood cells are deposited like control wells.

Data Analysis

The *T*-test was used to compare the productivity of the IPB-D2 and IPB-D3 chickens. Estimating the phenotypic correlation between ND titers and production performance using statistical correlation analysis allows the evaluation of the linear relationship between the two variables. The repeatability value can be estimated using the results of the variety analysis presented in Table 1 (Becker 1985). The repeatability (*R*) was calculated as follows:

$$\sigma^2 w = \frac{MS_w - MS_e}{K}$$

$$R = \frac{\sigma^2 w}{\sigma^2 w + \sigma^2 e}$$

$$\sigma^2 e = MS_e$$

Table 1 List of variance of repeatability estimation

Source of variance	Df	SS	MS	EMS
Among the broodstock (w)	n-1	SS_w	MS_w	$\sigma^2_\varepsilon + k\sigma^2_w$
Between the broodstock (e)	n(m-1)	SS_e	Mse	σ^2_ε

Remarks: df (degree of freedom), SS (sum of square), MS (mean of square), EMS (expected mean of square), n (total of sire), m (total of observed), σ^2_ε (Variance total of individual), σ^2_w variance between of individual).

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The standard deviation was calculated using the following formula:

$$SE(r) = \sqrt{\frac{2\,[(1-R)^2(1-r]^2(1+(k-1)R^2}{k(k-1)(N-1)}}$$ (Becker 1985)

RESULTS AND DISCUSSION

Pro ductivity of IPB-D2 and IPB-D3 chickens

The productivity of the IPB-D2 and IPB-D3 chickens is presented in Table 2. IPB-D2 chicken egg production was 44.70%, and IPB-D2 was 44.80%. Egg production was lower than that reported by Habiburahman *et al.* (2020), where IPB-D1 chicken production was 49.2%. IPB-D1 chickens were the elders of IPB-D2 and IPB-D3 chickens. IPB-D3 chicken egg production was the same as the results of Hawari *et al.* (2024) research on IPB-D3 chicken egg production (44.80%). The difference in egg production among IPB-D1 chickens is suspected to be due to differences in maintenance management and environmental influences. In addition, the nature of incubation is suspected to cause low egg production.

The fertility of IPB-D2 chickens was 80%, that of IPB-D3 chickens was 82%, that of IPB-D2 chickens was 67%, and that of IPB-D3 chickens was 70%. Fertility of IPB-D3 chickens was higher than that of IPB-D2 chickens. In addition, Fitriyani *et al*. (2023) The fertility of IPB-D1 chickens was grouped based on low to high concentrations of yolk immunoglobulin (IgY) (85% and 73.3%, respectively). IgY stored in the yolk during incubation is a source of antibodies produced by chickens to protect them after hatching. Hatchability in IPB-D1 chickens was based on IgY concentrations of 65% and 70%, respectively. This difference is suspected to affect egg storage time and egg fertility, and differences in mating intensity of each chicken and hatchability are influenced by hatchery management.

ND Titer of Hens and Males of IPB-D2 and IPB-D3 Chickens

The ND titers of IPB-D3 hens and males at 27 weeks of age are presented in Table 3. The ND titers in the IPB-D2 and IPB-D3 chickens were 0.57 and 0.46. ND titers in IPB-D2 and IPB-D3 chickens were 1.92 and 1.82. The re search conducted by Touko *et al.* (2021) reported that no ND titer was found in bare-neck chicks and normal feathered chickens aged 29 weeks, even though the elders had been vaccinated and selected with high antibody titers. The reduction in ND titer at 27 weeks is suspected to be due to passively acquired antibodies, IgY, which has been depleted with age. According to Tauko *et al.* (2015), antibodies from the mother can only protect chicks 10 d after hatching. The absence of ND titers suggests that proteins obtained from egg yolks are not only used as antibodies but also as a source of energy in the embryonic phase.

ND titer of IPB-D2 chicks and IPB-D3 chickens

ND titers in IPB-D2 and IPB-D3 DOC chickens at 14 d are presented in Table 4. The ND DOC titers in IPB-D2 and IPB-D3 chickens was 1.61 and 1.34. The ND titer of 14-day-old IPB-D3 chickens was 1.02 and 1.37, respectively. The ND titer of IPB-D1 chickens without vaccination in the first week was 2.3 and then increased in the 3rd week to 4.43 ND antibody titers formed in all chickens due to subclinical infections in nature (Setyaningsih *et al.*2020). According to the OIE (2021),

Table 2 Performance of IPB-D2 and D-3 chickens

Variable	IPB-D2	IPB-D3	Remarks
Egg production (%)	44.70±3.2	44.80±5.0	NS
Fertility (%)	80.20±8.7[a]	82.10±3.5[b]	*
Hatchability (%)	67.70±6.7[a]	70.10 ± 2.5[b]	*
Embryo mortality (%)	23.60±1.5[a]	28.50±3.7[a]	NS

Description: NS shows no significant difference P>0.05, *: significant difference.

Table 3 Newcastle disease titers of chickens at 27-weeks of age

Types of chickens	Rooster	Hen
IPB-D2	0.57±0.53	1.92±0.75
IPB-D3	0.46±0.40	1.82±1.27

Description: The shows no significant difference P>0.05, GMT: Geometric mean titer LOG 2.

Table 4 Newcastle disease day old chicken titers and 14 day-old titers

Age (weeks)	IPB-D2	IPB-D3	*P* value
DOC	1.61 ± 1.10	1.34 ± 0.95	0.06
2	1.02 ±1.20	1.37 ± 0.95	0.47

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antibody titer 24 is positive for Newcastle Disease and is considered seronegative below 2^4.

The immunity of chickens to ND can be passive or active. Passive immunity is mediated by antibodies from parents (maternal antibodies). These maternal antibodies, in addition to inhibiting the formation of immunoglobulins in newborns, prevent the occurrence of immunoglobulins during vaccination. The duration of maternal antibodies in chickens is only 4–7 days; maternal antibodies in all species inhibit antibodies after vaccination, or a lack of antibodies reduces protection against disease (Niewiesk 2014). Okwor *et al.* (2014) showed that the ND GMT titer value without vaccination after hatching on the 7th day was 3.00, on the 14th day was 3.12, and on the 28th day was 1.49.

The chicks received antibodies from the mother through the yolk. Antibodies from hen serum are transferred to the yolk through a blood vessel when the egg is still in the ovary. The immune system in chickens, which involves the production of antibodies, plays a vital role in fighting diseases such as ND. Antibodies produced by chickens can bind to ND virus particles and prevent them from attaching to host cells, thereby preventing the spread of the disease.

Egg Yolk ND Titer and Phenotypic Correlation in IPB-D2 and IPB-D3 Chickens

The ND titer of the egg yolk was observed for the three periods(Table 5). The ND titer of the yolk of IPB-D2 chickens was 4.02, and that of IPB-D3 chickens was 3.75. The ND titer of IPB-D2 chicken eggs was higher than that of IPB-D3 chickens. The high ND titer in IPB-D2 chickens is due to the results of the initial selection of prospective IPB-D2 chicken strains that have an ND titer above 3 HI LOG 2. Yolk can protect the embryo during incubation if the virus attacks it.

The embryo mortality rates of the IPB-D2 and IPB-D3 chickens were 23.60% and 28.50%, respectively. The hatchability of IPB-D2 and IPB-D3 chickens without vaccination was 80% and 82% better than that of eggs vaccinated *in ovo*. Okwor *et al.* (2014) found that eggs in unvaccinated chickens had a hatchability percentage of 17%. *In-ovo* vaccination of 13-day-old embryonic eggs resulted in higher embryo mortality than that observed in vaccinated 18-day-old embryonic eggs.

The phenotypic correlations of ND egg yolk with several characteristics of egg production, hatchability, and embryo mortality are presented in Table 6. The results proven that the ND of the yolk was positively correlated with egg production, but negatively correlated with embryo mortality. This is suspected because the death of the embryo is not due to the ND virus but can be caused by failure at hatching. The chicks received antibodies from the mother through the yolk. Antibodies from hen serum are transferred to the yolk through a blood vessel when the egg is still in the ovary. The research by (Han *et al.* 2021) suggests that antibodies in egg yolks are a potential substitute for antibiotics that can prevent pathogens in human food and animals.

According to Khairiyah *et al.* (2023), chickens that develop antibodies in response to exposure to disease agents, known as antigens, have an active immune system, whereas chickens that receive antibodies from their mothers through eggs have a passive immune system.

Repeatability of IPB-D2 ND Titer and IPB-D3 chicken

The results of the calculation of the repeatability values are listed in Table 7. The repeatability of the IPB-D2 chicken ND titers was 0.49, and the repeatability of the IPB-D3 chicken ND titer was 0.39. Repeatability of the 14-day-old ND titer was 0.42 and 0.25. Repeatability of the ND titer in IPB-D2 chickens was higher than that in IPB-D3 chickens. This is suspected to be due to the selection of IPB-D2 chickens for disease resistance, whereas IPB-D3 chickens were selected based on body weight growth. In addition, the inheritance of IPB-D2 chickens and IPB-D3 chickens is due to the influence of

Table 5 Egg yolk newcastle disease titers

Observation	Titer ND kof egg yolk LOG 2 GMT	
	IPB-D2	IPB-D3
Period I	3.30±2.64	3.64±2.54
Period II	4.11±1.30	3.77±2.36
Period III	4.65±1.77	3.85±2.05
Average	4.02±1.94[a]	3.75±2.31[b]

Description: a,b shows significant different P<0.05, GMT: Geometric mean titer LOG 2.

Table 6 Phenotypic antibody newcastle disease of egg yolk

Observation	Phenotypic correlation of egg yolk ND			
	Egg production	Embryo mortality	Hatchability	Remarks
IPB-D2	0.20[a]	-0.20[a]	0.35[a]	NS
IPB-D3	0.15[a]	-0.35[a]	0.30[a]	NS

Description: NS shows no significant difference at *P*>0.05.

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Table 7 Maternal repeatability of newcastlle disease antibodies

Types of chickens	Repeatability value	
	DOC	14-day old
IPB-D2	0.49±0.30	0.42±0.33
IPB-D3	0.39±0.28	0.25±0.15

their elder traits, namely IPB-D1 chickens, which have good resistance to ND diseases. Setyaningsih*et al*. (2020) IPB-D1 chickens appear to have a good immune response to the ND virus.

Repeatability is used to determine the reproducibility of a trait possessed by an individual during his/her lifetime (Darwati *et al.* 2019). The study results showed that the value of maternal repeatability of antibodies against ND was moderate to high. Noor (2008) repeatability estimation range from 0 to 1. Repeatability can be expressed as a measure of the degree of relationship between production in the first period and production in the next period of record and that has more than one production record and can also be suspected of permanent environmental influences. Repeatability is the upper limit of rarity. According to Walugembe et *al*. (2019), The heritability of growth in Tanzanian local chickens in the maternal phase before vaccination was 0.35, and the heritability after vaccination was 0.21. Repeatability is the upper limit of rarity. The difference in the number of chickens used could have caused a difference in the genetic inheritance value.

CONCLUSION

The fertility and hatchability of IPB-D3 chickens were higher than those of IPB-D2 chickens. The ND titer in the yolk of IPB-D2 chickens was higher than that in IPB-D3 chickens. Repeatability of the ND titer of IPB-D2 chicks was higher than that of IPB-D3 chickens.

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