

Antibody titer uniformity in Indonesian Native Chickens against avian pathogens in a tropical environment: a three-time series study

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Abstract. Elevated temperatures in tropical environments detrimentally affect poultry immunity, increasing susceptibility to infectious diseases and compromising overall health. This study investigated the antibody titer uniformity in Indonesian native chickens (Kedu, Arab, and Lignan) against H5N1, H9N2, and ND-G-VII viruses within a tropical climate. The research involved a three-time series monitoring of log₂ antibody titers at ages 10, 30, and 45 days. The 90 Indonesian native chicken blood samples were collected to assess their immune system. Results revealed a consistent pattern of antibody titer uniformity for H5N1 and H9N2 throughout the early growth stages, indicating a consistent immune response in the tropical environment. Contrasting with ND-G-VII, the antibody titers increased significantly ($p < 0.05$: 4.90 ± 1.20 to 6.20 ± 1.23) at 10 to 45 days, reflecting a progressive immune response in Indonesian native chickens against ND-G-VII. The findings emphasize the importance of considering environmental context when interpreting immune responses in poultry reared in tropical climates, which is influenced by the uniformity of immune responses against different pathogens. Such knowledge could have implications for designing effective vaccination strategies and disease prevention measures tailored to the specific challenges of poultry farming in tropical regions.

1 Introduction

High temperatures in tropical environments can be detrimental to poultry antibody responses, as sustained heat stress has been shown to hinder the development and function of avian immune defenses [1]. In tropical regions such as Indonesia, where the poultry industry has seen significant growth in recent years, the presence of various avian pathogens has posed continuous challenges. Historically, Indonesia has faced outbreaks of avian influenza and

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other infectious diseases that have had profound economic and public health implications. The progress made in mitigating these pathogens and the associated losses has been substantial, yet the persistent high temperatures in the region present an ongoing challenge, potentially exacerbating the susceptibility of poultry to these infections. Given this context, it becomes increasingly important to understand the specific impact of heat stress on poultry immunity in tropical environments.

Chronic heat stress (CHS) is a condition that arises when animals are exposed to prolonged periods of elevated temperatures, such as during a hot summer. While this extended exposure to high temperatures may not result in immediate death, it can have adverse effects on the animal's growth, immune system function, and ability to resist diseases [2]. The tropical climate can be characterized as being both warm and humid, and there is extensive documentation regarding how ambient temperature and relative humidity (RH) serve as significant stressors for chickens [3].

Vaccination is a successful method for stimulating the adaptive immune system to prevent the occurrence of infectious diseases caused by harmful bacteria or viruses. This widely employed immunization approach has been applied to industrial animals, including chickens. In the poultry sector, numerous vaccines have been employed, particularly to manage Avian Influenza and Newcastle disease. Nevertheless, when chickens raised for egg production are subjected to chronic heat stress, the levels of antibodies that typically rise in response to these vaccinations do not increase as anticipated [1].

It is important to examine the proper vaccine administration management to minimize the possibility of virus infection in local Indonesian chickens. This research will evaluate antibody titer responses to Avian Influenza and Newcastle Disease, considering the vaccine management that have been implemented on Kedu, Lignan, and Arab chickens at the Breeding Center for Maron Chickens, Temanggung, which are Indonesian Native Chickens breed. This research contributes to understanding the immune development of Indonesian native chickens under tropical conditions, providing insights into their responses to specific viral infections at different stages of growth.

2 Material and methods

2.1 Animal testing and sample collection

This experiment uses 3 breeds of Indonesian native chicken, including Kedu, Lignan, and Arab reared in the breeding center for Maron chickens, Temanggung. Chickens are kept in colony cages for each breed and each age. A total of 3 groups of chickens: Kedu, Lignan and Arab were vaccinated with NDIB killed and NDIB Live Vaccine at the time of hatching, then given a NDIB Live booster at 21 days and NDAI killed at 35 days ages. Data were collected at 10, 35, and 45 days. Basal feed is obtained from a mixture of corn, rice bran, soybean meal, and several other feedstuffs and premixes to meet NRC standards. 1.2 g/kg betaine supplementation (Excential Beta-key, Orffa ©) was given in feed to improve quality [4]. Drinks were given to the chickens ad-libitum.

The Avian influenza and Newcastle Disease vaccine we use medivac NDAI H5N1 Subtype 2.3 and ND G7 Strain. We measured antibody titers using haemagglutination and hemagglutination inhibition test following the OIE standards chapter 3.3.4 for avian influenza [5] and chapter 3.3.14 for Newcastle disease [6] which were carried out at Japfa Comfeed's Agrilab.

2.2 Environment microclimate data

To assess the microclimate conditions in the Temanggung region, we have accessed climatological information from the Meteorology and Geophysics Agency of Indonesia in April 2023 [7], focusing particularly on temperature and humidity data for the Temanggung

area. We provide microclimate data in the form of THI (Temperature Humidity Index). THI was calculated based on Zulovich and DeShazer's [8]:

$$THI = db^{\circ}C - [(0.31 - 0.31 RH)(db^{\circ}C - 14.4)]$$

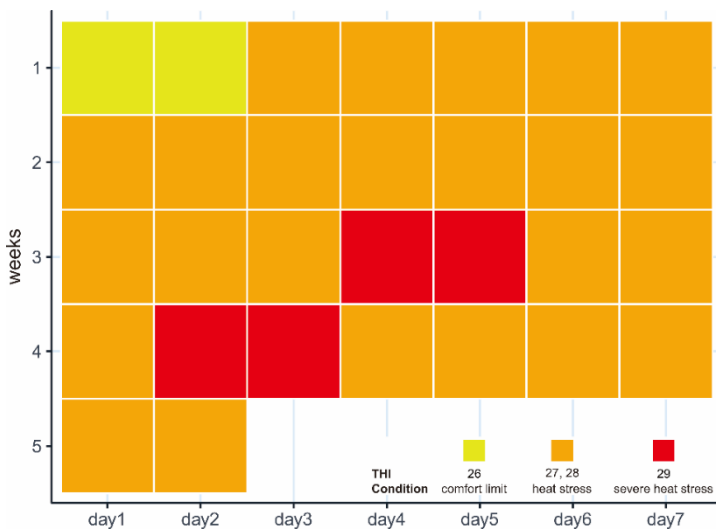
where THI: temperature humidity index, db°C: dry bulb temperature in Celsius, and RH: relative humidity in percentage.

2.3 Experimental design and statistical analysis

A completely randomized design and analysis of variance were used in this experiment to evaluate the antibody titers in every sample collected (10, 35, and 45 days of age). Duncan test was used if there were significant differences in ANOVA test results. Software R version 4.1.2 performs statistical data analysis according to the R Core Team procedure. Package ‘agricolae’ and ‘ggplot2’ are used to support the analysis process in R software.

3 Result and discussion

Following our analysis of the weather conditions in the Temanggung region using data from BMKG (Meteorology, Climatology, and Geophysics Agency), it can be deduced that chickens experience constant exposure to heat stress on a daily basis, as indicated by Figure 1.



Note: data retrieved from BMKG 2022.

Fig. 1. Heatmap of THI condition.

The values applicable to animals like poultry and pigs that do not have sweat glands are as follows: normal when less than 27.8, moderate between 27.8 and 28.8, severe within the range of 28.9 to 29.9, and very severe (reaching emergency levels) when equal to or greater than 30.0 [9]. Elevated heat stress conditions have been linked to negative effects on the immune system's response [1]. Recent research has revealed that the anticipated increase in antigen-specific antibody response following immunization with a commercial vaccine is not observed in heat-stressed broiler chicks, particularly those with heightened levels of the stress hormone corticosterone (CORT) [10]. Base on figure 2, antibody titers for H5N1, H9N2, and ND G VII were examined in Indonesian native chickens at three different ages: 10 days, 35 days, and 45 days.

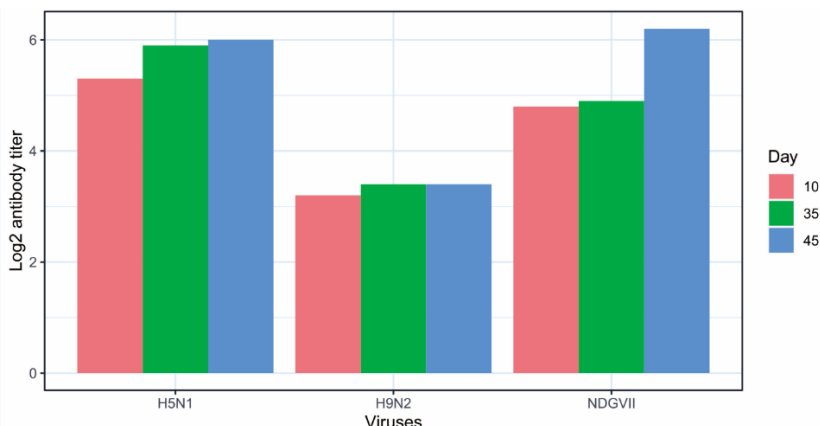


Fig. 2. Boxplot of Log 2 antibody titers in native chicken

The results indicated that there were no significant differences in antibody titers for H5N1 and H9N2 among the age groups. However, for ND G VII, a significant difference was observed, with higher antibody titers at 45 days of age compared to 10 and 35 days. This suggests that age has an impact on the antibody response to ND G VII in these chickens. These findings provide valuable insights into the immune responses of Indonesian native chickens to specific pathogens at different stages of development, which can have implications for poultry health management and vaccination strategies. The OIE (World Organization for Animal Health) has established a threshold for immunity levels against ND (Newcastle Disease) and AI (Avian Influenza) viruses. If the antibody level is greater than 4 HI log 2, it is considered protective. However, if the antibody level is less than 4 HI log 2, it is deemed not protective against the virus [11]. The results of the antibody titer examination at 10 days of age indicated a protective antibody level for the specific antibody against AI strain H5N1, with a value of 5.30 ± 0.95 (table 1).

Table 1. Log 2 antibody titers of H5N1, H9N2, dan ND G VII in Indonesian native chicken.

Age	n	H5N1	H9N2	ND G VII
10-day	10	5.30±0.95	3.20±0.42	4.80±1.48 ^b
35-day	10	5.90±0.74	3.40±0.70	4.90±1.20 ^b
45-day	10	6.00±1.05	3.40±0.52	6.20±1.23 ^a
P-Value		0.205	0.656	0.042

Note: ^{a,b} difference superscript in the same column indicate significant difference ($P < 0.05$).

Similarly, the antibody levels against ND strain G7 also showed a protective level at 4.80 ± 1.48 . Observations on day 35, following the Live ND vaccine booster administered on day 21, revealed that the levels of AI and ND antibodies remained protective. During the third observation on day 45, following the NDAI vaccine booster given on day 35, protective titer results were still observed. However, after conducting three monitoring sessions, it became evident that the antibody level against H9N2 did not reach a protective threshold. This occurred because the killed vaccine administered did not include a combination with the AI H9N2 strain.

When we consider the influence of heat stress on immunity, it becomes evident that native traditional chickens in Temanggung exhibit an ability to adapt to challenging environmental conditions. Upon closer examination, it is suggested that providing betaine to chickens of all ages may have a positive impact on coping with environmental fluctuations [12–14]. Research conducted on this matter affirms that administering betaine can effectively counteract the detrimental effects of heat stress [14]. Many studies state that betaine plays a

role as an osmoprotectant for poultry [15]. In hot environmental conditions, it can reduce excess digestive tract cell fluids. Furthermore, the stable condition of the digestive tract cells will trigger the metabolic system to run optimally, and the more influential the body can absorb the nutrients [12,16,17]. On the other hand, nutrients in the digestive tract are also a significant factor in determining the microbial composition of the digestive tract [13]. Digestive tract microbes directly play a role in many processes. One of the main ones is in the immune system by producing anti-inflammatory products that can reduce pathogens in poultry [13,18]. In addition, betaine has been found to elevate lymphocyte count by up to 22.59% and enhance immunity against the ND virus by up to 103.48% in terms of increased antibody levels [19]. Furthermore, betaine supplementation has been found to decrease the heterophil percentage and increase the lymphocyte percentage, leading to a decrease in the heterophil/lymphocyte ratio [15].

4 Conclusion

In conclusion, this study demonstrates that native chickens exhibit an ability to adapt to heat stress conditions. Additionally, the research highlights the adaptability of native traditional chickens in Temanggung to challenging environmental conditions. It suggests that betaine supplementation, especially for all age groups, may positively impact their ability to cope with environmental fluctuations. Administering betaine was found to mitigate the adverse effects of heat stress and significantly enhance immune responses following vaccination.

These findings contribute to a deeper understanding of the immune development of Indonesian native chickens in tropical conditions, providing valuable insights into their responses to specific viral infections at different growth stages. Furthermore, it underscores the importance of considering the environmental context when interpreting immune responses in poultry reared in tropical climates. This knowledge holds potential implications for designing effective vaccination strategies and disease prevention measures tailored to the unique challenges of poultry farming in tropical regions.

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