

Stability of the Broiler Antibody Under Heat Stress Using Dry Rice

Rusli Tonda^{1,2}, Roy Hendroko Setyobudi³, Adi Sutanto^{3,*}, Lili Zalizar³,
Nonok Supartini¹, Endang Dwi Purbajanti⁴, Zane Vincevica-Gaile⁵,
Ivar Zekker⁶, Wahyu Widodo³, Muhammad Basyaruddin⁷, Ahmad Fauzi³,
Delfy Diah Sulistyoningrum⁸, and Wira Wirawan¹

¹University of Tribhuwana Tungadewi, Malang 65144, East Java, Indonesia

²PT Zakiyah Jaya Mandiri, Lumajang 67381, East Java, Indonesia

³University of Muhammadiyah Malang, Malang 65144, East Java, Indonesia

⁴University of Diponegoro, Semarang 50275, Central Java, Indonesia

⁵University of Latvia, Riga LV-1004, Latvia

⁶Institute of Chemistry, University of Tartu, 50411 Tartu, Estonia

⁷The State Islamic University of Maulana Malik Ibrahim, Malang 65144, Indonesia

⁸University of Brawijaya, Malang 65145, East Java, Indonesia

Abstract. This study examines the impact of using dry rice, characterized by low calories and high resistant starch content, on broiler performance under heat stress conditions in Indonesia. Broilers face challenges related to stress, particularly heat stress, which can compromise their immunity. The research investigates the potential of resistant starch in dry rice to enhance broiler digestion performance and immunity. The experiment, conducted at Zakiyah Farm and the Laboratory of the Wates Veterinary Center, Yogyakarta, involved three treatments (T1, T2, and T3) with five replications each. Observed variables included water consumption, carcass weight, Packed Cell Volume (PCV), bursa fabricius weight, and Hi-Test Nd antibody levels. Results showed no significant impact on water consumption, carcass weight, and PCV across all treatments. However, a decrease in bursa weight in T1 and T3 indicated an increased level of stress. The lowest seropositive Hi-Test Nd values in T1 and T3 suggested that the use of dry rice through the sowing method might alleviate heat stress on broilers. In conclusion, this research emphasizes the potential of dry rice to maintain the stability of broiler antibodies under heat stress conditions, emphasizing the need for further exploration of alternative dietary components.

Keywords: Alternative feed, anti-heat stress, environmentally friendly feed, functional feed.

* Corresponding author: sutanto@umm.ac.id

1 Introduction

Besides the high price of feed, other obstacle that must be faced by the broiler breeder is an extreme climatic change. High temperature will make the chicken have heat stress and cause bad performance [1–5]. Heat stress on broiler can be perceived directly by sudden death and indirectly by disease infection. As a result, morbidity and mortality will increase and lead to the decrease of the broiler's productivity [6, 7] which finally have negative impact on the breeder's income [8].

Heat stress will disturb digestion process of the broiler due to the jejunum may be damaged in response to heat stress [9, 10]. As the intestines are damaged, they will disturb the metabolism process in the body [11, 12]. Low consumption of feed will decrease nutrient intake. The decrease in nutrient intake may cause lack of nutrient such as protein and it causes the protein level in blood will be lower. Low protein level in the blood will reduce the livestock ability to form antibodies [13–15].

A way to avoid the chicken from digestive disorders under heat stress condition is by providing low calories and high resistant starch feeds [16–18]. It will reduce calories of the body and improve the digestive organs. The resistant starch will not be digested in the intestines, thereby reducing the function and work of the intestines in the digestive process. The resistant starch will function optimally as microbial food in the process of forming organic acids in the body [19, 21]. The feed material which includes to the source of resistant starch is dry rice. Dry rice is made of rice which has been cooked, dried, and has gelatinization process.

Dry rice is a food waste processed product. The product is not only used as an alternative feed, but also helps to reduce environmental pollution due to organic waste [22, 23]. Dry rice has some advantages because it has low calories and contains high resistant starch [21]. The previous research as reported by Tonda *et al.* [24] showed that dry rice has advantages such as high palatability. Dry rice not only has high palatability, but it can also be used as a substitute for corn and rice bran [25]. The use of dry rice in a broiler may reduce the heat stress as result of the research by Tonda *et al.* [26]. But there has been no research to test the use of dry rice to maintain stability of immune by measuring variables of water consumption, weight of carcass, PCV value, weight of bursa fabricius and broiler antibody. However, the research was conducted to study the use of dry rice to maintain immune stability of the broiler. It is hoped that the research will not only provide benefits in the livestock sector but also help to provide an alternative feed which is environmentally friendly feed.

2 Materials and methods

2.1 Materials

The research has received ethical permission from the Medical Faculty, University of Muhammadiyah Malang (E.5.a/222/KEPK-UMM/X/ 2022). The research was conducted at PT Zakiyah Jaya Mandiri (S 8°9'4.3416" E 113°14'53.6352") and antibody testing was conducted at the Center of Veterinary Wates Yogyakarta (S 7°52'1.4592" E 110°10'3.6444"). Objects of the research were 200 d old chicken (DOC) of platinum type (PT Multibreeder Adirama Indonesia, tbk.) with an average weight of 42 g. Maintenance during the starter period took place from 1 d old that includes the feeding BR1 type BR1 (PT Wonokoyo) and

battery cage is 100 cm × 100 cm × 100 cm per unit treatment. Feed and water are given in ad libitum. Treatment on the dry rice was started at 21 d.

The used dry rice has met the requirements as feed for the broiler. The test has been conducted at the Laboratory of Nutrition University of Muhammadiyah Malang, East Java, Indonesia (S 7°55'4.8792" E 112°35'37.0644") which resulted 0.83 % ash, 12.58 % water, 8.96 % coarse protein, 0.43 % coarse fats, 0.59 % coarse fibers, and 87.42 % dry matters.

2.2 Methods

Method of the research was experiments with Randomized Complete Design (RCD) that comprised of three treatments and five replications so that there were 15 experimental units. Each experimental unit comprised of 12 broilers so that a total of 180 healthy broilers aged 21 d were needed. Treatments used in the research are as follow: T1 (full base feed without dry rice as control), T2 (base feed with composition of 20 % dry rice was given by sowing it on the base feed), and T3 (base feed with composition of 20 % dry rice was given by mixing it evenly with basal feed). Treatment T2 used the method of sowing on the feed in hot weather (10 a.m to 4 p.m) with the aim that the chicken will continue to consume feed without having excess calories because dry rice contains lower calories. Besides that, the water absorption capacity of the dry rice makes the chicken feel full after consuming it. Meanwhile, in the treatment T3, dry rice was not given specifically in hot weather conditions because it was mixed evenly with the feed so that the dry rice was consumed within 24 h.

Feed and drink are given in ad libitum. The remaining feed is weighed every day to determine the amount of chicken consumption. Dry rice is given at the age of 21 d to 35 d in accordance with each treatment. It is given every day at 10 a.m to 4 p.m under heat stress (30 °C to 33 °C). The temperature of the closed house is conditioned to the heat stress temperature by installing a 100 W lamp in each experimental unit. The data obtained from the observation be tabulated using Excel program.

2.2.1 Water consumption

Heat stress may affect water consumption of the chicken. The chicken experiencing heat stress tends to reduce water consumption because the chicken's body tries to conserve water to maintain proper fluid and electrolyte balance in the body [27]. It may cause dehydration on chicken and worsen the condition of the heat stress. Therefore, high quality and sufficient water availability is very essential and must be concerned to keep them healthy. Water consumption for chicken is calculated using the Equation (1).

$$\text{Water consumption per head} = \frac{\text{Amount of water consumed (mL)}}{\text{Number of chickens at that time}} \quad (1)$$

2.2.2 Weight of carcass

Weight of carcass refers to total weight of the animal's body parts after removing the inedible parts. Weight of carcass refers to total weight of the animal's body after the slaughtering process and removing the inedible parts such as feather, head, feet, internal organs, and other parts that are not included in the meat products sold [28, 8].

Weight of carcass is essential in the animal husbandry industries and meat processing because it is an important measure to assess productivity, efficient maintenance, and profit. The breeder (poultryman) and the producer of meat use the weight of carcass to measure the yield of the poultries that have been raised and processed. Weight of carcass for broiler is calculated using the Equation (2).

$$\text{Carcass weight (\%)} = \text{Body weight} - (\text{head, feather, internal organs, and feet}) \quad (2)$$

2.2.3 Packed cell volume

Packed Cell Volume (PCV) is a term used in blood analysis, especially for cattle/poultry and animal. PCV indicates proportion of the blood volume occupied by red blood cells (erythrocytes) compared to total blood volume [29, 30]. Normal value of PCV for chicken is 22 % to 35 % [29].

According to Nwogor *et al.* [31, 30] Packed Cell Volume (PCV) can be calculated as follow: blood sample is prepared in a proper tube to ensure that the blood may not be contaminated by other substances. Then, a hematocrit pipette or capillary tube is fully filled with blood. One of the hematocrit pipette ends is closed with finger, while the other end is put into the hematocrit tube which has been filled with blood. After that, the hematocrit tube containing blood is placed vertically in a centrifuge and must be done carefully to avoid any damage during the process. The next process is to put the blood into a centrifuge and operate it at high speed for a few minutes. It will separate the blood components into three different layers, namely erythrocyte (red blood cells), plasma, and buffy coat layers, which contain leucocyte and platelet. Results of the process can be read using a hematocrit reader or alike which usually show the column height of the erythrocyte in the hematocrit tube in percentage. The readable results are the hematocrit values (PCV) as percentage of the blood volume filled by erythrocytes.

2.2.4 Weight of bursa fabricius

Bursa fabricius is an organ which is only had by aves, and it lies between two lobes of the kidney. Bursa fabricius is a part of immune system in aves, which plays important role in producing immune cells, such as B cells or B lymphocyte cells. These B cells will produce antibodies that fight against infection or disease in aves [32, 33].

It is important to maintain the health of bursa fabricius in chicken because the organ plays an important role in maintaining health and immune system of the chicken against disease. Factors that may affect the health of bursa fabricius in chicken are the environmental condition, nutrition, and good management of the closed house. Equation to calculate the relative weight of bursa Fabricius is given in the Equation (3) below [34]:

$$\text{Relative weight of bursa fabricius} = \frac{\text{Weight of bursa fabricius}}{\text{Body weight}} \times 100 \% \quad (3)$$

2.2.5 Antibody

As human and other animals, chicken has immune system which protects the body from infection and disease. The main component of the immune system in the chicken is antibody [14].

Hi-Test ND (Haemagglutination Inhibition Test) is a method to detect the existence of antibody against Newcastle Disease (ND) virus in chicken. The following is how to measure Hi-Test ND in chicken [35, 14]: (i) Prepare the samples: prepare a small amount of blood serum from the chicken and separate it from the blood cells by letting the blood settle or using a centrifuge. Then take 25 μL to 50 μL serum and put it into the test tube. (ii) Prepare the antigen: prepare the antigen of newcastle disease virus that has been standardized. (iii) Antigen dissolution: antigen is dissolved in a buffer solution and then drops a little of antigen into the test tube which has been filled with the chicken's blood serum. (iv) Stirring: antigen and serum are mixed well using pipette and sterile stirrer. (v) Incubation: the mixture of

antigen and serum is incubated for 30 min to 45 min at room temperature. (vi) Addition of erythrocyte: after being incubated, a little erythrocyte is added into the test tube, then shake or stir evenly. (vii) Second incubation: re-incubation is carried out in the test tube for 30 min to 45 min at room temperature. (viii) Read the results: data of the test result is read by paying attention to the results of erythrocyte deposition or clumping.

2.3 Statistical analysis

The data obtained from panting frequency and heart weight examinations as well as hematology analysis were tabulated in Microsoft Excel and then run through ANOVA [36, 37]. Any significance in treatments called for LSD test [38, 23].

3 Result and discussion

Results of the research showed that water consumption ($\text{mL head}^{-1} \text{d}^{-1}$) of the chicken ranged 135 to 150. Weight of carcass (%) ranged 70 % to 73 %. Relative weight (%) ranged 0.12 to 0.20. PCV ranged 27 % to 29 %. Seropositive Hi-Test ND ranged 40 % to 80 %. Results of the research that relate to the application of dry rice toward physiological condition and antibody of the broiler under heat stress are presented in Table 1 below.

Table 1. Results of observation on variable.

Treatment	Water consumption ($\text{mL head}^{-1} \text{d}^{-1}$)	Weight of carcass (%)	Relative weight of bursa fabricius ($\text{g } 100 \text{ g}^{-1}$)	PCV (%)	Hi test ND (seropositive)
T1	145.06 ± 9.04	73.06 ± 1.71	0.14 ± 0.06	29 ± 2.97	40 %
T2	150.40 ± 9.40	71.32 ± 1.24	0.20 ± 0.05	27 ± 2.88	80 %
T3	137.90 ± 13.6	70.69 ± 1.23	0.12 ± 0.06	28 ± 2.07	40 %

3.1 Water consumption

Water consumption in Table 1 shows that there is no influence among the three treatments. Results of ANOVA show that P value > 0.05. Respectively, the highest water consumption is T2 ($\text{mL head}^{-1} \text{d}^{-1}$) (150.40), T1 (145.06) and the lowest is T3 (137.90). Water consumption levels are presented in Figure 1. Data shows that the highest water consumption is T2 although it doesn't show any significant effect.

According to Jahejo *et al.* [39]; Lin *et al.* [40] heat stress may have significant effect on water consumption in broiler. When the chickens have heat stress, they will try to manage the temperature of their bodies to prevent overheating. A natural way the chickens use to cool off their bodies is consuming much water. Broilers will change their water consuming pattern during the heat stress period. If the water consumption is insufficient, the chickens may have dehydration, heat stress, inhibited growth, and reduce their productivities [29, 40]. Therefore, appropriate water management is very important to maintain welfare and performance of the broilers during heat period [41–43].

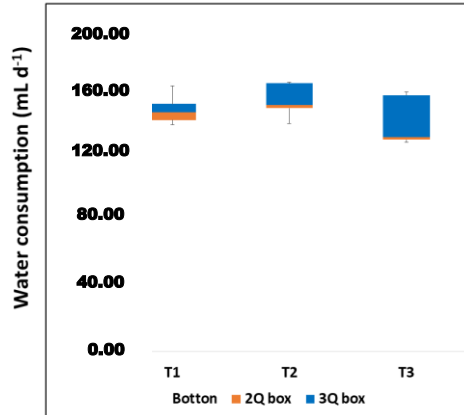


Fig. 1. Water consumption.

3.2 Weight of carcass

Based on the statistical results, the use of dry rice as feed in ration does not have significant effect ($P > 0.05$) on percentage of the broiler carcass as well as toward the control. According Zuidhof *et al.* [44] and Ripon *et al.* [28] weight of carcass closely related to cutting weight and body weight gain of the chicken. The average percentage of carcass resulted in the research (Table 1 and Figure 2) is still normal. The percentage of carcass for the body parts of the broiler ranges from 65 % to 75 % of the live weight and percentage of carcass is affected by final body weight and carcass weight [45, 46].

The use of dry rice showed the same yield of carcass weight as all treatments. It indicated that dry rice has the same ability in terms of carcass weight both with dry rice and without dry rice. On this basis, the use of dry rice has no side effects on reducing carcass weight.

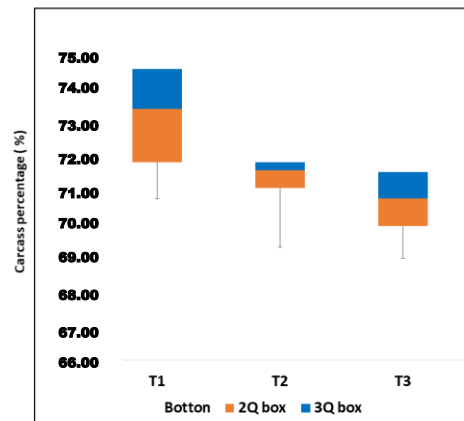


Fig. 2. Carcass percentage (%).

3.3 Packed cell volume (PCV)

PCV values (%) in the research are T1 29; T2 27; and T3 28, respectively. The lowest PCV was shown by treatment T2, 27 %. The result showed that the use of dry rice by sowing

method did not increase the PCV value above the normal limit. Normal values of PCV for chicken range from 22 % to 35 % [47, 29]. Even though T2 did not increase PCV but the whole treatments were normal. The increase of PCV values is highly affected by nutrition adsorption in the broiler's body, especially protein. The nutrient plays important role to maintain metabolism process in the body. Nwogor *et al.* [31] suggested that PCV value is useful in assessing the protein status and finding out supplementation level of protein in different physiological conditions.

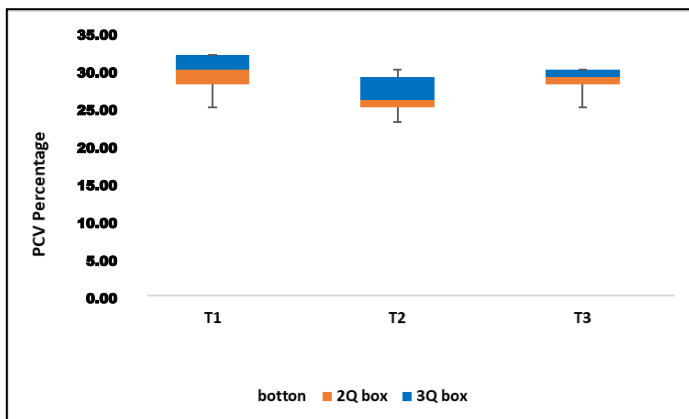


Fig. 3. PCV values.

According to Londok *et al.* [47] PCV value is an indicator of blood ability to carry oxygen. The increase of PCV value may indicate the increase of blood viscosity due to impaired blood circulation. However, the increase of PCV value that is still within the standard indicates the health condition of the poultry is normal.

PCV is an essential parameter in monitoring the animal health because it provides an insight about health of the whole blood circulation systems in animal and oxygen carrier capacity [48]. The decrease in PCV may indicate specific conditions such as anemia, bleeding, or blood loss that are caused by various factors such as parasites, malnutrition, or disease. The increase in PCV may indicate dehydration or certain medical conditions. Monitoring PCV values over time will help to diagnose and manage health problems in animals [29, 31].

3.4 Weight of bursa fabricius

Results of the research showed relative weight of bursa fabricius (%) as follow T1 0.14; T2 0.20; and T3 0.12, respectively. Treatment T2 showed the yield in normal condition, while T1 and T3 showed under normal conditions. This result is the same as the findings of the research by Sulistiyanto *et al.* [49]; Cazaban *et al.* [34] who suggested that relative weight of bursa fabricius ranged from 0.17 % to 0.24 % of the live weight as well as research by Hirakawa *et al.* [50] suggested that relative weight of bursa fabricius ranged from 0.20 % to 0.22 % of the live weight.

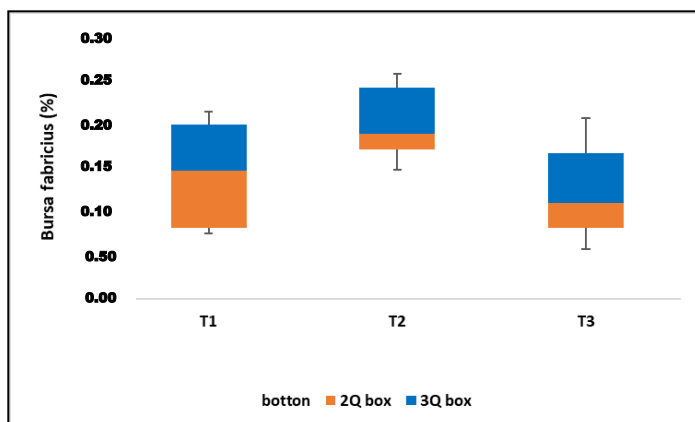


Fig. 4. Weight of bursa fabricius ($\text{mg } 100 \text{ g}^{-1}$).

Data (Table 1 and Figure 4) shows relative weight of bursa fabricius T2 better than T1 and T3 that have decreased. Hakim *et al.* [51] stated that chickens that are raised under stress conditions, such as the increase of room temperature and high density of the closed house, will reduce the relative weight of bursa fabricius. The same statement conveyed by Pamok *et al.* [52] that in general, bursa fabricius in chicken that has heat stress may become an atrophy and reduce the number of lymphocytes. The phenomena occurred in chickens which were raised in an environment with high temperature, corticosterone that derived from adrenal cortex will go into the blood circulation to increase metabolism in chicken. A decrease in feed consumption resulted in reduced nutrients so that chicken may have prolonged stress which caused atrophy of the thymus. The loss weight of bursa fabricius apparently reduced the numbers of lymphocyte so that the antibody, such as gamma globulin, which is important in immune system of the body, will be low [53].

The application of dry rice under heat stress will be able to maintain the weight values of bursa fabricius. It is due to the dry rice given by sowing method at 10 a.m will suppress the stress level in chicken as shown by relative weight of bursa fabricius 0.20 % of live weight. Tonda *et al.* [21] suggested that dry rice is good for broiler. Low calories in dry rice caused the body's calory production would not be excessive, thereby it would reduce the stress level in broiler under heat stress condition. Moreover, other benefit of dry rice is increasing the intestinal performance because the apathogenic bacteria will grow optimally in the intestines. Such apathogenic bacteria produce an enzyme to maintain stability of the absorbed functions in the chicken's intestines [26, 37].

3.5 Antibody

Results of ND antibody titer examination for each treatment are Seropositive; T1 40 %, T2 80 % and T3 40 %, respectively. Seronegative; T1 60 %, T2 20 %, and T3 60 %, respectively. The data shows that the highest immune response was indicated by treatment T2 (Figure 5). The standard protective antibody titer against the ND virus that prevails in ASEAN is 24 HI unit [54].

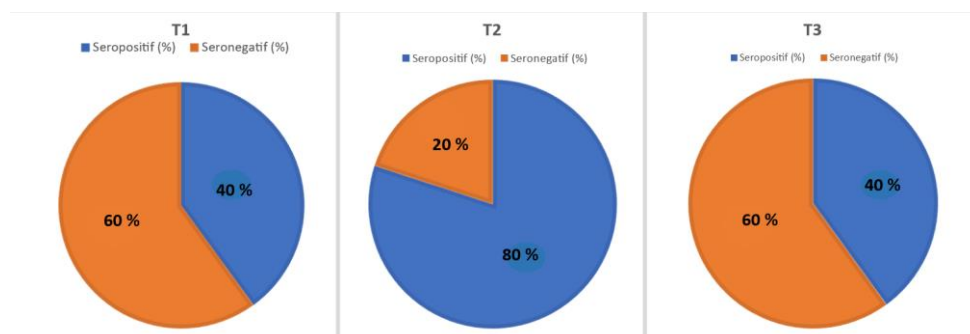


Fig. 5. Hi test ND.

Results of the research (Figure 5) show that the protective level of the chicken under heat stress showed low immune response. Low immune level in broiler as the treatment of temperature $> 30^{\circ}\text{C}$ will weaken the body endurance. It is triggered by low nutrition intake due to heat stress [49, 53]. Moreover, under heat stress, the digestive system would not function optimally, and the broiler may have wet dropping and increase the ammonia level. High ammonia will promote the growth of bacteria and cause respiratory problems in the chicken. If the heat stress is left longer, the chicken's condition will be increasingly weak. The weaker condition of the chicken, its body endurance will increasingly decrease.

Even though results of the antibody test showed low values, the treatment T2 showed higher value compared to other treatments. The application of dry rice under heat stress by sowing method at 10 a.m will suppress the stress level in chicken as shown by the immune level which is not decreased. The same statement conveyed by Tonda *et al.* [24, 25] that dry rice is good for broilers. Low calories in dry rice caused the body's calory production would not be excessive, thereby it would reduce the stress level in broiler, reduce painting, and avoid the wet dropping. Moreover, high resistant starch in dry rice will trigger the optimal growth of apathogenic microorganisms. Resistant starch is a nutrition that can be digested by apathogenic bacteria but not pathogenic bacteria [55, 21]. The apathogenic bacteria will grow optimally and produce enzymes to improve and maintain stability of the absorptive functions in the chicken's intestines [56, 57, 19, 20]. The increase in nutrition absorption in the chicken's intestines will increase the chicken's performance. The increase performance of the chicken will increase the breeder (poultryman) welfare [58, 36].

4 Conclusion and recommendation

The research concluded that the use of dry rice whether evenly mixed or sown over the feed would not increase the water consumption and do not affect on weight of carcass. Nevertheless, the use of dry rice by sowing it over the feed under heat stress will maintain health and stability of the chicken's immune as shown by normal amount of PCV, bursa fabricius is not reduced, and Hi-Test ND also has higher percentage.

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