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The Effect of Administration of Immune Herbal Probiotics on Oxidative Stress Profile and Performance of KUB 2 Chickens

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ABSTRACT

Immune herbal probiotics are nutrients containing probiotics and enzymes. The purpose of this study was to evaluate the effect of administering immune herbal probiotics on the oxidative stress profile and performance of KUB 2 chickens. The research materials were 192 KUB-2 male chickens aged 21 days and immune herbal probiotics. The research method used was an experimental method. There were 4 treatments with 4 repetitions. The treatments given were P0 = Control, P1 = 2 ml/L immune herbal probiotics, P2 = 4 ml/L, P3 = 6 ml/L. The variables measured were Oxidative Stress Profile and net gain. Data were analyzed using ANOVA. The results showed that administering immune herbal probiotics had a very significant effect ($P < 0.01$) on SOD activity and MDA levels and had no significant effect ($P > 0.05$) on Net Gain. The average value of SOD activity (mmol/L) was P0: 47.00a, P1: 14.33b P2: 31.72bc and P3: 43.94c. The average MDA level (mmol/L) was P0: 6.28a, P1: 48.69a, P2: 41.11b and P3: 14.51b. The average body weight gain was P0: 0.73 grams, P1: 0.76 grams, P2: 0.81 grams and P3: 0.77 grams. The conclusion of the study was that the administration of Immune Herbal Probiotics affected SOD activity and MDA levels in KUB 2 Chickens.

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Introduction

Ayam Kampung Unggul Balitbangtan (KUB) 2 is the result of selection from the kampung chicken group for 6 generations. The selection criteria used are increasing egg production by reducing brooding. The average egg production reaches 180 eggs/year, with

this result it is expected to produce Day Old Chick (DOC) in large quantities Iswanto, [1] . One of the organs that plays a role in the chicken's immune system is the lymphoid organ. Male native chickens have faster and larger lymphoid organ growth compared to female native chickens. Therefore, it is necessary to minimize the use of antibiotics and maintain the immune system in native chickens by providing natural ingredients (herbs) as immunomodulators of Rahmawati et al .[2].

Probiotics are defined as live microbes that are used as supplementary feed and can benefit their hosts by improving the balance of their digestive microbes of Vester and Fahey. [3]. Most of the microbes in probiotics come from lactic acid bacteria. Lactic acid bacteria are a group of gram-positive bacteria, round or rod-shaped, do not produce spores and have a final product in the form of lactic acid of Detha. [4]. Giving 6 ml/liter of probiotic herbal medicine *Lactobacillus fermentum* in drinking water plus amino acids has a good effect on body weight and FCR values of broiler finishers of Halan et al. [5]. Vaccination and antibiotics have not been fully able to help livestock immunity because many management factors are not optimal. High antibody titers at the time of vaccination can neutralize the vaccine antigen used, thereby reducing the response to the vaccine given and can result in vaccination failure of Kencana, et al .[6]. Continuous uncontrolled use of antibiotics as AGP (antibiotic growth promoters) to stimulate chicken growth causes antibiotics to no longer be effective as therapeutic drugs in livestock of Putra and Humaidah. [7]. Herbal immunity derived from rhizome plants contains active compounds including essential oils as antibacterials and anti-inflammatories, and contains antioxidant compounds that can ward off free radicals that enter the body of Nurhayati. [8].

Super Oxide Dismutase (SOD) is a primary antioxidant that can prevent the formation of new free radicals. This is because SOD is able to change free radicals into molecules that have a reduced negative impact before they have a chance to react with Nisa. [9]. *Malondialdehyde* (MDA) is a compound that can describe the activity of free radicals in cells so that it can be used as an indication of oxidative stress due to free radicals, Herry. [10]. Based on the description above, it is necessary to evaluate the effect of giving immune herbal probiotics on the oxidative stress profile and performance of KUB 2 chickens.

State of the Art

Research into improving poultry performance and health through functional nutrition approaches continues to grow in line with restrictions on the use of antibiotic growth promoters (AGPs) in the livestock industry. Recent trends indicate a shift away from synthetic antibiotics toward probiotics, phytobiotics, and herbal immunomodulators as safer and more sustainable alternatives. Several previous studies have reported that lactic acid bacteria-based probiotics can improve gut microflora balance and increase feed efficiency, while certain herbs serve as sources of bioactive compounds with antioxidant and immunostimulatory activities. However, most previous research has focused on production performance parameters such as body weight gain, feed conversion ratio (FCR), and feed consumption, particularly in commercial broiler chickens. Studies that integrate biochemical indicators of oxidative stress, such as Superoxide Dismutase (SOD) activity and protein levels, have been limited. Malondialdehyde (MDA), particularly in superior local chickens such as the Balitbangtan Superior Village Chicken (KUB) 2, is still relatively limited. However, oxidative stress is a crucial physiological factor that directly impacts immunity, cellular health, and sustainable livestock performance, particularly during the early growth phase.

This study provides an important contribution in filling this research gap by examining the effects of immune herbal probiotics on the oxidative stress profile (SOD and MDA) and growth performance of KUB 2 chickens. The main novelty of this study lies in the integrative approach that combines probiotics and various immunomodulatory herbal plants in one formulation, and evaluates their impact quantitatively through oxidative stress biomarkers, not only production indicators. The results showed that the administration of immune herbal probiotics had a significant effect on increasing SOD activity and reducing MDA levels, which indicates an increase in endogenous antioxidant capacity and a decrease in oxidative stress in KUB 2 chickens, although it did not have a significant effect on body weight gain.

Thus, this study reinforces the paradigm that the success of nutritional interventions is not always directly reflected in growth performance, but also in the physiological balance and cellular health of livestock. This study positions immune herbal probiotics as a preventive and supportive strategy in maintaining the immunity of superior local chickens, while also opening up further research directions related to dosage optimization, specific

herbal formulations, and their relationship to long-term production performance and livestock product quality. Therefore, this research holds a crucial position in the development of herbal-probiotic-based functional nutrition in sustainable poultry farming systems.

Method

The materials used in the study were immune herbal probiotics and 192 21-day-old male KUB 2 chickens divided into 16 cages. Each treatment consisted of four replications, each containing 12 KUB 2 chickens.

The research method used in this research is experimental. with use Design Random Complete (RAL) which consists of 4 treatments with 4 repetition. The treatment in this study is as follows:

P0 : Control (Without Immune Herbal Probiotic)

P1 : Administration of Immune Herbal Probiotics as much as 2 ml/L in drinking water

P2 : Administration of Immune Herbal Probiotics at 4 ml/L in drinking water

P3 : Administration of Immune Herbal Probiotics as much as 6 ml/L in drinking water

The daily drinking water requirement for KUB 2 chickens is 300 ml/head/day according to the opinion of (Tikan, 2022).

The variables observed in this study were Daily Body Weight Gain, *Mortality* , SOD Activity, MDA Levels and Immunoglobulin G (IgG) Levels. DOC maintenance was in accordance with the KUB 2 Chicken maintenance standards (Sartika, 2016). When the KUB 2 chickens were 20 days old, they were weighed first using a digital scale to obtain uniform body weight. KUB 2 chickens were placed in 16 battery cages and each cage consisted of 12 KUB 2 chickens.

The type of feed is given according to the KUB 2 chicken phase using JAPFA *broiler comfeed factory feed* . Feed is given 2 times/day and drinking water is changed every day. Cage

cleaning is done every day. The treatment process as follows with the administration of Immune Herbal Probiotics is carried out when the KUB chicken is in the grower phase, namely 3 weeks of age. The treatment of providing drinking water as much as 250 ml/head/day using 3 different concentrations for each treatment, namely 2 ml, 4 ml and 6 ml. This treatment stage is carried out for 5 weeks. Every day there must be a check to ensure that the drinking water is consumed so that the chicken gets the Immune Herbal dose according to the treatment. The research data were analyzed using *Analysis Of Variance* (ANOVA). The results of the analysis of variance that show a real or very real effect will be continued with the Least Significant Difference (LSD) test to determine the differences between treatments.

Results and Discussion

Results

ANOVA (*Analysis Of Variance*) test of giving herbal immune probiotics in drinking water had a very significant effect ($P < 0.01$) on the SOD activity of KUB 2 Chickens. Giving herbal immune probiotics has an effect on increasing SOD activity. Herbal immune probiotics are a mixture of lactic acid bacteria and herbal plants such as turmeric, curcuma, ginger, kencur, temu ireng, Kunci, galangal, garlic, noni, betel leaves, and neem leaves. According to Zulfanita et al. [11] . In medicinal plants there are active compounds such as alkaloids, phenolics, tripenoids, glycoside essential oils and so on which act as antivirals, antibacterials and immunomodulators. Livestock herbal medicine can also increase appetite in livestock so that livestock are healthier and not easily attacked by disease. Turmeric or turmeric is a plant that contains active compounds including essential oils as antibacterial and anti-inflammatory, and contains antioxidant compounds that can ward off free radicals that enter the body of Nurhayati, [8] .

KUB 2 chickens at the age of 3 weeks are in the growing phase. Chickens in the growing phase require optimal cellular performance so that metabolism can work well to prepare for the early *egg-laying* period. If external and internal conditions are not supportive, chickens are susceptible to stress. Stressful conditions can cause disruption to several physiological parameters which can ultimately reduce production performance when approaching *egg-laying* Fradinata, et al. [12] . Giving herbal immune probiotics in drinking

water can increase SOD concentration. The increase in SOD activity as a result of giving herbal immune probiotics means that KUB 2 chickens have a stronger capacity to ward off antigens. This is due to the synthesis of SOD as a body mechanism to reduce the possibility of exposure to antigens. Astuti [13] . states that SOD catalyzes superoxide anions into hydrogen peroxide and prevents the formation of free radicals that cause cell damage.

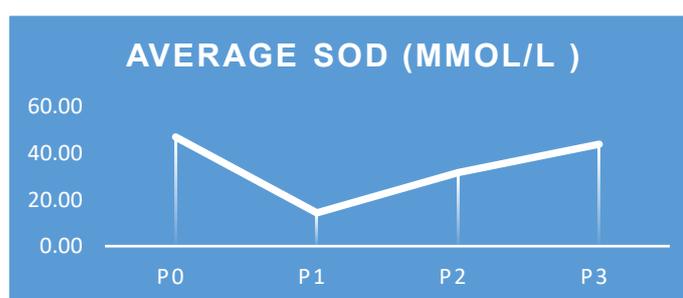


Figure . 1. Mean values of *Superoxide dismutase* (SOD) .

Based on the graph above, it shows that the more herbal immune probiotics are given, the more SOD activity will increase. P0 as a control has the highest SOD activity. The highest SOD activity at P0, namely without giving herbal immune probiotics but is not different from P3, namely giving 6 ml of herbal immunity. SOD activity without giving herbal immunity is the same as giving 6 ml of herbal immunity. This is thought to be because SOD has a similar activity ability in counteracting free radicals in KUB 2 Chicken cells during the growing period. The role of SOD is to prevent free radicals that are formed due to cell stress. SOD is an endogenous antioxidant enzyme that has an important role in directly protecting cells from free radical interference and directly maintaining the balance of toxic oxygen of Putri. [14]. SOD is an antioxidant that works by cleaning free radicals or *reactive oxygen species* (ROS) with enzymatic reactions and converting them into more stable products Rarangsari, [15] .

Discussion

ANOVA (*Analysis Of Variance*) test of giving herbal immune probiotics in drinking water had a very significant effect ($P < 0.01$) on the MDA activity of KUB 2 Chicken. Giving herbal immune probiotics had an effect on reducing MDA levels. Herbal immune probiotics are a mixture of lactic acid bacteria and herbal plants such as turmeric, ginger, ginger, kencur, temu ireng, Kunci, galangal, garlic, noni, betel leaves, and neem leaves. According to Zulfanita et al. [11] . In medicinal plants there are active compounds such as alkaloids, phenolics, tripenoids, glycoside essential oils and so on which act as antivirals, antibacterials and immunomodulators. Turmeric or turmeric is a plant that contains active compounds including

essential oils as antibacterial and anti-inflammatory, and contains antioxidant compounds that can ward off free radicals that enter the body Nurhayati, [8] . According to Ratnayani, [16] . MDA levels are due to the alleged role of herbal immune probiotics as antioxidants that prevent the lipid peroxidation propagation process and eventually lead to the termination step. The termination step is the final process of stopping lipid peroxidation so that it stops the formation of MDA.

KUB 2 chickens at the age of 3 weeks are in the growing phase. Chickens in the growing phase require optimal cellular performance so that metabolism can work well to prepare for the early egg-laying period. Internal and external conditions that do not support stress cells which result in increased free radicals in the body. Free radicals can attack DNA, protein or fat. If the fat attacked by free radicals is *unsaturated fatty acids* or *polyunsaturated fatty acids* (PUFA), then in the propagation stage a lipid radical will be formed and then in the termination stage it will become a lipid peroxidation which produces MDA. High MDA indicates high free radicals in cells which results in cell damage. Cell damage starts from the cell membrane, resulting in disrupted metabolism in the body. Herbal immune probiotics are needed for KUB 2 chickens that experience stress during the growing period. Herbal immune probiotics contain high antioxidants and can reduce MDA levels. According to Muparijin, [17] . High MDA concentrations indicate that there is an oxidation process in the cell membrane and are experiencing stress. High antioxidant status is usually followed by a decrease in MDA levels of Samdihutami, Desmiaty and Anbar , [18] . According to Saleema, [19] . The MDA levels of stunning chicken spleen $3,021 \pm 0.232 \mu\text{g}/\text{gr}$ sample were also higher when compared to non-stunning chicken spleen MDA $2,412 \pm 0.336 \mu\text{g}/\text{gr}$ sample.

The MDA levels of the stunning chicken spleen $3.021 \pm 0.232 \mu\text{g} / \text{gr}$ sample were also higher when compared to the MDA of the non-stunning chicken spleen $2.412 \pm 0.336 \mu\text{g} / \text{gr}$ sample. The role of MDA in the livestock body is as a marker of oxidative stress, so MDA can see the stress in KUB 2 chickens. Malondialdehyde (MDA) is the final production of lipid peroxidation in the body through an enzymatic process if a high concentration of MDA indicates an oxidation process in the cell membrane. Mulianto , [20] . Oxidative stress has the potential to cause damage if ROS production exceeds antioxidant capacity. Rahmawati, [2] .

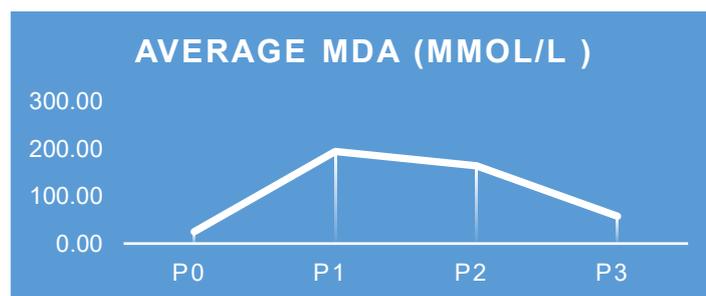


Figure . 2 . Mean value of *Malondialdehyde* (MDA)

Based on the graph above, it can be seen that increasing the addition of herbal immune probiotics has an effect on decreasing MDA. P0 as a control, aka no addition of herbal immune probiotics, shows the lowest MDA levels. P3, namely treatment with herbal immune probiotics, can reduce MDA levels to 14.51 ± 58.06 mmol/L. Herbal immune probiotics with their bioactive substances, namely carotenoids and flavonoids, contain high antioxidants. Antioxidants are able to donate hydrogen atoms quickly to free radicals, and are able to act as scavengers (dampeners) of free radicals directly. Herbal immune probiotics with their antioxidants will be able to reduce the formation of free radical compounds due to the presence of toxic compounds such as ROS and reduce peroxidation which ultimately decreases MDA levels. Setyarini, [21] . ANOVA (*Analysis of Variance*) test of giving herbal immune probiotics in drinking water did not have a significant effect ($P > 0.01$) on the weight gain of KUB 2 Chickens.

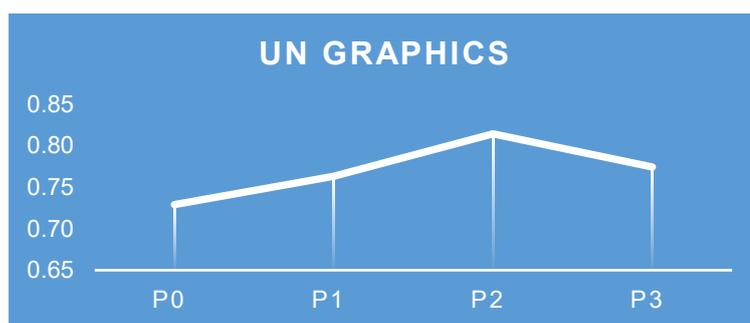


Figure . 3 . Mean value of Weight Gain

KUB 2 chickens have a relatively slow growth rate when compared to broiler chickens, this is because these chickens have not been selected for specific business purposes, whether as layers or meat. The weight gain of KUB 2 chickens per week is recorded with an increase in P0: 0.73 grams, P1: 0.76 grams, P2: 0.81 grams and P3: 0.77 grams. The factor that affects the weight gain is not optimal is stress. Stress is responded to by the body by mobilizing glucose to be converted into energy and used to suppress the stress itself. As a result, only a little

energy is directed to weight gain. Comfortable conditions in KUB 2 chickens allow the weight gain process to run well, unlike if KUB 2 chickens are in a stressful condition. Fattah et al. [22] . explained that stress will arise when chickens cannot release heat from their bodies because of the high temperature levels in the cage. The higher the humidity, the more humid the atmosphere in the cage will be and result in an increase in ammonia levels. This can disrupt the health of the chicken and the chicken growth process for more optimal nutrient absorption. Ibrahim, et al [23] .

Conclusions

Based on the research results, the immune status of KUB 2 chickens can be improved by adding Immune Herbal Probiotics. Immunity functioned optimally based on a mortality rate of 0 during the study period from 21 days to 56 days of age. Further research is needed on unvaccinated KUB 2 chickens to obtain more concrete results on the benefits of Immune Herbal Probiotics.

References

- [1]. Astuti, S., 2012. Soybean Isoflavones and Their Potential as Free Radical Scavengers. *Journal of Agricultural Technology & Industry* , 13 (2), 126-136.
- [2]. Detha, A. (2019). Characteristics of lactic acid bacteria isolated from Sumba mare's milk . *Journal of Veterinary Studies* , 7 (1), 85-92.
- [3]. Fattah, HA, Faridah, R., Amalia, NHA, and Khaerudin., 2023. The Effect of Temperature and Humidity Settings in Closed House Cages on Broiler Performance. Faculty of Agriculture. Sinjai University. South Sulawesi.
- [4]. Fradinata, E., Yaman, A. and Dasrul, D., 2022. Education on Open House Laying Hen Cultivation in Blang Bintang. *Journal of Independent Service* , 1 (8), 1405-1412.
- [5]. Halan, M. 2020. The Effect of Giving Probiotic Herbal Medicine Plus Amino Acids on Feed Consumption, Body Weight Gain and Feed Conversion of Finisher Phase Broilers. Faculty of Animal Husbandry. Islamic University of Malang.
- [6]. Herry, S. 2016, Analysis of Body Weight Gain and Molecular Profile of Heat Stress in Ongol Crossbred Cattle at Different Altitudes. Faculty of Animal Husbandry, Islamic University of Malang. Malang.

- [7]. Ibrahim, S., & Allaily, A. (2012). The effect of various litter materials on the concentration of ammonia in ambient air in the cage and the performance of broiler chickens. *Agripet Journal* , 12(1), 47-51.
- [8]. Iswanto, H. 2018. Getting to Know Free-Range Broiler Chickens Closer. Agro Media Pustaka. Yogyakarta.
- [9]. Kencana, GAY, Suartha, NMA S, Paramita, and Handayani, AN 2016. Combination Vaccine of Newcastle Disease with Avian Influenza Triggers Protective Immunity in Laying Hens Against Tetelo Disease and Bird Flu. *Veterinary Journal* , 17 (2), 257-264.
- [10]. Nurhayati., CU Wirawati and DD Putri. 2015. The Use of Fermented Products and Turmeric in Feed on Broiler Performance and Income Feed Ang Chick Cost. *Jurnal ZooteK* . Vol. 35 (2): 379-389.
- [11]. Muparijin, AD, 2023. The Effect of Radiofrequency Exposure on Malondialdehyde Levels and Liver Histology of Mice (*Mus musculus*) (*Doctoral Dissertation, State Islamic University of Maulana Malik Ibrahim*), Malang.
- [12]. Mulianto, N., 2020. *Malondialdehyde* as a Marker of Oxidative Stress in Various Skin Diseases. *Mirror of the World of Medicine* , 47 (1), 39-44
- [13]. Nisa, K., 2017. The Effect of 70% Ethanol Extract of Rambutan Seeds (*Nephelium lappaceum L*) on the Increase in SOD Levels and the Decrease in MDA Levels in the Pancreas of Mice (*Mus musculus*) Induced by *Streptozotocin* (*Doctoral dissertation, State Islamic University of Maulana Malik Ibrahim*). Malang.
- [14]. Putra, DC, and Humaidah, N. (2022). Effectiveness of Probiotics as a Replacement for Antibiotic Growth Promoters (AGP) in Poultry (Review Article). *Animal Engineering Dynamics: Scientific Journal (e-Journal)* , 5(02).
- [15]. Putri, IAMB, 2021. The Effect of Giving Moringa Leaf Powder (Moringa oleifera) on SOD and MDA Levels in Pregnant White Mice with Diabetes Mellitus (*Doctoral dissertation, Wijaya Kusuma Surabaya University*), Surabaya.

- [16]. Rahmawati, G., FN Rachmawati, and H. Winarsi., 2022. *Superoxide Dismutase Activity of Diabetic Rats Given Cardamom Stem Extract and Glibenclamide. Scripta Biologica* , 1 (3), 197-201.
- [17]. Rarangsari, NE, 2015. The Effect of Soursop Leaf Extract (*Annona muricata L.*) on SOD and Liver Histology of Rats (*Rattus norvegicus*) Induced by Alloxan (*Doctoral dissertation, State Islamic University of Maulana Malik Ibrahim*), Malang.
- [18]. Ratnayani, R., 2013. Analysis of Cytoglobin Expression and Its Relationship to Oxidative Stress in Blood and Brain Tissue of Hemorrhagic Stroke Patients (*Doctoral dissertation, University of Indonesia*), Depok.
- [19]. Sandhiutami, NMD, Desmiaty, Y. and Anbar, A., 2017. Antioxidant Effect of Papaya Seed Ethanol Extract (*Carica papaya L.*) on *Superoxide Dismutase Enzyme Activity and Malondialdehyde Levels* in Mice Under Oxidative Stress with Swimming. *Indonesian Journal of Pharmaceutical Sciences* , 14 (1), 26-23.
- [20]. Saleema, AK, 2018. Malondialdehyde (MDA) Levels in the Heart and Spleen of Stunned and Non-Stunned Broiler Chickens.
- [21]. Setyarini, AI, 2016. The Effect of *Vigna Unguiculata* Extract on SOD Levels, MDA Levels, MAPK Activity and Proliferation of Vaginal Fibroblasts of *Rattus norvegicus* Post-Ovariectomy (*Doctoral dissertation, Brawijaya University*), Malang.
- [22]. Vester, BB Fahey, GC 2010. Prebiotics and Probiotics in Companion Animal Nutrition. In: Cho SS, Finocchiaro ET (eds). Handbook of Prebiotics and Probiotics Ingredients. CRC Press. Boca Raton.
- [23]. Zulfanita, R. Eny and MDP Utami. 2017. Ration restrictions affect broiler chicken body weight gain during the growth period. *Journal of Agricultural Sciences*. 7(1): 59-67.