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The effects of native chicken strains and feed addives on immunity, kidney functions, and blood protein

I. Ismoyowati*, A. Darmanto, E. Tugiyanti, F. M. Suhartati, W. Suryapratama, A. Sodiq, D. M. Saleh, and M. Y. Sumaryadi

Universitas Jenderal Soedirman, Purwokerto - Indonesia
**Corresponding email : moyowati@gmail.com*

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ABSTRACT

This study aimed to investigate the interactions between chicken strains and supplementation of feed additives, and the efficacy of the interactions for improving immunity, kidney function, and blood protein of native chicken. Research materials were 480 chickens of different strains including kampung chicken, KUB chicken (Balitbangtan superior native), and Kedu chicken. Basal feed offered to the chickens contained 3,118.95 kcal/kg energy and 19.2% crude protein. The research was conducted in a factorial completely randomized design, utilizing 12 treatments and four replicates. The data were subjected to analysis of variance, followed by the Honestly Significant Difference Test (HSD). The analysis of variance showed that the interactions between chicken strains and 1% supplementation of feed additives were not significantly different ($P>0.05$) across all parameters, but chicken strain significantly affected ($P<0.05$) antibody titers against AI and ND, as well as lymphocyte infiltration in the lamina propia ileum. KUB chickens had a higher level of immunity than that Kedu chickens. Supplementation of 1% feed additives tends to improve the level of immunity as reflected by the increased titers against AI and ND after vaccination, as well as the undisturbed kidney functions. Conclusively, the interactions between chicken strains and the supplementation of 1% feed additives into basal feed produced relatively similar results. Meanwhile, KUB chickens showed better immunity than kampung chickens and Kedu chickens. Supplementing feed additives (garlic and kalimun) showed relatively effective results in improving antibody titers against AI and ND, so it is necessary to increase the supplementation dosage to enhance significant immunity.

Keywords: Feed additives, Native chicken strains, Immunity, Kidney functions, Blood protein

INTRODUCTION

Native chickens are spread in almost all parts of Indonesia and are in rural areas. Most of the native chicken was reared extensively and semi-intensively. Maintenance of native chickens with minimal handling causes the immune system of chickens to be low so that it can cause disease and death. Extreme environmental conditions, disease, nutritional deficiencies, and com-

petition for feed cause death (Ismoyowati *et al.*, 2021). The availability of superior native DOC with a high level of immunity against disease germs remains an issue among poultry breeders. Extensive system maintenance has an impact on suboptimal growth performance, high mortality, and low meat production and quality. The low quality of feed and the absence of disease prevention causes poor productivity of native chickens (Dal Bosco, *et al.*, 2021). Native chickens with high levels of immunity and normal kidney functions will produce optimum product performance. Body immune status is reflected in the conditions of antibody titers, total leukocyte and white blood cell differential, and infiltration of lymphocyte and macrophage. Blood proteins, such as total protein plasma, blood urea nitrogen, and albumin – are other indicators of poultry welfare (Saki *et al.*, 2018).

The productivity of native chickens is significantly influenced by the feed offered by farmers. Feed is a vital factor in the success of native chicken breeding. Therefore, feed supplemented with different feed additives is expected to produce effective results in improving the immunity, kidney function, and blood protein of native chicken. Indonesia is home to rich biodiversity in which many floras and fauna are available to provide feed additives for livestock including native chicken. Garlic (*Allium sativum*) and green chiretta (*Andrographis paniculata*) are among the local herbal products that have been supplemented to improve the health, performance, and well-being of native chicken in Indonesia (Sugiharto, 2021). Garlic contains pharmacological substances that are beneficial for the body's immune, such as Alliin, Allicin, E-Ajoene, Z-Ajoene, 2-Vinyl-4H-1,3-dithiin, Diallyl sulfide (DAS), Diallyl disulfide (DADS), Diallyl trisulfide (DATS), and Allyl methyl sulfide. Garlic is one of the most important bulb vegetables that have a pungent Antibacterial, Antifungal, and Anti-Protozoal Activity. Garlic is also investigated to have immunomodulatory, anti-inflammatory, and antioxidant effects and this focused on the question of whether the known effect of processed garlic and its related compounds mainly allicin in inhibiting ache and buche enzymes (El-Saber Batiha *et al.*, 2020).

Green chiretta is commonly used as medicine to treat infections in the respiratory tract and diarrhoea because it contains antimicrobial properties and active components like andrographolide (Mishra *et al.*, 2021). Compared to tetracycline antibiotics, green chiretta is evidently more effective against Shigella bacteria (Benoy *et al.*, 2012). Furthermore, Deepak *et al.*, (2014) reported that green chiretta shows the best antibacterial activity against gram-positive bacteria, i.e., S. aureus and S. pyogenes. Besides garlic and bitter, there are feed additives from manufacturers such as kalimun (PT. Kalbe Farma Tbk.) which are mostly given to poultry. Kalimun contains vitamin E, selenium, and zinc, which play a role in increasing immunity, performance, reproductive organs and fertility. The novelty of this research is to examine differences in immunity in native chicken strains and whether feed additive supplementation can increase immunity based on hematological and blood protein parameters, AI and ND antibody titer values.The objective of this research is to investigate the interactions between chicken strains and the supplementation of different feed additives, as well as the impact of the interactions on immunity, kidney function, and blood protein of different strains of native chicken (*Gallus domesticus*).

MATERIALS AND METHODS

Research Materials

This study was conducted under the requirements of animal ethics set forth by the Ethical Clearance Commission, Faculty of Veterinary Medicine, Gadjah Mada University (No. 021/EC-FKH/Eks.2022). The research materials were 480 unsex day-old-chicks from three strains, namely kampung chickens, Superior Balitnak Native chickens (KUB), and Kedu chickens. The basal feed was composed of different compositions and nutrient contents as presented in Table 1. The feed additives consisted of green chiretta meal (*Andrographis paniculate*), garlic powder, and kalimun (PT. Kalbe Farma Tbk). Each kilo-

Table 1. Feed Composition and Nutrient Content of Basal Feed*

Feed Composition	Percentage (%)
Cornmeal	53
Ricebran	21
Soybean kernel	10
Fishmeal	13
Palm oil	0,5
CaCO3	1
Top mix	0,5
Lysin	0,5
Methionine	0,5
Total	**100**
Feed Nutrients	**Total**
Energy (kcal/kg)	3118.95
Crude Protein (%)	19.28
Ash (%)	6.3
Fat (%)	6.15
Crude Fat (%)	3.99
Calcium (%)	1.1
Phosphor (%)	0.85
Pavl (%)	0.5
Lysine (%)	1.48
Methionine (%)	0.92

*Nutrient content of basal feed is calculated based on the Table of Feed Composition in Indonesia (Hartadi *et al.*, 1980).

gram of kalimun contains 10,000 IU of vitamin E, 100 mg of selenium and 40,000 zinc. The equipment used consisted of 48 units of 1m2 litter cage, complete with a feeding and drinking system. The other equipment we used for the experiment includes scalpels, surgical scissors, digital scales with 0.1g increments, 3ml syringes, EDTA and non-EDTA tubes, the container for intestine samples, spectrometer, equipment for Hemagglutination Inhibition (HI) test, and microscopes.

Research Method

The experiments were conducted in a Factorial Completely Randomized Design applied to three strains of chickens (kampung chickens, KUB chickens, and Kedu chickens) and 4 treatment feeds (control, 1% green chiretta, 1% garlic, and 1% kalimun), so there were 12 treatments with four replicates, accounting for 48 experimental units. The treatments were as follows: A1B1 = kampung chickens offered with basal feed, A1B2 = kampung chickens offered with basal feed + 1% green chiretta meal, A1B3 = kampung chickens offered with basal feed + 1% garlic powder, A1B4 = kampung chickens offered with basal feed + 1% kalimun, A2B1 = KUB chickens offered with basal feed, A2B2 = KUB chickens offered with basal feed + 1% green chiretta meal, A2B3 = KUB chickens offered with basal feed + 1% garlic powder, A2B4 = KUB chickens offered with basal feed + 1% kalimun, A3B1 = Kedu chickens offered with basal feed, A3B2 = Kedu chickens offered with basal feed + 1% green chiretta meal, A3B3 = Kedu chickens offered with basal feed + 1% garlic powder, and A3B4 = Kedu chickens basal feed + 1% kalimun.

Procedure of Research

The experimental management was conducted on Day Old Chicks (DOC). Until 21 days, the DOC was offered with commercial feed for broilers containing 12% water, 22.5% crude protein, 3-7% crude fat, 5% crude fibre, 7% ash, calcium 0.9 - 1.1%, phosphorus 0.6-0.9% and metabolic energy of 3000 kcal/kg. Vitamin and vaccination (ND-AI) were given to the native

chicken at the age of 4 and 28 days. Treatment feed adaptation was performed at the age of 22-27 days. On the 28th day, the native chickens were divided into 12 treatments (10 chickens), each with four replicates, hence 480 native chickens. These chickens were offered the same feed, i.e., basal feed supplemented with feed additives, until the age of 12 weeks.

Data collection for research variables was performed when the chickens already reached 12 weeks of age. The samples used for measuring the level of immunity were two chickens from each experimental unit, and one chicken was sacrificed to observe the infiltration of lymphocytes and macrophages in the lamina propia ileum. The samples were collected randomly from male chickens in each experimental unit. Data collection included total leukocyte and white blood cell differential, antibody titers against AI and ND, infiltration of lymphocyte and macrophage in lamina propia ileum, total plasma protein level, blood urea nitrogen (BUN), and blood albumin.

The blood sample was drawn from the vena pectoralis under the wing, using a syringe. Prior to injection, the area was cleansed with a cotton ball drenched in alcohol, after that the syringe was injected into the vena pectoralis. From each chicken, 3ml of blood sample was drawn and then put into a minitube+EDTA (whole blood) and non-EDTA (serum) for further observation. Blood protein was measured using a spectrometer (Purnomo *et al.*, 2016), and the antibody titers against AI and ND were measured under the hemagglutination inhibition test (Toro and Tang, 2009).

The ileum sample for infiltration of lymphocyte and macrophage in lamina propia was taken by first euthanizing the chicken, then the chicken was sacrificed and cut open at the abdominal part to remove the intestines which were then soaked in 10% formalin liquid. The measurement of lymphocyte and macrophage inside the lamina propia ileum was performed histologically using the hematoxylin-eosin (HE) colouring method, using a microscope with 10x ocular and 100x objective magnification. Each preparation was observed for any changes from 10 different angles. Lymphocyte and macrophage count in lamina propia ileum of each microscopic slide was converted into percentages and the divisor value is the number of other cells from each angle (Pratiwi and Nurhajati, 2017).

Data Analysis

The obtained data were subjected to analysis of variance in a factorial Completely Randomized Design. If the analysis showed a significant difference ($P<0.05$) in the measured variables, an Honestly Significant Difference test (HSD) ensued using SPSS 25.

RESULTS AND DISCUSSION

Total Leukocyte and White Blood Cell Differential

Analysis of variance showed that the interactions between chicken strains and the supplementation of 1% feed additives showed a non-significant difference ($P>0.05$) in the total leukocyte and white blood cell differential of native chicken (Table 2). A leukocyte is a cell that plays a role in the body's immune against many diseases through phagocyte mechanism and antibody production. The mean leukocyte number in broiler chickens and thermal environmental data of the pen under the natural light-dark cycle was 13.78 ± 1.8 (x 10^3/ml) (Makeri *et al.*, 2017). According to Tugiyanti *et al.*, (2016) the total and types of leukocytes may serve as the parameter for poultry diagnostic and infection status. A high number of leukocytes reflects an optimal body immune. A high white blood cell count usually indicates increased production of white blood cells to fight infection.

Chicken strain and feed additives had no significant effect on total leukocytes in the blood. The three chicken strains (Kampung, KUB and Kedu chickens) genetically have the same origin, namely the descendants of the red jungle fowl. Hatta *et al.* (2021) reported that local chickens in the Southeast Asia region originated from the Red Jungle Fowl, which spread in various regions to form various local chicken clumps ac-

Table 2. Average of Total Leukocyte and White Blood Cell Differential of Native Chicken Offered with Basal Feed Supplemented with Feed Additives.

Treatments	Leukocyte (x 10^3 / ml) ^{ns}	Heterophils (%)^{ns}	Eosinophils (%)^{ns}	Lymphocyte (%)^{ns}	Monocyte (%)^{ns}
A1B1	8.73 ± 1.70	30.70 ± 8.04	2.50 ± 1.11	61.70 ± 8.52	5.0 ± 1.58
A1B2	9.52 ± 2.22	28.20 ± 5.26	2.20 ± 1.92	62.20 ± 7.56	7.2 ± 2.16
A1B3	9.71 ± 0.54	30.50 ± 8.13	3.00 ± 1.00	58.20 ± 5.44	8.2 ± 2.38
A1B4	9.70 ± 2.34	33.50 ± 8.55	1.50 ± 0.86	58.70 ± 7.08	6.2 ± 2.58
A2B1	10.35± 1.70	23.20 ± 4.65	4.20 ± 1.47	65.20 ± 6.83	7.2 ± 2.16
A2B2	10.60 ± 1.50	34.00 ± 8.09	3.00 ± 1.00	57.50 ± 9.93	5.5 ± 1.5
A2B3	8.50 ± 1.31	30.50 ± 10.5	2.50 ± 1.65	59.70 ± 11.7	7.2 ± 2.77
A2B4	9.42 ± 0.84	35.50 ± 8.29	2.70 ± 0.43	54.00 ± 9.67	7.7 ± 1.78
A3B1	11.10 ± 1.0	33.50 ± 10.8	4.00 ± 1.41	57.50 ± 9.83	5.0 ± 1.41
A3B2	10.31 ± 1.7	27.20 ± 10.1	4.00 ± 1.58	61.20 ± 10.1	7.5 ± 2.17
A3B3	10.45 ± 1.6	29.00 ± 9.027	3.20 ± 1.90	60.70 ± 9.80	7.0 ± 1.87
A3B4	9.15 ± 0.88	36.70 ± 9.36	3.00 ± 1.22	55.50 ± 8.61	4.7 ± 1.92

Note: A1 = kampung chickens, A2 = KUB chicken, A3 = Kedu chickens, B1 = Control Feed, B2 = Feed + 1% green chiretta meal, B3 = feed + 1% garlic powder, B4 = Feed + kalimun powder.
^{ns} = Interactive effects between chicken strains and feed additives were not significantly different (P>0,05).

cording to their respective geographic locations. Supplementation of feed additives did not respond to the number of leukocytes because the chickens were in good health. According to Sumadi *et al.*, (2019) that the concentration of leukocytes in the blood will increase sharply along with the presence of infection in the body. Islam *et al.*, (2004) added that total blood leukocytes are influenced by sex, age, strain, climate, geographical location, and nutritional status.

White blood cell differential refers to the unity of two groups of white blood cells, namely granulocytes (heterosinophils, eosinophils, and basophils) and agranulocytes (lymphocytes and monocytes). The results of our experiment with native chickens showed that the white blood cell differential was within the normal range which, 20-40% heterophils, 2-8% eosinophils, 24-84% lymphocyte, and 3-10% monocyte (Purnomo *et al.*, 2016). These results were similar to those by Islam *et al.*, (2004) that native chicken farmed in Sylhet Bangladesh had 19.50 ± 0.51% heterophils, 3.75 ± 0.44% eosinophils, 71.00 ± 0.73% lymphocyte, and 4.75 ± 0.44% monocyte.

Healthy native chickens may be the cause of the non-significant effect of 1% supplementation of feed additives on the percentage of white blood cell differential because we did not observe a significant difference during the phagocytosis against the foreign bodies. The average percentage of heterophils in this study was 31.06 ± 9.34% which belongs to the normal range (20-

Table 3. The Average of Antibody Titers against AI, ND, and Infiltration of Lymphocyte and Macrophage in lamina Propia Ileum of Native chicken Offered with Basal feed Supplemented with Feed Additives.

Treatments	Antibody AI ^{ns}	Antibody ND ^{ns}	Lymphocyte Infiltration (%)^{ns}	Macrophage (%)^{ns}
A1B1	28 ± 6.9	144 ± 69.7	1.75 ± 0.15	1.06 ± 0.26
A1B2	72 ± 34.8	416 ± 354	1.66 ± 0.08	0.80 ± 0.81
A1B3	64 ± 39.1	272 ± 159	2.12 ± 0.60	0.69 ± 0.10
A1B4	176 ± 83.1	296 ± 218	2.05 ± 0.44	0.98 ± 0.16
A2B1	96 ± 95.5	804 ± 817	2.74 ± 0.55	0.85 ± 0.15
A2B2	24 ± 8.0	624 ± 825	2.31 ± 0.40	0.69 ± 0.11
A2B3	192 ± 186	808 ± 813	2.31 ± 0.70	0.91 ± 0.27
A2B4	98 ± 51.9	168 ± 94.3	2.17 ± 0.60	0.74 ± 0.11
A3B1	12 ± 13.2	48 ± 16	1.61 ± 0.33	0.86 ± 0.14
A3B2	16 ± 11.3	48 ± 47.5	2.13 ± 0.38	0.83 ± 0.21
A3B3	36 ± 53.5	274 ± 238	1.77 ± 0.37	0.76 ± 0.04
A3B4	22 ± 24.8	208 ± 177	2.15 ± 0.34	0.91 ± 0.13

A1 = kampung chickens, A2 = KUB chicken, A3 = Kedu chickens, B1 = Control Feed, B2 = Feed + 1% green chiretta meal, B3 = feed + 1% garlic powder, B4 = Feed + kalimun powder.
^{ns} = Interactive effects between chicken strains and feed additives were not significantly different (P>0,05).

40%). Heterophils are engaged in phagocytosis against foreign bodies or dead tissues. Heterophils carry phagocytosis characteristics and become the frontier in fighting infectious diseases (Wamboi *et al.*, 2020). The average percentage of eosinophils in this study was 3 ± 1.56% or within the normal range (2-8%). Eosinophils are formed in the bone marrow and serve as the response system against allergies, parasites, and inflammation. The average percentage of lymphocytes in native chicken was 59.37 ± 9.22% or within the normal range (42-6%), and the average percentage of monocyte was 6.56 ± 2.35%, also within the normal range (3-5%). The role of lymphocytes is to respond to antigens and stress by increasing antibody circulations for improving the immune system of the animal. Meanwhile, monocyte exhibits the ability to perform phagocytosis against 100 cells of pathogenic bacteria and to regulate inflammation (Makeri, *et al.* 2017).

Antibody Titers against Avian Influenza and Newcastle Disease

The result of the analysis of variance indicated that the interactions between chicken strains and 1% feed additives showed a non-significantly different effect (P>0.05) (Table 3), but chicken strains significantly affected (P<0.05) antibody titers against AI and ND (Table 4). Avian influenza (AI) is one of the viral diseases among poultry with a very high level of a pathogen. This study showed a higher average of antibody titers against AI than that reported by Eid and Iraqi (2014) who studied broilers offered with garlic powder as much as 100, 150 and 200 g garlic powder/tonne, namely log 5.49-6.81. The slight increase in AI antibody titer observed in the garlic supplement group may be due to the immunostimulating effect of garlic. The result of antibody titers against AI showed relatively high titers above the standard log 16 of protective titers. It may be because the testing occurred four weeks after the AI vaccination, so the antibody titer of native chicken was at its maximum level. Toro and Tang (2008) explained that the survival rate of AI-vaccinated chickens is 100%, while the non-vaccinated is 13.2%. A study by Talebi *et al.* (2015) indicated that offering synbiotic biomin would enable the antibody titers to reach their peak on the 35th day. Jafarpour *et al.* (2016) added that the use of feed additives could increase the number of immunoglobulins (IgA, IgM, and IgG) in the intestines and serum of broiler chickens.

Honestly Significant Difference Test (HSD) showed that KUB chickens (102.62 ± 127.2) were significantly different (P<0.05) from Kedu chickens (21.50 ± 33.14) (Table 5). It is because the chicken strains significantly affected the antibody titers against AI in native chickens. Kedu chickens offered with several feed additives tend

Table 4. The Average of Total Protein Plasma (TPP), Blood Urea Nitrigen (BUN), and Blood Albumin of Native chicken Offered with Basal feed Supplemented with Feed Additives.

Treatments	Average of TPP, BUN and Blood albumin		
	TPP (g/dL) ^{ns}	BUN (mg/dL) ^{ns}	Albumin (g/dL) ^{ns}
A1B1	2.50 ± 0.30	6.38 ± 1.42	3.01 ± 0.51
A1B2	2.45± 0.38	4.80 ± 1.12	3.36 ± 0.66
A1B3	2.35 ± 0.21	5.20 ± 1.44	3.49 ± 0.49
A1B4	2.60 ± 0.50	5.65 ± 1.19	2.92 ± 0.57
A2B1	2.80 ± 0.34	6.18 ± 1.13	2.84 ± 0.37
A2B2	2.50 ± 0.36	4.93 ± 1.37	3.14 ± 0.49
A2B3	2.55 ± 0.45	5.85 ± 1.21	3.19 ± 0.44
A2B4	2.70 ± 0.41	4.40 ± 0.68	3.10 ± 0.54
A3B1	2.40 ± 0.37	4.80 ± 1.12	2.93 ± 0.38
A3B2	2.65± 0.35	5.78 ± 1.81	2.67 ± 0.51
A3B3	2.55 ± 0.45	4.80 ± 0.88	2.80 ± 0.39
A3B4	2.65 ± 0.35	4.60 ± 0.43	2.84 ± 0.14

A1 = kampung chickens. A2 = KUB chicken. A3 = Kedu chickens. B1 = Control Feed. B2 = Feed + 1% green chiretta meal. B3 = feed + 1% garlic powder. B4 = Feed + kalimun powder.
^{ns} = Interactive effects between chicken strains and feed additives were not significantly different (P>0.05).

to produce the lowest antibody titers against AI. Genetic variations in native chickens and a high immune system in KUB chickens cause the high titer of ND antibodies produced, making them more resistant to ND virus attacks. Rehman *et al.*, (2021) stated that the inherited immunity system depends on the antigen-presenting cell (APC) and phagocyte cells including dendritic cells (DC), granulocytes, and macrophages.

Offering feed additives such as kalimun and 1% garlic to native chicken tends to improve the average antibody titers against AI, namely 98.66 ± 89.6 and 97.33 ± 138.8 compared to green chiretta and control. Feed additives of kalimun and 1% garlic are the most effective for improving the formation of antibody titers against AI four weeks after vaccination. Each kilogram of kalimun contains 10,000 IU of vitamin E, 100 mg of selenium and 40,000 zinc. The active compounds of kalimun improve animal antibody by increasing the activities of immunostimulants and multiplying the immunomodulator cells such as macrophages, B cells, and T cells. Garlic contains bioactive compounds that serve as antibacterial agents, such as allicin, diallyl disulfide, and diallyl trisulfide. Antibacterial activities in garlic can control pathogenic bacteria of both gram-positive and negative (Rychen *et al.*, 2017). However, offering feed additives of 1% green chiretta (37.33 ± 34.33) showed a lower level of antibody titers against AI than that of the control feed (45.5 ± 69.73). It is allegedly due to the non-optimum performance of green chiretta in forming the antibody but rather acted as the immunosuppressant that lowers antibody titers against AI. It was in line with Rychen *et al.*, (2017) that feed additives may serve as the immunostimulator, immunoregulator, or immunosuppressor.

Newcastle disease (ND) is one of the viral diseases among poultry with a very high mortality rate of up to 80% (Boakye *et al.*, 2016). The results of this study indicate that the mean antibody titer against ND is close to the study reported by Oberlander *et al.* (2020) who reported the antibody titer of the free-ranging bird population averaged log 4.84 using the HI test method. The result of antibody titers against ND among native chicken was still above the protective titers, i.e., log 4 (16). Chickens that have protective antibody titers protektif will be able to fight against antigen virus during infection. Chicken with antibody titers more than log 6 (64) will be able to divert ND 100%. Antibody titers against ND will reach their maximum level 3-4 weeks after vaccination. It is in line with Qiu *et al.*, (2007) that on days 21-35 of each treatment group, the animals reached the highest level of antibody titers against ND.

The Honestly Significant Difference Test (HSD) showed that KUB chickens (601.0 ± 781.85) had higher antibody titers ($P<0.05$) than Kedu chickens (144.6 ± 186.45) (Table 5). Genetically, KUB chickens had the highest level of immunity as reflected by the formation of antibody titers against ND. Rehman, *et al.*, (2021) stated that the inherited immunity system depends on the antigen-presenting cell (APC) and phagocyte cells including dendritic cells (DC), granulocytes, and macrophages activated by microbial components such as Gram-negative bacteria lipopolysaccharides (LPS). Offering feed additives of 1% garlic (451.33 ± 583.22) resulted in the highest average of antibody titers against ND than green chiretta (362.83 ± 596.58), kalimun (224.00 ± 187.58), and control (332.00 ± 606.7). Feed additives of 1% garlic were shown to be the most effective in improving the formation of antibody titers against ND in 4 weeks after vaccination. According to Qiu et al (2007), bioactive compounds that play a role as antibacterial in garlic are allicin, diallyl disulfide, and diallyl trisulfide. Antibacterial activities in garlic can control pathogenic bacteria of both gram-positive and negative.

Infiltration of Lymphocyte and Macrophage in lamina propia ileum

The analysis of variance showed that the interactions between chicken strains and supplementation of feed additives did not significantly affect ($P>0.05$) total lymphocyte and macrophage (Table 3), but the chicken strains alone significantly affected ($P<0.05$) the percentage of total lymphocyte in lamina propia ileum (Table

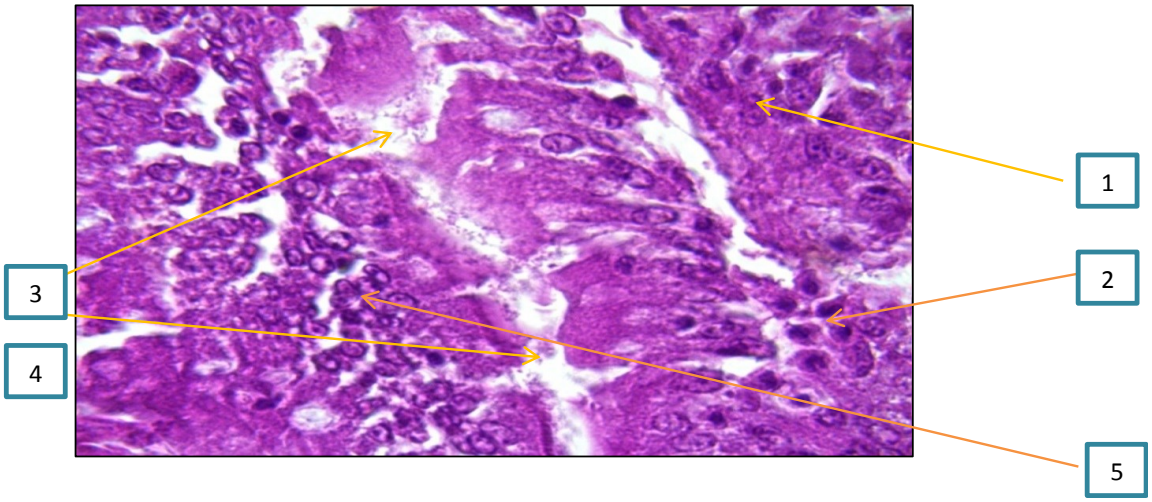

Figure 1. Lymphocyte infiltration in lamina propia ileum at 1000X magnification

(1) – (4) Lymphocyte cells. (5) Other cells in lamina propia ileum.

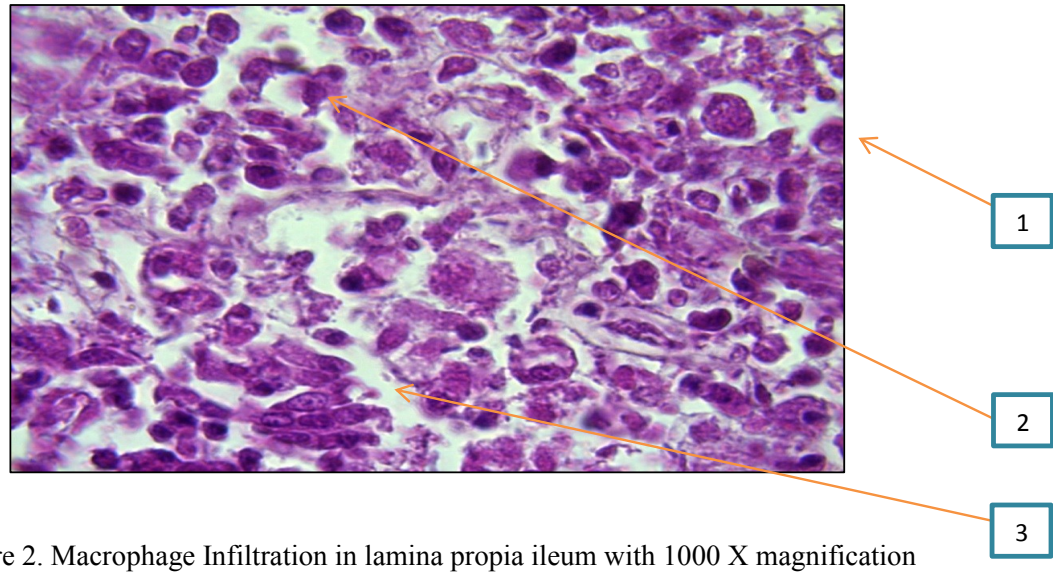

Figure 2. Macrophage Infiltration in lamina propia ileum with 1000 X magnification

Note: (1) – (2) Macrophage cells. (3) Other cells in lamina propia ileum.

5). A lymphocyte is the main cell in the lymphatic system, and its size is relatively smaller than macrophage and neutrophil (Pratiwi and Nurhajati, 2017). The lymphocyte is mostly found in the intraepithelial layer and in lamina propia ileum (small intestines). Lymphocyte cells commonly found in the digestive tract are the lymphocyte T-cells that play a role in the recognition of antigens and transduction signals (Han *et al.*, 2017). The average percentage of the total lymphocyte in lamina propia ileum was 2.068 ± 0.54% with a range between 1.61 ± 0.33% - 2.74 ± 0.55% (see Table 3 and Figure 1). These results reflect those by Pratiwi and Nurhajati (2017) who examined broilers offered with aloe vera supplemented into feed and reported that the average percentage of total lymphocytes in lamina propia ileum was 1.69 ± 0.007% - 2.14 ± 0.30%.

The physiological condition of healthy chickens reflects the weakness of cell B stimulations to produce antibodies because of the absence of foreign bodies that infect the animal. The honestly significant difference test (HSD) showed that KUB chickens (2.898 ± 0.617%) were significantly different (P<0.05) from kampung chickens (1.898 ± 0,429%) and Kedu chickens (1.920 ± 0.429%) (Table 6). KUB chickens had the highest percentage of lymphocyte in lamina propia ileum which may be due to the high level of immunity that stimulates the production of more lymphocyte than that in kampung chickens and Kedu chickens. The higher the lymphocyte infiltration in lamina propia ileum, the higher the production of lymphocyte, and therefore granting the chickens better immunity against diseases.

Supplementation of 1% feed additives did not provide a good response to both specific and non-specific defense system. The active substances in several feed additives can disturb the transduction process (tranfer of genetic material of virus and bacteria) that is very effective against infection agents that disturbs animal (Rychen *et al.*, 2017). Han *et al.*, (2017) explained that a high number of lymphocyte cells in lamina propia ileum mikroskopis is indicative of te migration of lymphocyte cells to chicken's lamina propia. According to Iheukwuemere *et al.*, (2006) lymphocyte cells in lamina propia ileum illustrates the phagocytosis and immune functions performed by lymphocyte cells.

Macrophage is the big-size white blood cells that digest antigen, microbe, and other substances. Macrophage plays a role in cellular and pathogenic phagocytosis as well as stimulating lymphocyte and other body immune cells to be responsive to pathogen (Pratiwi and Nurhajati, 2017). The average of total macrophage in lamina propia ileum of native chicken was overal 0.843 ± 0.20% within the range of 0.69 ± 0.10% to 1.06 ± 0.26% (See Table 3 and Figure 2). The physological condition of healthy chickens reflects the weakness of cell B stimulations to produce antibody because the absence of foreign bodies that infect the animal. We found that the strains of native chicken and several feed additives could not improve the number of macrophage because native chickens genetically have the same capacity of macrophage activity in lamina propia ileum. Also, supplementation of 1% feed additives did not show an increasing effect on both specific and non-specific defense system, particularly in the activities of macrophage cells. Feed additives contributes in surpressing detrimental microorganism. Rychen *et al.*, (2017) stated that bioactive compounds in feed additives can disturb the transduction process of viral genetic materials that may infect the animal body.

Total Protein Plasma (TPP), Blood Urea Nitrogen, and Blood Albumin

Protein plasma is an organic compound of blood plasma consisting of simple and conjugated protein such as glycoprotein and lipoprotein. The result of the present study showed that the total protein plasma among native chickens was within the normal range. The analysis of variance indicated an interaction between the strains of native chickens and 1% supplementation of several feed additives had a non-significant effect (P>0.05) on the total protein plasma, BUN and blood albumin levels of native chicken (Table 4).

Table 5. Average Antibody titers against AI. ND and lymphocyte infiltration in various strains of native chicken

Chicken strains	Antibody titers against AI	Antibody titers against ND	Infiltration of Lymphocyte (%)
Kampung chickens	85.00 ± 76.33 [ab]	282.0 ± 253.64 [ab]	1.898 ± 0.429 [a]
Superior Native Chickens of Balitnak (KUB)	102.62 ± 127.2 [b]	601.0 ± 781.85 [b]	2.898 ± 0.617 [b]
Kedu chickens	21.50 ± 33.14 [a]	144.6 ± 186.45 [a]	1.920 ± 0.429 [a]

Different superscripts show significant difference (P<0.05).

The chickens showed healthy psychological conditions as they were offered the same feed with equal level of protein and Fe, thus the total protein plasma remain unchanged.

Abun *et al.* (2018) stated that total protein plasma is physiologically affected by age, hormone, growth, sex, nutrition intake, parasitic infection, and lost fluid in the body. Similarly, Wamboi *et al.*, (2020) stated that the increase of the total protein plasma was found in chickens infected with Haemoproteus sp. Elagib *et al.*, (2012) added that the concentration of protein plasma would decrease as the environmental temperature increased.

Blood urea nitrogen (BUN) is the main product of protein metabolism. The level of urea in the blood serum is significantly affected by protein degradation in the liver that is excreted into the urine through kidneys (Subayo et al, 2013). The average level of blood urea nitrogen in native chickens was overall 5.31 ± 1.36 with a range of 4.40 ± 0.68 – 6.38 ± 1.42 (Table 4). According to Subayo *et al.*, (2013) the BUN level of monogastric animal is affected by the quality and quantity of the feed consumed. The imbalance amino acids in the feed may affect the concentration of urea in the blood. Chicken strains and 1% supplementation of feed additives did not significantly affect the BUN level; therefore, there is no disturbance in kidney functions, and BUN is produced at normal level. According to Suman *et al.*, (2018) the increase of BUN in blood may also be attributed to several factors including the increased protein catabolism followed by balanced negative nitrogen, overdegradation of blood protein, the decreased rate of glomerular filtration, and toxic chemicals that potentially damage the kidney.

Albumin is the most protein found in the plasma, and therefore, the decrease in albumin is the cause of hypoproteinemia (Kanu *et al.*, 2016). The average level of blood albumin in native chicken is overall 3.02 ± 0.31 in the range of 2.67 ± 0.51 – 3.49 ± 0.49 (Table 4). This result demonstrates similar level of blood albumin with that reported by Ladokun *et al.*, (2008) on their experiment to Nigerian native chickens that live in humid subtropics, namely 3.23–3.34. The level of blood albumin in this study was remain the normal range, which illustrates normal deposition of meat protein. Kanu *et al.*, (2016) reported that the decrease of albumin concentration in the blood may cause a decreasing productivity in chickens because the protein deposition in the body is low. The strains of native chickens and 1% supplementation of feed additives did not significantly affect the level of blood albumin. However, while supplementing feed additives of 1% garlic tends to increase the level of blood albumin compared to control feed, green chiretta, and kalimun. The increase of blood albumin indicates the presence of infection in animal body. The bioactive compounds in several feed additives would also improve body immune system by inhibiting inflammation and body immune cells, like T cells, B cells, and macrophage.

CONCLUSION AND RECOMMENDATION

The interactions between chicken strains and 1% supplementation of different feed additives into basal feed produced relatively similar response to imunity, kidney functions, and blood protein level of native chickens. KUB chicken strain showed a higher level of body immune than that of kampung chickens and Kedu chickens. Supplementing 1% feed additives (garlic and kalimun) showed relatively effective results in improving antibody titers against AI and ND, so it is necessary to increase the supplementation dosage to enhance significant immunity.

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