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Effect of Breadfruit Leaf Powder (*Artocarpus altilis*) on Performance, Fat and Meat Cholesterol Level and Body Immune of Male Native Tegal Duck

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Abstract: This research was aimed to investigate the effect of the breadfruit leaf powder on performance, fat and meat cholesterol level and body immune of male Tegal duck. Completely Randomized Design with six treatments includes b_0: feed without breadfruit leaf powder, b_1: feed + 3%/kg feed breadfruit leaf powder, b_2: feed + 6%/kg feed breadfruit leaf powder b_3: feed + 9%/kg feed breadfruit leaves powder, b_4: feed + 12%/kg feed breadfruit leaves powder, b_5: feed + 15%/kg feed breadfruit leaves powder had applied in this study. Each treatment had 5 replicates with 4 ducks, comprising 120 male Tegal ducks in total. Treatments were given for 5 weeks from one month old to slaughter age of 9 weeks old. Data were subject to analysis of variance and should differ be observed across treatments, Honestly Significant Difference test ensued. Analysis of variance showed that supplementation of breadfruit leaf powder did not significantly affect ($p>0.05$) on body weight, feed conversion and carcass percentage of 9-week old male Tegal duck, but significantly affected ($p<0.05$) fat level, meat cholesterol, total of protein plasma, hemoglobin level and ratio of H/L. Conclusively, breadfruit leaf powder had a strong antioxidant activity and was safe for male Tegal ducks. Supplementing 9% breadfruit leaf powder was effective to lower fat level and meat cholesterol and improve the wellness of male Tegal duck.

Key words: Antioxidant, immunity, native duck, breadfruit leaf

INTRODUCTION

Indonesia has high duck population approximately 46,875,310, scored number three in the world after China and Vietnam (Directorate General of Livestock and Animal Health Services, 2015). Tegal ducks are native Indonesia duck that mostly kept in Tegal Region, Central Java, Indonesia. Female ducks produce eggs and the male, mainly produces meat. Ingram *et al.* (2000) stated that livestock management, feed nutrient and environment condition might induce stress in poultry. Indonesia environment with 20.4-36.8°C day temperature, 18.4-4.2°C evening temperature and 55.3-85.8% humidity (Indonesian Statistics, 2015) is not optimum for duck farming because it can induce stress (El-Badry *et al*., 2009). Oxidative stress is an occurring response when livestock thrives in a hot environment, marked by the depleting appetite and high water intake, then negatively affect physiological process and body immunity (Syahruddin *et al*., 2013; Subekti, 2005). One contributing factor to duck growth and productivity is body immune, which is the indicator of body defense against foreign matters such as bacteria or a virus and protection from free radicals formed within the body. Duck is one of poultry disease vectors on poultry. Immunity system is closely related to the body immune supported by immune cell and antibody. Good immunity will affect performance. Improving body immune is through the utilization of antioxidant mainly found in plant. The health status of duck can be seen from the blood profile such total plasma protein, hemoglobin and the ratio of H/L of blood. Plasma protein synthesized in the liver. Blood plasma protein functions is to regulate the osmotic pressure of the blood, maintain normal blood pressure and helps balance acid base (Nelson and Cox, 2008). Total protein plasma is measured to give an indication of total immunoglobulin concentration and liver function.

One of the antioxidant-rich plants are breadfruit. The breadfruit leaf contains beneficial substances, for example hydrocyanic, acetylcholine, tannin, riboflavin, phenol, saponin, flavonoid and compounds derived from flavonoid type pyranoflavonoid namely cyclocommunin, cyclocommunol and cycloartocarpin (Hakim, 2007; Mulyati, 2009). Flavonoid synthesis of a plant is derived from phenylalanine amino acid and tyrosine yielded from shikimate pathway (Heldt, 2005). High fat and cholesterol level in duck meat are expected to decrease by the utilization of the breadfruit leaf powder. Flavonoid positively affects the prevention of cardiovascular disease and is effective to lower total serum cholesterol, thereby preventing coronary heart disease (Kesuma, 2011). Flavonoids present in the leaf of breadfruit (*Artocarpus altilis)* are quercertin, DS 6, artoindocianin F and cycloaltisilin. Flavonoids have the ability to counteract excessive free radicals generated by the synthesis of bile acids, thereby increasing LPL activity

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due to the occurrence of lipid peroxidation can be inhibited by flavonoids which act as antioxidants. Increased activity of LPL enzyme will hydrolyze triglycerides into free fatty acids and glycerol and can be stored in adipose tissue and muscle tissue. The ability of breadfruit leaf in treating some diseases was allegedly closely associated with antioxidant phytochemicals in these plants are phenolic compounds (phenolic acids, flavonoids, tannins, lignans) and non-phenolic (carotenoids, vitamin C), which have antioxidant substances and activities anti radical. Breadfruit leaf as antibacterial herbs can diversely affect duck health, as observed from hemoglobin and H/L ratio that eventually affects duck performance.

MATERIALS AND METHODS

General procedures: Research was conducted in a 5 x 15 m^2 tile-roofed open cage in Sokaraja Kulon village. The cage consisted of 30 treatment units measuring 1 x 1 m^2 furnished with fan, temperature and humidity meter with litter floor. Each cage has one trough and one water container for 4 ducks. Litter was changed if wet or clumpy. The materials used were four-week-old Tegal ducks, one of Indonesian native ducks. Ducks were fed twice a day and water was provided *ad libitum*. Treatments were given for 5 weeks with one week adaptation period. The daily observed feed consumption was by calculating the amount feed given minus the residue. Body weight was scaled weekly to observe body weight gain, whereas blood sampling through vena brachialis was conducted at 9 weeks old to observe a total of protein plasma, hemoglobin level and heterophyle and lymphocyte level. Ducks were then slaughtered to obtain the carcass.

This experimental research was subject to Completely Randomized Design, each treatment had 5 replicates each with 4 ducks, or 120 male ducks in total. Six treatments include feed without breadfruit leaf powder, feed + 3%/kg feed breadfruit leaf powder, feed + 6%/kg feed breadfruit leaf powder, feed + 9%/kg feed breadfruit leaf powder, feed + 12%/kg feed breadfruit leaf powder and feed + 15%/kg feed breadfruit leaf powder.

The observed variables were duck performances (body weight, feed conversion and carcass percentage), fat level and meat cholesterol and immunity (total of protein plasma, Hb level and H/L ratio) of male Tegal duck. The obtained data were subject to analysis of variance, followed by Orthogonal Polynomial Test if difference across treatments was observed (Steel and Torrie, 2001).

Procedure determining meat fat level: Fat flask was oven-dried at 105°C for 12 h, cooled in a desiccator and weighted. A total of 5 g dry sample wrapped in paper strainer was then stored in soxhlet extractor. The condenser was then placed on top and the flask below. Afterward, the sufficient hexane solvent was poured into the flask followed by extraction for a minimum of 6 hours resulted in the descended of clear solvent to the bottom flask. A flask containing post-extraction fat was then oven-dried at 105°C to vaporize the solvent residue to obtain constant weight and then cooled in desiccator. Fat flask was then weighed to know the fat weight.

Cholesterol analysis: Meat cholesterol analysis was subject to the Lieberman-Buchard method. The principle was cholesterol-bearing chloroform extract would react to anhydride acetic acid and solid sulfate acid to form a colored reaction. The absorption was measured at 420 nm wavelength. The absorption was linear to a cholesterol concentration. Meat was oven-dried at 60°C and 0.02 g was dissolved in alcohol-ether (3:1). The tube was firmly sealed for 1 min shaking, let sit for 30 min and then centrifuged at 5000 rpm for 3 min. Supernatant was poured into a goblet glass and vaporized in boiling water bath. The formed residue was dissolved with chloroform to obtain 5 ml volume. Meanwhile, 5 ml cholesterol standard and 5 ml blanco chloroform were prepared. All tubes were added with 2 ml anhydride acetic and 0.1 solid sulfate acids then shaken hard. Tubes were stored in a dark room for 15 minutes and the solution was measured for absorbability at 420 nm wavelength.

Determining total plasma protein: Analysis of plasma total protein content, was performed using the principle of biuret test. Biuret reagent is pipetted into 62 tubes consisting of a blank tube, a standard tube and 60 test tubes, each as much as 8 ml. Then, 50 μL of distilled water was added into the blank tube. Similarly, 100 μL of standard solution was added to standard tubes whereas 100 μL of protein and plasma to sample tubes. All tubes were then homogenized and then let stand for 30 min at room temperature. The absorbance of the solution of the test samples, standards and blanks was read at a wavelength of 540 nm. The reading values were analyzed by the following formula:

$$\text{Levels of protein} = \frac{\text{Au-Ab}}{\text{As} - \text{Ab}} \times 6 \text{ g/dL/10}$$

Determining hemoglobin: Erythrocyte obtained from blood sampling was sucked using standard erythrocyte pipette to 0.5 level, then a Rees Ecker solution was sucked to 101 level. Erythrocyte pipette was shaken to 8 level so the blood mixed well. Three to four drops of blood solution was then stored in a hem cytometer sealed with glass lid. Erythrocyte was calculated under 45 x magnified microscope. Hemoglobin was determined by the spectrophotometer method by pouring from 0.02 ml blood sample into the test tube

containing 5 ml Drabbing, then shook to homogenous and measured using spectrophotometer.

Determining heterophil and lymphocyte: The number of heterophil and lymphocyte was calculated from the preparations blood smear. Duck blood was collected at the end of treatments from vena brachialis and stored in 2 ml tube for blood smear preparat. Blood smear preparations were started with smearing blood on glass object, then fixated with methanol, colored with giemsa, cleaned with water and left to dry at room temperature. Upon drying, the preparat was observed under microscope to count the percentage of heterophil and lymphocyte (Bain and Path, 2005). The percentage was multiplied with the number of leukocytes to obtain the number of heterophil and lymphocyte. Leukocytes number was calculated using improved neubeur counting chamber after blood was diluted with a Turk solution (Koen Praseno *et al*., 2013). Determining the heterophil/lymphocyte ratio was obtained by dividing the number of heterophil by the number of lymphocyte.

RESULTS AND DISCUSSION

Body weight of 9-week-old duck: Table 1 showed that the body weight of the male Tegal duck that consume the feed added with breadfruit leaf powder was 1334.40± 50.04-1449.40±75.36 g. The result of this study was higher than Ismoyowati and Sumarmono (2011) that reported Tegal ducks has a weight of 1181 g at the age of 9 weeks and feed conversion of 2.4. Breadfruit leaf powder contained flavonoid, vitamin E and C (antioxidant) that very effective on humoral immunity and health status (Tugiyanti *et al*., 2014) but analysis of variance result showed that breadfruit leaf powder supplementation did not significantly affect ($p>0.05$) slaughter weight of 9-week-old male Tegal duck. Vitamin E and C. It indicated that male Tegal duck fed with breadfruit leaf powder supplementation were still able to digest feed well because breadfruit leaf contained not only phosphor, calcium, vitamin C, vitamin B, flavonoid and flavonoid derivation (Umar *et al*., 2007; Hakim, 2007) that improve livestock health, but also high crude fiber digestibility to duck. It was in line with Sutrisna (2012) that 5% crude fiber was significantly different ($p<0.05$) from 10 and 20%, but not significantly different from 15%. Moreover, ducks advantage over chicken was their ability to digest higher crude fiber so as not affecting body weight gain (Purba and Prasetyo, 2014). It showed that duck could still digest feed crude fiber up to 20%, but higher level would decrease daily weight gain that eventually resulted in low slaughter weight.

Feed conversion ratio (FCR): Tugiyanti *et al*. (2013) stated that feed conversion which is strongly related to feed consumption and body weight gain and it could be used to measure feed efficiency and fowl productivity. Average FCR of 9-week-old duck supplemented with breadfruit leaf powder was 2.14±0.17 (b_1) to 2.65±0.03 (b_5). Analysis of variance indicated that supplementation of breadfruit leaf powder did not significantly affect ($p>0.05$) feed conversion of 9-week-old male Tegal duck. FCR in meat duck was closely related with feed efficiency or converting feed into meat. Breadfruit leaf insignificantly affected feed digestibility and conversion into meat. Breadfruit leaf was one of potential herbal plants containing chemical compounds which derived from the plant metabolism. Chemical compounds from primary metabolism, such as carbohydrate, protein and fat are used by the plant in its growth, while secondary metabolite compounds like terpenoid, steroid, cumarin, flavonoid and alkaloid are plant natural product as protection against diseases (Heldt, 2005). Alex (2010) stated that medicine plant is exceptionally useful to improve body immune, to cure many disease and to reduce stress in chicken.

Breadfruit leaf contained higher crude fiber (15.56%), tannin and saponin, but the content did not affect FCR, assumedly due to the high digestibility of crude fiber in the feed. On the other hand, Mohiti *et al*. (2012a,b) reported decreased weight gain as the crude fiber increased. High content of crude fiber is correlated with digestibility and absorbability of ducks' digestive organs. Digestive process of crude fiber in duck occurred in the cecum and the yield of the digestive process was volatile fatty acid (VFA). Fermentative digestive system in male duck digestive tract fed with 15% crude fiber feed contained 295.80 µmol/ml VFA in the ileum, cecum and colon as a potential energy source. Male ducks were tolerant to ration with crude fiber up to 15% and yielded optimum performance. Crude fiber in feed served as bacteria substrate in the fermentative digestive system and the major population of cellulotic bacterial or 5.01 Log 10 CFU/g was in the cecum, thereby degrading crude fiber (Sutrisna, 2010).

Carcass percentage: Analysis of variance result indicated that supplementing breadfruit leaf powder did not significantly ($p>0.05$) affect the carcass percentage of 9-week-old male Tegal duck. As the supplementation could not produce different slaughter weight so the carcass weight was relatively similar to broiler chicken. The relatively similar and linear carcass components to body weight gain would result in similar carcass percentage. Carcass percentage was determined by the amount of body part wasted such as head, neck, feet, viscera, feather and blood. Mahfudz (2009) stated that the carcass percentage of broiler chicken was 65-75% of live weight Randa *et al*. (2002) stated that supplementing higher crude fiber to lower fat content in duck farming also caused the significant decrease of carcass and non-carcass weight; besides, higher crude fiber affected digestion process and nutrient

Table 1: Performance, fat and meat cholesterol level of 9-week-old male tegal duck fed with breadfruit leaves powder supplemented feed

	Performance			Fat and meat cholesterol level	
Treatments	Slaughter weight (g)	Feed conversion (g)	Carcass percentage (g)	Fat (%)	Meat cholesterol (mg/100 g)
b_0	1356.00±0.30	2.39±0.07	68.61±5.54	5.56±0.19^b	174.82±0.03^b
b_1	1449.40±75.36	2.14±0.17	70.09±5.51	5.03±0.07^a	170.45±0.23^b
b_2	1437.60±1.00	2.31±0.19	73.28±2.09	4.78±0.41^a	166.78±0.41^a
b_3	1334.40±50.04	2.61±0.07	78.26±8.72	5.00±0.12^a	157.69±0.49^a
b_4	1365.20±8.04	2.57±0.17	72.96±2.92	5.05±0.02^a	154.88±0.38^a
b_5	1423.60±21.65	2.65±0.03	69.03±6.44	5.40±0.05^b	158.03±0.42^a

b_0: feed without breadfruit leaves powder
b_1: feed + 3%/kg feed breadfruit leaves powder
b_2: feed + 6%/kg feed breadfruit leaves powder
b_3: feed + 9%/kg feed breadfruit leaves powder
b_4: feed + 12%/kg feed breadfruit leaves powder
b_5: feed + 15%/kg feed breadfruit leaves powder

Table 2: Body Immune (Total Protein Plasma, hemoglobin level and H/L ratio) of 9-week old male Tegal Duck fed with breadfruit leaves powder supplemented feed

	Body Immune		
Treatments	Total plasma protein (g/dL)	Hb level (g/100 ml)	H/L ratio
b_0	2.96±0.38	11.08±1.48^a	1.80±1.03^{ab}
b_1	3.04±0.47	11.88±1.09^{ab}	0.65±0.19^a
b_2	3.20±0.37	12.42±1.24^{ab}	0.97±0.10^{ab}
b_3	3.48±0.23	13.12±0.61^b	0.82±0.36^{ab}
b_4	2.72±0.30	11.08±0.87^a	2.43±1.75^b
b_5	2.76±0.48	11.06±1.51^a	1.88±1.19^{ab}

b_0: feed without breadfruit leaves powder
b_1: feed + 3%/kg feed breadfruit leaves powder
b_2: feed + 6%/kg feed breadfruit leaves powder
b_3: feed + 9%/kg feed breadfruit leaves powder
b_4: feed + 12%/kg feed breadfruit leaves powder
b_5: feed + 15%/kg feed breadfruit leaves powder

absorbability. Contributing factors of carcass weight were types of livestock, age and feed. Feed quality, breed and stress during slaughter also significantly affected meat quality (Givens, 2005; Liu and Niu, 2008).

Meat fat content: The average meat fat content across treatment was 4.87±0.19% (control/b_0); 5.03±0.07% (b_1); 4.87±0.19% (b_2); 4.78±0.41% (b_3); 5.00±0.125% b_4); 5.05±0.02% (b_5) and 5.40±0.05 (b_5). Analysis of variance result showed that supplementing breadfruit leaf powder significantly affected ($p<0.05$) meat fat content of 9-week-old male Tegal duck. Duck meat contained 21.4% protein, 8.2% fat, 1.2% ash and 15.900 Kcal/kg energy metabolizable (Amirudin *et al*., 2011). Ismoyowati and Widiyastuti (2003) implied that duck breast meat contained 4.55%, while the leg was 4.77%. Supplementing breadfruit leaf powder to feed lowered the fat content because antioxidant in the leaf could directly blockade HMG-CoA reductase activity and beta oxidation of mitochondria fatty acid because of the density of *carnitine palmitoyltransferase* (CPT-I) as the gate of long-chain fatty acids into mitochondria. Vitamin E, carotenoid, flavonoid and omega-3 protected against the fat-soluble free radical. Therefore, the protective effect was thorough. Consuming antioxidant will protect cytoplasm and the cells against free radicals exposure. High fat content in meat ducks can be reduced by 6% the bay leaf powder supplementation in the diet. Decreased levels of fat due to the activity of bioactive compounds in the bay leaf include flavonoids, tannins and saponins (Ismoyowati *et al*., 2016).

Antioxidant performance in the breadfruit leaf was significantly supported by essential fat-omega-3 ALA. This healthy fat not only serves as an antioxidant but also improves the capacity of other antioxidants to be optimized by cattle body. Body can utilize several carotenoid of breadfruit leaf only when transporting compounds or essential fat distribute it throughout the body. Therefore, bioactive in breadfruit leaf is utilizable to decrease fat.

Furthermore, most electrons serve in the respiratory chain and migrate across the respiratory chain to cytochrome c oxidase. Imbalance between high electron input and electron limitation causes an excessive reduction in complex I and III of the respiratory chain. It serves as the basis of reduced complex reaction with oxygen to form reactive oxygen species (ROS). Antioxidant is a molecule that blockade or prevent the oxidation of other molecules. Oxidative reaction results in free radicals that can damage cells.

The decrease of fat content in fresh duck meat was most likely contributed to antioxidant nature in breadfruit leaf powder. Fat accumulation is related to supplementation of breadfruit leaf powder containing antioxidant and fat-soluble flavonoid, thereby causing a tendency to fat accumulation in body tissue. Supplementing antioxidant at a certain dosage might increase meat fat content, because Gurr *et al*. (2002) and Linder (2006) stated that fat-soluble vitamin tended to accumulate in tissue, causing a more intensive or increasing fat synthesis by liver tissue. Randa (2007) also reported that the effect of supplementing vitamin E and the combination of vitamin E and vitamin A in native duck increased fat content in liver, meat and skin.

Cholesterol level of duck meat: Analysis of variance result showed that supplementing breadfruit leaf powder significantly affected ($p<0.05$) meat cholesterol level of 9-week-old male Tegal duck. Duck meat contained up to

64.86 mg/100 g cholesterol (Muliani, 2014). Ismoyowati and Widiyastuti (2003) stated that cholesterol level in duck breast meat was 166.91 mg/100 g and the leg was 188.41 mg/100 g. Supplementing breadfruit leaf powder to feed lowered cholesterol level because antioxidant content in the breadfruit leaf directly blockade the activity of reductase HMG-CoA. Enzyme activity blockade made mevalonic was not formed from HMG-CoA, but converted into squalene, lanosterol, dihyrolanosterol, D 8-dimethylsterol, 7-dihydrocholesterol and eventually cholesterol. Antioxidant is furthermore able to lower VLDL, triacylglycerol and additional cholesterol in blood circulation. VLDL is IDL precursor and IDL is the precursor of LDL. LDL distributes cholesterol from the liver to body tissues. LDL binds with LDL receptor on membrane walls to enter cells. Bound LDL will undergo endocytosis and break inside ribosome. Cholesterol absorbed in cell will increase the number of LDL receptor which decreases the LDL amount in circulation (Aprila, 2010). Previous research reported that flavonoid work by blocking cholesterol synthesis through reductase HMG CoA inhibitor. Saponin content in breadfruit can form complex bound that does not dissolve in cholesterol, therefore cholesterol cannot be absorbed in the intestines and tannin serves in blocking fat absorption.

Lectin in breadfruit leaf can prevent protein synthesis on cell walls and vitamin C as antioxidant in lectin reduces the risk of atherosclerosis by blocking LDL metabolism in secondary atherosclerosis lesion to prevent LDL oxidation in atherosclerosis lesion. Vitamin C also serves in cholesterol metabolism by: (1) increasing cholesterol rate that is discarded in the form of bile acid, (2) increasing HDL and (3) decreasing reabsorption of bile acid and the conversion into cholesterol and forming collagen to prevent atherosclerosis. The atherosclerosis blockage through LDL oxidation resistance is indicated by VCAM-1 secretion in endothil due to oxidized LDL, part of which is preventable by supplementing antioxidant (vitamin E and probucol) in endothil cells. Antioxidant also reduces LDL toxicity that is oxidized against endothil, smooth muscle and macrophage, decrease oxidative degradation due to nitric oxide, limit vasoconstriction and lower blood pressure (Suresh *et al*., 2012; Middleton *et al*., 2000).

Total plasma protein: Mean total plasma protein ducks in each treatment was b_0: 2.96±0.38, b_1: 3.04±0.48, b_2: 3.2±0.37, b_3: 3.48±0.23, b_4: 2.72±0.30 and b_5: 2.76±0.48 g/dl. Ismoyowati *et al*. (2006) reported that the total protein in the blood plasma of drake was 3.47±0.50 g/dL. Analysis of variance result demonstrated that supplementation of breadfruit leaf powder significantly affected ($p<0.05$) on total plasma protein of 9-week-old male Tegal duck. It showed that the breadfruit leaf powder supplementation improves the health status and suppressed the incidence of disease. Epidemiological studies have reported that breadfruit leaf phenolic compounds are effective against chronic fatal diseases (Ahmad and Beg, 2013; Hseu, 2008). The antioxidants contained in the breadfruit leaf are phenolic compounds (phenolic acids, flavonoids, tannins, lignans) and non-phenolic (carotenoids, vitamin C), which have antioxidant substances and activities antiradical highly effective in preventing animal disease.

Plasma protein serves to maintain the osmotic pressure, as a source amino acids for the network, transport nutrients to the cells and waste products to organ secretions and maintain acid-base balance of the body (buffer) (Frandson, 1992). Proteins found in plasma comprising of albumin, globulin and fibrinogen. Albumin has the ability to bind a variety of ligands and is responsible 80% of the osmotic pressure (Ganong, 1999). Globulin associated with the immune system (Kaneko *et al*., 1997).

Hemoglobin level: Hemoglobin is the red pigment that serves importantly to transport oxygen and nutrient to body tissues. Schlam *et al*. (1986) reported that each erythrocyte contained 400 million hemoglobin. Contributing factors to erythropoiesis and the amount of red blood cell also affect the hemoglobin level. Analysis of variance result demonstrated that supplementation of breadfruit leaf powder significantly affected ($p<0.05$) hemoglobin level of 9-week-old male Tegal duck. It showed that the supplementation did not interfere duck's hemoglobin level, instead improving the system of transporting oxygen and nutrients into body tissues. It was because breadfruit leaf contained antioxidant (flavonoid and vitamin A, B, C). Antioxidants reduce free radicals and maintain Fe 2^+ reduction status in hemoglobin, thereby improving oxygen and nutrient transport (Suryanto and Wehantouw, 2009; Shahidi, 1997). Supplementing 9% breadfruit leaf powder resulted in the highest hemoglobin level, 13.12±0.61 g/100 ml. Duck hemoglobin level in this research was 11.06±1.51-13.12±0.61 g/100 ml blood, or within the normal range. Ali *et al*. (2013) reported that blood hemoglobin in the male Tegal duck was 10.27±1.15-11.23±1.23 g/100 ml blood, Sturkie (1976) reported 14.2 and 12.7 g/100 ml blood in mature male and female Pekin duck, respectively (Sahli method), 13.3 and 12.7 g/100 ml blood in mature native male and female Indian duck, respectively (Wong or iron method). The amount of hemoglobin greatly varied in several literature as a result of different observation method.

H/L ratio: Body immune indicator as a duck response to factors causing stress and disease can be observed from blood components such as a heterophil-lymphocyte ratio (H/L). Analysis of variance result indicated that supplementing breadfruit leaf powder

significantly affected ($p<0.05$) H/L ratio of 9-week-old male Tegal duck. It showed that supplementing breadfruit leaf powder could improve duck body immunity. Active compounds such as alkaloid, flavonoid, tannin, saponin, glycoside and steroid/tripenoid that serve as antioxidants, antibacterial, antibiotic and anti-inflammation in the breadfruit leaf can improve duck health and body immunity.

Heterophil is an essential component in innate body immune, works at the initial infection, immediately detects and kills pathogens and directs signal to the other immune response mechanism. Heterophil also detects bacteria molecule through receptor which stimulates heterophil to conduct phagocytosis and induce cytokine expression. Heterophil contains antimicrobial that is released through degranulation to kill bacteria through phagocytosis process (Ferro *et al*., 2004; Redmond *et al*., 2011). Heterophil defense mechanism is the first hand-activated defense during inflammation response, thereby running important role in poultry immunity against disease (Harmon, 1998). Heterophil can respond to pathogen in 30 min during the initial inflammatory phase. The increasing response of innate immunity will reduce disease possibility, thus increasing productivity (Farnell *et al*., 2006). Result demonstrated that the increasing H/L ratio in duck indicated no infection that caused physiological stress. Lymphocyte status in duck served importantly to determine duck wellness. The duck has two main lymphocytes, lymphocyte B and lymphocyte T. Some microorganism thrives and breed intracellular, which difficult for antibody to reach. The T cell is needed to fight against the intracellular microorganism.

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