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Blood Profile and Performance of Native Chicken with Functional Feed

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Abstract: Native chicken quality performances could be improved through high quality feed. Thus, their growth, body weight and egg production could be optimized. This research used 60 hens of 6 weeks old native chicken with functional feed which consist of : menhaden fish oil, N-3 isolate of Histamine Methyl Transferase enzyme producer, *Lactobacillus* and *Bacillus* sp. at 1:1. The treatment consisted of 4 groups i.e., R0 (control), R1 (the use of functional feed at 2.5%), R2 (the use of functional feed at 5.0%); R3 (the use of functional feed at 7.5%). These are arranged based on a Completely Randomized Design with 4 replications, each replications with 5 hens, respectively. The measured variables were blood profile (cholesterol, triglycerides, LDL, HDL, erythrocyt, leucocyte, hematocrit and plasma protein) and performance (feed consumption, lipid consumption, energy consumption and carcass percentage). The results revealed that functional feed has increased very significantly ($p<0.01$) on lipid consumption, has increased significantly ($p<0.05$) to carcasses percentage. However, not significant difference ($p>0.05$) was observed on blood profile. A subsequent test was done with Honestly Significant Differences test to show that using functional feed up to 7.5% in fed could reduce lipid consumption, increased carcasses percentage but still produced relatively similar blood profile.

Key words: Functional feed, blood profile, native chicken

INTRODUCTION

Native chicken is a kind of Indonesian local chicken which is believed to be the most qualified sources for healthy eggs and chicken meat among Indonesians. Chicken's health is supported by the existence of balanced physiological condition of the blood, remembering the importance of blood in absorbing and transporting nutrient, hormones and enzymes. Blood also has a role in water turn-over, metabolism residue transport and homeostasis balance control. To keep the balance of blood physiological condition we can control the feed and give safe feed additives. One of the additives that can be used is functional feed.

Functional feed is a kind of feed which is aimed to improve the poultry health as well as production performances. This kind of feed contains probiotics (*Lactobacillus*: *Bacillus* sp. 1:1), menhaden fish oil and antihistamine producer bacterium N3 This functional feed is addressed to functional food definition. Functional food is naturally or through series of process containing one or more substance (s) which is approved scientifically to have certain physiological functions. Those physiological functions are mainly in health aspects. Feeding functional feed to broiler improved the poultry health status, reducing histamine content and fishy smell and producing higher quality of broiler meat. Functional feed material other than PUFA-omega-3 which is able to be used as immunostimulant is probiotic. The use of *Lactobacillus* sp. and *Bacillus* sp. as probiotic at ratio 1:1 had been proved improving broiler and egg layer performance, productivity and their immune response toward diseases (Iriyanti *et al*., 2009). *Lactobacillus* acts as immunomodulator through improving natural and adaptive immune responses (Wells, 2011). Motawe *et al*. (2014) showed the effect of probiotic plus yeast as a potential protective agent against aflatoxin toxicity which decrease the risk of occurrence of liver and kidney dysfunction. Some probiotic microorganisms may be reduced or eliminated by the low pH in the gizzard and thus have little effect in the lower intestinal tract where pathogens pose problems. Vargas-Rodriguez *et al*. (2013) showed that the results of this study indicated that, 0.1% probiotic (1×10^7 cfu/g of *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei* and *Streptococcus faecium)* increased the lean leg meat yield and the population density of 16 birds per m^2 generates a higher fat deposit in broiler chickens.

Healthy feed materials such as menhaden fish oil contains high concentration of essential fatty acids such as EPA and DHA. Wildan (2000) reported that various menhaden fish oil contained EPA 10.58-14.38% and DHA 5.62-5.73%. Others function of omega-3 fatty acids (PUFA)-enriched eggs are decreases the risk of heart disease, inhibits the growth of prostate and breast cancer and is required for normal fetal brain and visual development (Lewis *et al*., 2000).

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Menhaden fish oil could reduce very low density lipoprotein (VLDL) cholesterol content and triglycerides in cock blood. In addition, it also could reduce triacylglycerols, cholesterol and lipoprotein in blood serum (Aydin *et al*., 2006) by reducing lipogenesis process in liver and VLDL secretion (Du and Ahn, 2002; Tabeidian *et al*., 2005), as well as suppressed lipid synthesis (Cortinas *et al*., 2005). However, menhaden fish oil is not only containing omega-3 but also histamine which potential to trigger allergic reaction. Histamine synthesized by the present of histidine decarboxylase which produce by microorganisms (Kim *et al*., 1997). It was produced in mast cells in peritoneal and connective tissues (Marieb, 2001). Histamine concentration could be suppressed by controlling histidine decarboxylase activity with Histamine Methyl Transferase (HMT) and Diamine Oxidase (DAO).

MATERIALS AND METHODS

Experiment livestock: This research used 60 hens of three months old native chicken, were reared during four months (3-6 months) and randomly placed at bamboo battery cage sized 25 x 35 x 35 cm^3 with the feed and water were placed in the front of cages.

Experiment feed: The functional feed consisted of: 10% menhaden fish oil and 30% N-3 Isolate, 30%, *Lactobacillus* sp. and 30% *Bacillus* sp. suspension of 10^6 cells/ml, respectively. All experimental diets were isonutrient and isocaloric states, was arranged to contain feed protein at 19% and metabolic energy at 2800 cal/kg, based rice bran, corn, fish meal, soybean meal, palm oil, $CaCO_3$ and top mix (Table 1). The chemical composition of the feed was analyzed according to AOAC (2006). Feed and water were given *ad libitum*.

Research procedures: This research was conducted for four months, including a 2-weeks period to adaptation to the experiment feed by using a Complete Randomized Design (Steel and Torrie, 1994). There were 60 hen native chickens divided randomly in four treatments and five repetition with each trial consists of three hen native chickens. The treatments consisted of four groups i.e. R_0: Control, R_1: Use of functional feed at 2.5%; R_2: Use of functional feed at 5.0%; R_3: Use of functional feed at 7.5%.

Variables observed were blood profile (cholesterol, triglycerides, LDL, HDL, erythrocytes, leucocyte, hematocrit and plasma protein) and performance (feed consumption, lipid consumption, energy consumption and carcass percentage). After four months observation, blood samples were taken from vessels under the wings (vena axillaries), Three mililiters of blood samples were filled into tubes containing blood anticoagulants (EDTA/Ethylene Di-amine Tetra Acetic acid).

Blood profile: Cholesterol was determinate by cholesterol oxidase para-amino phenazone (CHOD-PAP) enzymatic. Triglyceride was determined by "gliycerol-3-phosphate-oxidase para-aminophenazone" (GPO-PAP) "enzymatic colorimetric" method (DiaSys, Germany). HDL cholesterol was second tube determined by enzymatic colorimetric after precipitation of lipoprotein with phosphotungstate-$MgCl_2$ acid method (DiaSys, Germany). serum VLDL+LDL cholesterol was calculated by subtracting total cholesterol with HDL cholesterol.

Hematological profile: Enumeration of erythrocytes was carried out by diluting blood 1:101 in a red blood cell pipette with Natt Herrick solution and counting the number of Red Blood Cells (RBC) using an improved in a haemocytometer chamber.

Total leukocyte count was obtained using an haemocytometer with Natt and Henrick's diluent to obtain an 1:200 blood dilution. The number of leukocytes were thereafter estimated as total WBC µl = number of cells to total WBC x 200. Packed cell volume (PCV) was measured by the micro-hematocrit method with 75 x 16 mm capillary tubes filled with blood and Hettich hematocrit centrifuge at 3000 rpm for 5 min and than Micro-capillary Reader and Total Protein Plasma values were determined by use of a refractometer (Chowdhury *et al*., 2005).

Sampling was performed in four months after the hens treated by feed consumption, lipid consumption is feed consumption x fat of ration (%) and energy consumption = feed consumption x energy of ration (calorie). Carcass percentage evaluation, taken from 5 selected chickens per treatment, with a subsample of 20 chickens per treatment for a total of 60 chickens.

Statistical analyses: Data were analyzed using Nested ANOVA Random Complete Design (CRD) If the there are any significant different between treatments. It was followed the Honestly Significant Differences test (Steel and Torrie, 1994).

RESULTS AND DISCUSSION

The treatments did not significantly affect ($p>0.05$) to blood profile and hematological levels (Table 2). These figures indicated that chicken was healthy, there was not any abnormality in blood as well as lipid metabolic products in blood. The feed trial up to 7.5% content could be used as mix feed in ration without any disturbances in nutrient metabolism and health.

The cholesterol level in result of this study shows decreasing level from 170.27 mg/dl to 123.04 mg/dl (27.74%) although statistically it was not that significant. Murwani *et al*. (2011) showed broiler chicken blood cholesterol was around 94.19-135.61 (mg/dl). Addition of menhaden fish oil up to 1.5% could reduce blood

Table 1: Ingredients and composition of feed treatment

	Treatment			
Feed ingredients (%)	Ro	R_1	R_2	R_3
Corn	47.5	45.0	42.5	40.0
Rice Bran	26.0	26.0	26.0	26.0
Soybean meal	14.0	14.0	14.0	14.0
Fish meal	10.0	10.0	10.0	10.0
Functional feed	0.0	2.5	5.0	7.5
Oil	0.2	0.2	0.2	0.2
$CaCO_3$	2.0	2.0	2.0	2.0
Premix	0.3	0.3	0.3	0.3
Total	100.0	100.0	100.0	100.0
Calculated analysis				
Protein (%)	19.19	19.27	19.35	19.42
ME (calori/kg)	2709.10	2745.45	2757.30	2880.50
Crude fiber (%)	5.02	5.51	6.11	6.08
Crude fat (%)	6.62	8.42	8.60	6.85
Calcium (%)	2.45	2.45	2.46	2.46
Phosphor (%)	1.23	1.23	1.23	1.23

ME: Metabolic energy

cholesterol total from 223.30±3.63 mg/dl (in control) to 125.83±4.23 mg/dl (Chashnidel *et al*., 2010).

Cholesterol is an important molecule that has roles in membrane structure as well as being a precursor for the synthesis of molecules such as steroid hormones, vitamin D and bile acids. Cholesterol can be obtained directly from the diet, or it can be synthesized in cells from 2-carbon acetate groups of acetyl-coenzyme A. Because the synthetic pathway is under feedback control from dietary cholesterol, the percentage of cholesterol arising from de novo biosynthesis or from the diet depends upon the amount of cholesterol that is ingested. Even when cholesterol intake is very low, de novo biosynthesis will enable the production of the cholesterol required to supply the large variety of biological processes in which this molecule is involved (Ponte *et al*., 2004).

Lipid consumed as triglyceride will be hydrolyzed in digestive system to monoglycerides, free fatty acids and glycerol. Free fatty acids is re esterification into intestine cells before excreted into blood (Lanori, 2002). Esterification fatty acids would produce triglyceride, cholesterol and cholesterol ether. But, non-esterification fatty acids would produce free fatty acids (Martin *et al*., 1985). Essential fatty acids have found in cells lipid structure, connected into integrity of mitochondrion membrane structure and found at high concentration in reproduction organs, phospholipids and they have acted as cholesterol development precursor (Harper *et al*., 1977).

Fish oil could reduce lipogenesis in liver and VLDL secretion (Du and Ahn, 2002; Tabeidian *et al*., 2005), suppress lipid synthesis (Cortinas *et al*., 2005), coordinator and regulator of lipid oxidation and fatty acids synthesis (Clarke, 2001). Fish oil also controlled hepatic enzymes concentration in glucose metabolism and fatty acids biosynthesis. Omega-3 could reduce triacylglycerols, cholesterol and lipoprotein levels in blood serum (Aydin *et al*., 2006). It also increased β-oxidation and receptor expression for LDL (Belzung *et al*., 1993).

After 7.5% functional feed given, the cholesterol level decreased but the fat level increased. This happened because the fish oil in the functional feed can inhibit the formation of HMG-CoA reductase enzyme that can inhibit the formation of cholesterol. Chashnidel *et al*. (2010) stated that cholesterol biosynthesis can be pressed with the existence of Omega-3 that can decrease the activation of HMG-CoA reductase and pyruvate kinase enzymes. Menhaden fish oil is long chain fatty acid that can decrease the level of fat and cholesterol in blood. Schreiner *et al*. (2004) stated that menhaden fish oil contains omega-9 (oleic acid) around 15.55%, omega-6 fatty acid around 8.91% and omega-3 around 26.29%.

The study result showed HDL level from 12.20±3.06 to 19.16±8.36 mg/dl and LDL level from 104.48±8.43 to 158.07±8.84 mg/dl. Although statistically there was no significant difference but the level of HDL after the usage of functional feed increased while the LDL level decreased. Murwani *et al*. (2011) found that broiler chicken serum had VLDL+LDL 23.17-55.33 mg/dl and HDL 40.43-113.94 mg/dl, respectively.

HDL role in blood cholesterol transport is bigger than LDL. Lehninger (1997) stated that if HDL level increased, cholesterol level decreased because HDL will transfer the cholesterol from muscle to liver and liver to tissue. Meyer *et al*. (2006) and Volek *et al*. (2005) stated that the increase of HDL cholesterol level can be affected by the increase of production from liver to intestinal mucosa.

Fish oil as omega-3 source can increase the role of HDL as antioxidant. Norata *et al*. (2003) stated that HDL cholesterol has a role in coagulation, fibrinolysis, platelet and molecules nearby attachment and protease expression that affect the antioxidant activities. That suits Lee and Lip (2003) who stated that fish oil supplementation can increase the HDL level and decrease triglyceride level.

Erythrocytes amount depends on gender, age, body condition, daily variation and stress condition. The amount of Erythrocytes also depends on the size of the blood cell itself (Schmidt and Nelson, 1990). Meanwhile King *et al*. (2010) show erythrocytes in Zambian helmeted guinea fowl (*Numida meleagris*) 2.44 ($x10^{12}$) cells/l. Erythrocytes in normal chicken was around 2.5-3.5 x 10^6/µl while the study showed the level around 2.24±0.47 x 10^6/µl to 2.74±0.81 x 10^6/µl. In the same path with this study, the erythrocytes in native chicken in Tamzil *et al*. (2014) study was around 2.46±0.04 ($x10^6/mm^3$). Whereas, Toghyani *et al*. (2006) mentioned that concentration of erythrocytes in commercial chickens kept at 33°C at the age of 21 days was $2.31x10^6/mm^3$.

One of the other factors that can affect the number of erythrocytes in circulation is hormone. The formation of erythrocytes is through some processes. The first cell as

Table 2: Blood profile native chicken hens

	Functional feed			
Variable	0%	2.5%	5.0%	7.5%
Cholesterol (mg/dl)^{ns}	170.27±9.68	135.68±5.76	134.05±2.42	123.04±7.07
HDL (mg/dl)^{ns}	12.20±3.06	13.72±3.53	19.16±8.36	18.56±5.60
LDL (mg/dl)^{ns}	158.07±8.84	121.96±5.33	114.89±4.00	104.48±8.43
Erythrocyte (x10^6/mm)	2.74±0.81	2.70±0.80	2.74±0.56	2.24±0.47
Leucocyte (x10^3/l) ^{ns}	9.34±1.96	8.74±1.24	9.91±3.14	8.13±2.16
Hematocrit (%)^{ns}	28.8±8.11	30.4±6.27	28.8±5.54	26.2±4.44
Plasma protein (g/dl)^{ns}	3.12±0.61	4.08±1.27	2.8±1.30	3.46±1.21
Effect	ns	ns	ns	ns

HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; Ns: Not significant

Table 3: Performances of native chicken hens

	Functional feed				
Variable	0%	2.5%	5.0%	7.5%	Affect
Feed consumption (g)	110.31±7.00	110.40±3.16	110.23±6.45	110.57±6.46	ns
Lipid consumption (g)	7.30±1.79	9.30±0.27	9.48±0.55	7.57±0.44	**
Energy consumption (cal)	298.83±73.30	303.09±8.68	302.59±17.7	318.49±18.61	ns
Carcass Yield (%)	50.51±4.66	51.55±2.29	52.13±1.55	50.60±2.53	*

Ns: Not significant; *: ($p<0.05$); **: ($p<0.01$)

part of the red blood cell is proerythroblast which will divide and form basophilic erythroblast. These first generation cells are called basophilic erythroblasts because it can be colored with base color, these cells contain very little amount of hemoglobin. In the next process, the cell is already full with hemoglobin at 34% concentration, so the nucleus become denser and smaller. In the meantime, reticulum endoplasm is being reabsorbed. In this stage, the cell is called reticulocyte because it still contains basophilic materials which normally will vanish and the cell will then become mature (Praseno, 2005).

Leucocytes levels after the treatment was 8.13-9.91 x 10^3 cells/µl, it considered lower than normal leucocytes level which commonly is 12-30x10^3 cells/µl. However, it is not indication of any health problem to the animals. This statement was supported by that fact there was not any sick and or mortality among animals during research. Lymphocyte is a leucocyte component in blood which responsible for immune system (Cahyaningsih *et al*., 2007). King *et al*. (2010) showed a haematological for Zambian helmeted guinea fowl (*Numida meleagris*) i.e. leukocyte was 21.42 (x10^9 cells/l). Ulupi *et al*. (2014) showed the leukocyte level in native chicken around 19.50±3.83 to 22.69±9.11 (x10^3 cells/mm^3) and 5.89±0.09 (x10^3 cells/mm^3) according to Tamzil *et al*. (2014).

Hematocrit level based on the study was around 26.2±4.44 to 30.4±6.27%. In the same path with the study result was Tamzil *et al*. (2014)'s study which showed the hematocrit level in native chicken around 32.46±0.55%. On the other hand, erythrocyte concentration in blood were positively correlated with hematocrit values (r = 0.8). This meant that the decrease in erythrocyte concentration would be followed by a decrease in hematocrit value. The normal level in chicken is around 30-50%, so it can be concluded that the number result of this study is nearly normal. Some factors that affect the hematocrit level are: erythrocyte, thrombocyte and leukocyte levels.

This research indicated plasma protein levels was slightly lower than normal which was 2.08-4.08 g/dl rather than 4.0-5.2 g/dl. According to Sutrisno (1985) The differences in plasma protein levels affected by protein content in feed, sex and age. Plasma protein will high if protein in feed is high. Younger animals tend to have higher plasma protein than the older ones. Murray *et al*. (2009) mentioned that plasma protein has several function such as controlling equillibrium body liquid and osmotic and intra vascular pressures.

Plasma protein level based on the study was lower than the normal plasma protein level i.e. 2.08-4.08 g/dl while the normal level are 4.0-5.2 g/dl. The difference in plasma protein level according to Sutrisno (1985) is influenced by the protein content in feed, gender and also the growth level. The higher protein contained in the feed will result in higher plasma protein level. At young animals, plasma protein is more likely higher. Plasma protein according to Murray *et al*. (2009) has some functions like control extracellular fluid balance, osmotic pressure and intravascular pressure. King *et al*. (2010) showed a haematological for Zambian helmeted guinea fowl (*Numida meleagris*) which were: plasma protein 4.03 g/dl, hematocrit 38.1% erythrocyte 2.44 (x10^{12}) cells/l, leukocyte 21.42 (x10^9) cells/l.

During the trial there was not any differences in chicken performances. It indicated that the feed trial did not affect physiological process in animals. The analysis of variance indicated that the treatments did no significantly affect ($p>0.05$) to feed and energy consumptions but significantly affect ($p<0.05$) to carcass yield and very significantly affect ($p<0.01$) to lipid consumption.

Feed consumption in this research was 110.23±6.45 up to 110.57±6.46 g each chicken per day. Ulupi *et al*.

(2014) showed that feed consumption of native chicken with AG and GG genotypes of TLR4 gene (92.19 and 91.20 g each chicken per day) and the feed consumption of AA genotyped chicken (91.70 g each chicken per day) was similar to the other genotypes of native chicken.

The study shows that actually, the increase of fat and energy consumption will be followed by the increase of resulted carcass. The average of the study shows lipid consumption as much as 7.30±1.79 (R_0) to 9.48±0.55 g each chicken per day (R_2) and energy consumption 298.83±73.3 to 318.49±18.61 calorie each chicken per day, while the carcass yield around 50.51±4.66% (R_0) to 52.13±1.55% (R_2).

Diwyanto (1999) showed that in comparison to the biological performance of improved chickens, native chickens are genetically capable of producing about half as many eggs and even less meat with dressing percentages at 12 weeks of age are about 63% of live weight, with a very low abdominal fat pad (0.82% of live weight).

An increasing focus on healthy food, low cholesterol and low fat meat, products might increase the demand for meat of native chickens, particularly by consumers who live in cities. Indications are that improved chickens selected for high growth rates could produce more meat with a considerable amount of body fat (about 3 or 4% abdominal fat, 1.3% breast fat, 6.8% thigh fat and 34.2% skin fat) compared to native chicken (about 0.82% abdominal fat, 0.8% breast fat, 4.4% thigh fat and 21.6% skin fat) (Triyantini *et al*., 1997).

Consumption of lipid in native chicken has strong effect to their growth especially to carcasses weight. Body lipid will be digested to be essential fatty acids and glycerol in intestine. It was absorbed as micelle and chylomicrons then metabolized to produce energy. Energy in chicken is used for their life such as growth, production and reproduction. Lipid consumed by chicken in form of triglycerides will be digested into monoglycerides, free fatty acids and glycerol in digestive system.

Essential fatty acids needed by body for growth and normal function of tissues and organs. It can not be synthesized by the animal itself. Some of those fatty acids are alpha linoleic acid (omega-6) and alpha linolenic acid (omega-3). Activity of linolenic acid was affected by the increase of denaturation activity. Synthesis of omega-3 and omega-6 are depended on fatty acid profile in feed (Raes *et al*., 2002). The ideal ratio of omega-3 and omega-6 is about 1:5 (Farrel, 1995). Omega-3 and omega-6 will interact well on reducing blood cholesterol levels if they are in optimal ratio.

Conclusions: The use of Functional Feed with menhaden fish oil, N-3 isolate of Histamine Methyl Transferase enzyme producer, *Lactobacillus* sp. and *Bacillus* sp. in local chicken up to 7.5% could reduce lipid consumption and increase carcass yield but the result as relatively similar effect to blood profile, feed and energy consumption.

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